DEDICATED TO THE FOND MEMORY
OF
Padma Bhushan
Prof. Dr H. NARASIMHAIAH

Former Vice-Chancellor
Bangalore University
FOREWORD BY THE VICE CHANCELLOR

Bangalore University is committed to excellence in research and teaching. The staff and students are our assets. The University views each one of its activities as an investment that helps in achieving its objectives.

The ultimate goal of the University, as laid down by the University Grants Commission, is to improve academic performance and aim for excellence. The Golden Jubilee volume on "A FRONTIER JOURNAL IN SCIENCE" is an attempt in this direction. The volume has contributions to a cutting-edge area from researchers within India. I understand that this Golden Jubilee volume contains compiled and reviewed papers on various facets of Biology covering microbiology, plant biotechnology, molecular entomology and ecology. I compliment the editorial team of Vijnana Bharathi for having come up with this volume and hope that the readers from within our University and outside benefit from this.

Prof. Dr B. Thimme Gowda
Vice Chancellor
Bangalore University
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Bangalore University, Bangalore, has introduced ‘The Science Journal’ in 1995 and has been catering to the needs of various disciplines of Science. The journal is published as a half yearly (with special issues on specific topics in between) and accepts scientific research articles based on original work, complying with the objectives and the impact on society and scientific community. It also publishes Basic and Applied science research articles. Vijnanabharathi also encourages articles with interdisciplinary approach. The articles are reviewed and published. Vijnanabharathi holds the copyright of articles it publishes, and any act of exact or altered versions will be considered as copyright violation.

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- Computer Sciences → Algorithm and computational complexity, Computer architecture and high performance computing, Distributed and grid computing, Human-computer interaction,
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**Review articles:** The journal publishes both original and invited review articles. The Editor-in-Chief, in consultation with the Editorial Board, identifies and invites reviews in specialized research areas. Additionally, unsolicited review articles can also be submitted.

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**Student articles:** Aimed to encourage and promote the participation of students, this section would also include reports of important scientific developments that will impact the ongoing frontier areas of research. The articles can either be experiment based or hypothetical approaches that can be adopted in frontier areas of research. All the content in this section will be published subject to peer review.

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The cover letter should indicate the corresponding author's full address and telephone/fax numbers and should be in an e-mail message sent to the Editor, with the file, whose name should begin with the first author's surname, as an attachment. The authors may also suggest two to four reviewers for the manuscript.

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These should not exceed 4000 words, 6 display items (tables and figures), should describe new and carefully confirmed findings, and experimental procedures should be given in sufficient detail for others to verify the work. The length of a full paper should be the minimum required to describe and interpret the work clearly.

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Order of manuscript

1. Title page,
2. Abstract,
3. Key words,
4. Text,
5. Acknowledgements,
6. References,
7. Tables,
8. Figures,
9. Legends (on separate page preceding the first figure),

Title page: The Title should be a brief phrase (capitalize first letter of each word in the title). The Title Page should include the authors' full names and affiliations, the name of the corresponding Author along with phone, fax and E-mail information.

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The Abstract should be informative and completely self-explanatory, briefly present the Topics, state the scope of the experiments, indicate significant data, and point out major findings and conclusions. No literature should be cited. Abbreviations should be avoided as much as possible. The Abstract should be 100 to 300 words in length.

Key words (2-6) should be provided below the Abstract to assist with indexing of the article.

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Acknowledgements should be as brief as possible.

I. Under Reference:
References citation should be superscripted in the text
Ex. (i) ........................ are the pioneers in the field of biosciences
Ex. (ii) Misra and Naha have identified two types of species
Ex. (iii) ...................... 1000 sq. km area has been occupied by these species.
The above superscripted citations have to be numbered & shown in the reference list as follows: (Ex...)
4. et al. – should be uniformly maintained in the text.

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(This should be changed accordingly in the cover page, as well as in the entire volume)
(ii) Give space between Vijnana Bharathi in all the pages of volume.
(iii) The bottom line in each page is to be thinned.

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(i) Except Figures / photographs / drawings / graphs pertaining to articles, all other features should be in black and white. Figures/ photos/ drawings/ graphs can also be in black and white if necessary.
(ii) However, cover page will be in colour.

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Studies On Indoor Airborne Fungal Spores Of Animal Rearing Houses At Hessaraghatta Village, Bangalore

Pavan R and Manjunath K

A comparative study of freshwater fish diversity of upstream, middle stream and downstream of Beiti River of Western Ghats region of Karnataka.

Sooryanarayanan S. Bhat and Dr. A. K. Hegde

Wolbachia association and its phylogenetic affiliation of Brugia malayi parasites from India.

Ravikumar H, Surendra N S, Prakash B M and Puttaraju H P.

In-vitro studies in Calamus prasinus, C. thwaitessi and C. vattayila


Cliff Swallows Are Good Mansions For Their Own Plan And Architecture Of Nest

Chaya H C, E Santosh, and Channaveerappa H

Survey On Outdoor Airborne Fungal Spores Of Bangalore City And Correlation With Meteorological Data

Pavan R, Narendra Babu S, Manjunath K and Nagendra Prakash B.S

Impact of mud puddling on reproductive success of Talicada nyseus L. (Lepidoptera: Lycaenidae) harboring Wolbachia infection

Kunal Ankola and Puttaraju H.P.

Evaluation of endophytic fungal spp. for Biodiesel production

Ravi Kumar K, B.R. Mrunalini and S.T. Girisha
STUDIES ON INDOOR AIRBORNE FUNGAL SPORES OF ANIMAL REARING HOUSES AT HESSARAGHATTA VILLAGE, BANGALORE

Pavan R* and Manjunath K
Department of Microbiology and Biotechnology, Bangalore University, Bangalore-56, India.

ABSTRACT:

Air pollution is one of the most serious problems to human health. Fungi are the causal agents for different diseases in animals, plants, and human beings. In rural areas of India a large number of people are occupationally involved with different types of animal sheds. In these sheds, a wide range of fungal growth substrates like moldy livestock foods, moldy hay, bedding of animals and their excreta are present, which could provide a huge airborne fungal spore load making these places unhygienic for the animal workers. The present study was carried out to investigate the indoor airborne fungi of four animal rearing houses viz., rabbit house, cow shed, poultry farm and swine house in Hessaraghatta village, Bangalore city. The samples was carried out by Andersen two stage sampler using an Malt Extract Agar (MEA) media were collected from January 2013 to December 2013. In our study, a total indoor airborne fungi of rabbit house (9682.79 CFU/m$^3$), cow shed (7808.36 CFU/m$^3$), poultry farm (8062.52 CFU/m$^3$) and swine house (6911.74 CFU/m$^3$) was recorded. The present investigation of four indoor animal rearing house would help in the finding out cause of fungal spores related problem affecting human health of animal rearing house workers.

INTRODUCTION

Aerobiology is a scientific discipline focusing on the study of the passive transport of organisms and particles of biological origin in the atmosphere (Isard and Gage, 2001). Air is an essential and important component of the ecosystem. Humans are in continuous exposure to air environment either outdoor or indoor, hence the air environment is a crucial factor that affects human health. Air mainly acts as dispersal or transport medium for the microorganisms. Air quality has been a concern for more than 100 years and started around 1850 during the hygienic revolution, followed by outdoor environmental issues (Spengler et al., 2000). The air breathed in most often comes from the enclosed buildings; good indoor air quality is therefore very essential and critical to human health. The airborne microbial quantity and quality vary with time of day, year and location (Lighthart, 2000). In recent years public interest has increasingly focused on released particulate matter from animal production facilities. This assumption is mainly based on experiences cited in occupational health studies, in which persons have been exposed to bioaerosols with subsequent deterioration of their health status (Douwes et al., 2003). During the present investigation, the aeromycological studies in indoor environments of four animal houses which would help in understanding the pattern of exposure to airborne fungi by the workers of various animal houses.
MATERIALS AND METHODS

The present study was carried out to investigate the indoor airborne fungi in rabbit house, cow shed, poultry farm and swine house in Hessaraghatta village, Bangalore. Sampling was carried out from the period January 2013 to December 2013 on each month fortnightly sampling in indoor animal rearing houses. Anderson two stage sampler was placed in the center of the animal house at 1.5 meter above the ground level. Malt Extract Agar (MEA) was used as sampling medium. Air flow was 28.3 L/min during the sampling and the sampling time was limited to 5 minutes (Andersen, 1958). Treatment of samples plates were placed in an incubator at 26°C and incubated for 5 days, then the colony count was recorded and the colony count was recorded again in 7 days. The results for each stage of the sampler were expressed as colony forming units per cubic meter of air (CFU/m$^3$) and total concentration was obtained by adding the CFU/m$^3$ from each plate. Identification of fungal colonies was based on morphological characteristics and microscopic observations with the help of Agarkar Research Institute, Pune (India). The data collected were statistically analyzed by one way and two way ANOVA and data was expressed in CFU/m$^3$.

RESULTS

The rabbit house, cow shed, poultry farm and swine house were situated at Hessaraghatta village, 10 km away from Bangalore city was selected as the site for sampling indoor air fungal samples. Sampling was carried out fortnightly for a period of 12 months from January 2013 to December 2013 and total fungal spores were recorded were shown in Table 1.

RABBIT HOUSE: The study period of rabbit house from indoor environment a total number of 9682.79 CFU/m$^3$ (colony forming units per cubic meter air) of fungal was isolated. Altogether 31 species belonging to 14 genera along with unidentified fungi were isolated, Among the total number of isolated fungal species of the rabbit house Penicillium (4.46%) was represented by 6 species viz., P. nigricans, P. griseofulvum, P. lilacinum, P. canescens, P. expansum and P. daleae followed by 6 species of Aspergillus (5.17%) viz., A. carbonarius, A. flavus, A. fumigatus, A. niger, A. terreus and A. versicolor, 3 species of Cladosporium (73.37 %) viz., C. herbarum, C. macrocarpum and C. cladosporioides, 3 species of Fusarium (6.3%) viz., F. moniliforme, F. oxysporum and F. chlamydospororium along with Absidia spp., (0.1%), Acremonium spp., (0.25%), Alternaria alternata (0.47%), Bipolaris spp., (0.21%), Curvularia spp., (0.32%), Mucor spp., (3.79%), Rhizopus spp., (0.25%), Phoma spp., (0.14%), Scopulariopsis spp., (0.07%) and Trichoderma spp., (0.69%). Dominant fungal species in indoor environment of rabbit house were Cladosporium herbarum (30.55%), Cladosporium spp., (17.24%), C. macrocarpum (15.67%), C. cladosporioides (9.91%) and Fusarium spp., (3.17%) contributed more but Absidia spp., (0.1%) and Bipolaris spp., (0.21%) were recorded less. The monthly variation of total fungal spores in the indoor environment of rabbit house showed maximum spore distribution in January (1606.15 CFU/m$^3$) followed by December (1104.89 CFU/m$^3$), June (1073.12 CFU/m$^3$) and March (1051.94 CFU/m$^3$) were isolated. Stastical analysis by Two-Way ANOVA for CFU and month of rabbit house were presented in Table 2.
### Table 1: Total fungal spores (CFU/m³) recorded from January 2013 to December 2013

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Genera and Species</th>
<th>Rabbit House</th>
<th>Cow Shed</th>
<th>Poultry Farm</th>
<th>Swine House</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Absidia sp.</td>
<td>10.59</td>
<td>-</td>
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<tr>
<td>2</td>
<td>Acremonium sp.</td>
<td>24.71</td>
<td>162.38</td>
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<td>49.42</td>
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<td>Alternaria sp.</td>
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<td>4</td>
<td>Alternaria alternata</td>
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<td>430.66</td>
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<td>Ascomycetes sp.</td>
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<td>67.07</td>
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<td>501.26</td>
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<td>342.41</td>
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<td>8</td>
<td>A. flavus</td>
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<td>462.43</td>
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<td>9</td>
<td>A. fumigatus</td>
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<td>11</td>
<td>A. oryzae</td>
<td>-</td>
<td>328.29</td>
<td>-</td>
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<td>12</td>
<td>A. ochraceus</td>
<td>-</td>
<td>49.42</td>
<td>-</td>
<td>-</td>
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<td>222.39</td>
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<tr>
<td>14</td>
<td>A. versicolor</td>
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<td>635.4</td>
<td>1683.81</td>
<td>1503.78</td>
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<td>70.6</td>
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<td>268.28</td>
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<td>P. versicolor</td>
<td>-</td>
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<td>250.63</td>
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<td>112.96</td>
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<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>46</td>
<td>Scopulariopsis sp.</td>
<td>7.06</td>
<td>31.77</td>
<td>-</td>
<td>21.18</td>
</tr>
<tr>
<td>47</td>
<td>Trichoderma sp.</td>
<td>67.07</td>
<td>250.63</td>
<td>420.07</td>
<td>165.91</td>
</tr>
<tr>
<td>48</td>
<td>Unidentified</td>
<td>413.01</td>
<td>218.86</td>
<td>289.46</td>
<td>356.53</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>9682.79</strong></td>
<td><strong>7808.36</strong></td>
<td><strong>8062.52</strong></td>
<td><strong>6911.74</strong></td>
<td></td>
</tr>
</tbody>
</table>
Table 2: Two-Way ANOVA for CFU and month of rabbit house

<table>
<thead>
<tr>
<th>Two-Way ANOVA</th>
<th>Sum of Squares</th>
<th>Degrees of Freedom</th>
<th>Mean Squares</th>
<th>F-Ratio</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colony</td>
<td>0.89</td>
<td>1</td>
<td>0.89</td>
<td>0.14</td>
<td>0.72</td>
</tr>
<tr>
<td>Month</td>
<td>88.52</td>
<td>11</td>
<td>8.05</td>
<td>1.24</td>
<td>0.36</td>
</tr>
<tr>
<td>Error</td>
<td>71.42</td>
<td>11</td>
<td>6.49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>160.84</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

COW SHED: During the present study period of cow shed contributed to 7808.36 CFU/m³ of fungal colonies from indoor environment. Altogether 29 species belonging to 13 genera with other unidentified fungi were isolated. Among the total number of isolated fungal species from indoor environment of the cow shed Aspergillus (27.36%) was represented by 6 species viz., A. flavus, A. niger, A. oryzae, A. ochraceus, A. fumigates and A. terreus followed by 3 species of Cladosporium (18.2%) viz., C. cladosporioides, C. herbarium and C. acremonium, 3 species of Fusarium (13.9%) viz., F. oxysporum, F. moniliforme and F. solani, 3 species of Penicillium (13.14%) viz., P. versicolor, P. citrinum and P. nigricans, 1 species of Curvularia (4.07%) viz., C. lunata along with Acremonium spp., (2.07%), Alternaria alternata (5.51%), Ascomycetes spp., (0.85%), Mucor spp., (2.66%), Neurospora spp., (0.72%), Rhizopus spp., (4.06%), Scopulariopsis spp., (0.4%) and Trichoderma spp., (3.2%) were isolated. Based on comparative analysis, dominant fungal species in indoor environment of the cow shed were Cladosporium (8.13%), Aspergillus (6.41%) and Aspergillus niger (6.1%) but Scopulariopsis (0.4%) and Aspergillus ochraceus (0.63%) were least recorded. Monthly variation of total fungal spores in the indoor environment of the cow shed showed maximum fungal spores distribution in May (780.13 CFU/m³) followed by February (773.07 CFU/m³) and January (755.42 CFU/m³). One-Way ANOVA for CFU’s of the cow shed between variation and within variation was not statistically significant in CFU’s for both groups when subjected to same conditions for the entire year as shown in Table 3.

Table 3: One-Way ANOVA for CFU of the cow shed

<table>
<thead>
<tr>
<th>One-Way ANOVA Table</th>
<th>Sum of Squares</th>
<th>Degrees of Freedom</th>
<th>Mean Squares</th>
<th>F-Ratio</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Variation</td>
<td>0.16</td>
<td>1</td>
<td>0.16</td>
<td>0.18</td>
<td>0.67</td>
</tr>
<tr>
<td>Within Variation</td>
<td>19.55</td>
<td>22</td>
<td>0.88</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Variation</td>
<td>19.72</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

POULTRY FARM: Air sampling studies in poultry farm revealed a total number of 8062.52 CFU/m³ of fungal species isolated from indoor environment. The qualitative analysis showed altogether 22 fungal species belonging to 12 genera with other unidentified fungi. Among the total number of isolated fungal species from indoor environment of poultry farm Aspergillus (5.80%) was represented by 4 species viz., A. flavus, A. fumigatus, A. niger and A. terreus followed by 2 species of Fusarium (14.87%) viz., F. moniliforme and F. oxysporum, 2 species of Penicillium (17.54%) viz., P. chrysogenum and P. griseofulvum, 1 species of Cladosporium (36.68%) viz., C. cladosporioides, 1 species of Curvularia (2.88%) viz., C. lunata along with Alternaria spp., (6.52%), Helminthosporium spp., (1.79%), Mucor spp., (0.96%), Neurospora spp., (0.87%), Phoma spp., (1.79%), Rhizopus spp., (1.4%) and Trichoderma species. The dominant fungal species in indoor environment of the poultry farm were Cladosporium spp., (20.88%), C. cladosporioides (15.8%) and Penicillium spp., (11.99%) but Aspergillus spp., (0.74%), A. flavus (0.74%), Neurospora spp., (0.87%) and Mucor spp., (0.96%) were least recorded. Monthly incidence in the indoor environment of the poultry farm
maximum spore distribution in May (886.03 CFU/m³) followed by February (741.3 CFU/m³) and November (702.47 CFU/m³) were isolated. Based on the Two-Way ANOVA for colony and month of the poultry farm were shown in Table 4.

Table 4: Two-Way ANOVA for colony and month of the poultry farm

<table>
<thead>
<tr>
<th>Two-Way ANOVA</th>
<th>Sum of Squares</th>
<th>Degrees of Freedom</th>
<th>Mean Squares</th>
<th>F-Ratio</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colony</td>
<td>2.58</td>
<td>1</td>
<td>2.58</td>
<td>1.78</td>
<td>0.21</td>
</tr>
<tr>
<td>Month</td>
<td>11.29</td>
<td>11</td>
<td>1.03</td>
<td>0.71</td>
<td>0.71</td>
</tr>
<tr>
<td>Error</td>
<td>15.96</td>
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<td>1.45</td>
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</tr>
<tr>
<td>Total</td>
<td>29.84</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SWINE HOUSE: In swine house a total number of 6911.74 CFU/m³ of fungal species were contributed from indoor environment. A total 25 fungal species belonging to 15 genera with other unidentified fungal form were isolated. Among the total number of isolated fungal species from indoor environment of swine house Aspergillus (16.63%) was represented by 3 species viz., A. flavus, A. fumigatus and A. niger followed by 3 species of Cladosporium (31.19%) viz., C. cladosporioides, C. herbarum and C. lunata, 2 species of Fusarium (11.78%) viz., F. moniliforme and F. oxysporum, 1 species of Alternaria (9.34%) viz., Alternaria alternata, 1 species of Penicillium (13.63%) viz., P. nigricans, 1 species of Rhizopus (1.22%) viz., R. oryzae along with Acremonium spp., (0.71%), Botrytis spp., (0.2%), Mucor spp., (1.73%), Curvularia spp., (1.68%), Nigrospora spp., (0.61%), Phoma spp., (0.66%), Scopulariopsis spp., (0.3%) and Trichoderma spp., (2.4%) were recorded. Monthly incidence of total fungal spores in the indoor environment showed maximum spore distribution in May followed by January 893.09 CFU/m³, December 861.32 CFU/m³ and February 840.14 CFU/m³ compared to other months of year. Dominant fungal species with their contribution in indoor environment of the swine house were Cladosporium spp., (21.75%) and Penicillium (12.66%) which contributed maximum but Botrytis spp., (0.2%) and Scopulariopsis spp., (0.3%) were least recorded. The statistically data were analyzed by Two-Way ANOVA for colony and month of the swine house were presented in Table 5.

Table 5: Two-Way ANOVA for colony and month of the swine house

<table>
<thead>
<tr>
<th>Two-Way ANOVA Table</th>
<th>Sum of Squares</th>
<th>Degrees of Freedom</th>
<th>Mean Squares</th>
<th>F-Ratio</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.79</td>
<td>1</td>
<td>0.79</td>
<td>1.11</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>91.67</td>
<td>11</td>
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<td>11.76</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>7.80</td>
<td>11</td>
<td>0.71</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>100.25</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

There are many reports available on the airborne fungal spores of animal houses conducted in rabbit house (Wang et al., 2007 and Miao et al., 2010), cow shed (Adhikari et al., 2004 and Ajoudanifar et al., 2011), poultry farm (Doris et al., 2005 and Hameed et al., 2010) and swine house (Martin et al., 1996; Black et al., 2001 and Milicevic et al., 2010). There are numerous reports of contamination of indoor air with fungal spore levels well in excess (Shelton et al., 2002 and Curtis et al., 2000). In India, indoor fungal concentrations are high in different occupational indoor environments as reported by Jain (2000) and Srikanth et al., (2008). Fungi aerosol which is procreated continually in animal raising house is not only endangered to the feeders and domestic animals, but also cause environment pollution (Yu and Che, 1998). Indoor air quality is imperative to...
maintain the health and productivity of farm workers and animals. There are many reports regarding health of farm workers coinciding with rapid changes from traditional farm to large intensive livestock operation.

The present study was carried out to identify the fungal contaminants present in the air inside selected animal rearing houses. Good indoor air quality depends on animal house management, feeding and manure handling, the ventilation system, as well as overall cleanliness of the animal house and type of animals kept. The animal houses showed 46 fungal types belonging to 18 genera. There are many allergenic fungi like *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Alternaria* sp., *Cladosporium acremonium*, *Fusarium moniliforme*, *Mucor* and *Rhizopus* species. Some of these fungal spores are immunotoxic and show adjuvant effects on humans. They cause many infections, diseases, allergies and some are opportunistic pathogens. The workers in animal rearing houses showed high prevalence of allergies like rhinitis, wheezing, skin rash, watering of eyes, cough and other related allergic symptoms. The study has shown that the animal rearing houses are contaminated with high concentrations of *Cladosporium* followed by *Aspergillus*, *Penicillium* and *Fusarium*.

Some veterinarians and feeders are very easily infected with fungal aerosol leading to respiratory diseases; 13% veterinarians are reported to be infected in this way (Che and Yu, 1998). Caretakers and sampling sites have prolonged exposure to such environment which result in occult infection or develop to chronic nosomycosis and lead to predisposition to other diseases. At present, there are no safe levels of airborne fungi concentration in indoor environments, but high concentration would result in threats to the health of human beings and animals.

**CONCLUSION**

This study was carried out in the animal rearing houses; it clearly revealed the concentration of different fungal species in the environment. The data of fungal spore content in indoor environment helped us to prepare the fungal spore calendar on this region and prediction model will be helpful to forecast the allergenic fungal spore load in the air of Hessaraghatta village. Respiratory allergic problems and hospital admission with relevant diseases of that zone are related to the presence of airborne allergenic fungal spores. The results of the present study could be incorporated while taking suitable measures to prevent health hazards of animals and workers, living or working in such infectious environments.

**ACKNOWLEDGEMENT**

This study was supported by the Department of Microbiology and Biotechnology, Bangalore University, Bangalore. The authors are grateful to University Grants Commission - Basic Science Research Fellowship, New Delhi, for financial support of this research. The authors appreciate the animal rearing houses workers for providing invaluable support and technical assistance during the sampling time.
REFERENCES


A COMPARATIVE STUDY OF FRESH WATER FISH DIVERSITY OF
UPSTREAM, MIDDLE STREAM AND DOWNSTREAM OF BEDTI RIVER
OF WESTERN GHATS REGION OF KARNATAKA

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1Department of Zoology, 2 Department of Biotechnology M.M.Arts & Science College,
SIRSI-581402, Uttara Kannada, Karnataka, India

ABSTRACT:

The present report describes the status of fish diversity in river Bedti of Western Ghats region of Karnataka. Fresh water offers very common and suitable habitats of the Biosphere. It has characteristic features in chemical, physical properties and hosts a large biodiversity which have adapted to dynamic environment. It has a well-defined food chain and food web through which energy is channelized and community. The present work was carried from June 2013 to June 2014. Fishes were caught with the gill net, cast net & drag net of suitable dimensions. The fishes were soon preserved and sent to the ZSI Kokatta for identification. The identified fishes were classified up to families. Highest fish diversity was recorded in downstream of the river.

This article is to be cited as:

INTRODUCTION

The study area is mainly located in Uttara Kannada district. Uttara Kannada district of Karnataka state has a geographic area of 10,291sq km and situated strategically in the middle of the Western Ghats. It is located between 13° 55’ to 15° 32’ N latitude and 74° 05’ to 75° 05’ E longitude (Yy et. al., 1998). It has a typical tropical climate with well-defined seasons and receives rainfall on an average 2500mm annually. The entire district is enriched in varied varieties of flora & fauna. The abundance of flora & fauna is mainly because of the four major rivers flowing in the district. The major rivers are 1) Bedti 2) Kali 3) Aghanashini and 4) Sharavati. Bedti is one of the west flowing rivers that originate in the Moist Deciduous forest areas of Dharwad district. The river is the outcome of hundreds of tributary streams which merge and become limited number of tributaries. The streams have their catchments covered with various types of Landscape element types ranging from dense forest to agricultural areas, scrubs and wasteland. The places selected for the present studies in upstream are Tamboor and Bedti bridge, in middle stream Gullapura and Kelase, in downstream are Hosakambi and Kallehwar. The objective of the present work is to reveal the fish species diversity with respect to the main three streams of river Bedthi.
MATERIALS AND METHODS

Fish sampling is the major fieldwork at all the specified locations. Fish sampling were made two times a year i.e. Pre monsoon & Post monsoon. For collecting the fish Gill nets, Cast Nets and Dragnets of different mesh size were used. The net fishing is one of the most popular fishing methods. The fishes caught alive and preserved in 4% Formaldehyde for the identification. The fishes caught in the net were immediately separated from the net and the numbers of fishes caught were counted and representative sample of every specimen were preserved in plastic jars using 4% formaldehyde solution. All colors, color patterns, spots blotches number and design of the fishes were carefully noted in the field note book and for identification different morphological characters were considered.

RESULTS

Species richness or diversity depends less on the characteristics of a single ecosystem than on the interactions between ecosystems, e.g. transport of living animals across the different gradient zones in the water body (Daniels, 2003; Sreekantha et al., 2007). Fish is captured in natural lakes, reservoirs, streams, tributaries, rivers and oceans. The worlds estimated total catches of fish is about seven million metric tons per annum. In many Asian countries inland catch make up 40 – 70 per cent of the total fish production (FAO, 1986). However, few species in spite of their great commercial interest have been comprehensively less studied to establish the importance of their distribution for their successful management (Daniels, 2003b). It is in this context, this study assumes importance reflecting the fish species diversity in river ecosystem.

The up Stream of Bedti River recorded 19 species under 6 families (Table-1). Among the families Cyprinidae dominated with 12 species, family Bagridae recorded 2 species, family Siluridae 2 species, family Claridae 1 species, family Ambassidae 1 species and family Aplocheilidae 1 species.

The upstream of Bedti documented a total of 476 individuals. The family Cyprinidae with 286 individuals contributed 60 per cent of the total fish catch. The high value was due to the abundance of *Garra gotyla stenorhynchos*, *Pseudoambasis ranga*, *Ompok bimaculatus* (Bloch) *Salmostoma boopis* (Day), *Puntius jerdoni* and *Puntius amphibius*. The genus *Puntius* appeared to be less abundant compared to other zones with only 65 individuals, which was estimated to be about 13to14 per cent of the total catch. The family Bagridae with 43 individuals contributed 9 per cent of the total catch. The family Ambassidae with 62 individuals contributed13 percent of the catch, Siluridae with 51 individuals contributed 11 percent of the total catch, Claridae with 32 individuals contributed 7 percent and Aplocheilidae with 2 individuals contributed very least per cent of the total catch. The dominant family Cyprinidae with 12 species classified under 8genera. The *Puntius* was the largest genus with 4 species. The genus Gara with 2 species and all other genera Labeo, Devario, *Tor*, *Salmostoma*, *Danio*, and *Rasbora* recorded only one species. The next family was *Bagridae* with 2 species under one genera. Two species of *Mystus* were recorded under family Bagridae. The family *Siluridae* was recorded 2 genera with one species each. All other families listed only one species each (Table –1).

The middle stream of Bedti River recorded 14 species under 6 families (Table-I). Among the families Cyprinidae dominated with 9 species. The family Belonidae, Aplocheilidae, Ambassidae, Gobiidae and Cichlidae recorded one species each.

The middle stream of Bedti documented a total of 440 individuals. The family Cyprinidae with 339 individuals contributed 77 per cent of the total fish catch. The high value was due to the abundance of *Salmostoma boopis* (Day), *Rasbora rasbora*, *Gara gotyla stenorhynchos*, *Puntius chola*, *Garra garra* and *Puntius...
amphibius. The genus *Puntius* appeared to be more abundant compared to upstream zones with 93 individuals, which was estimated to be about 22 per cent of the total catch. The family Belonidae with 49 individuals contributed 11 per cent of the total catch. The family Cichlidae with 23 individuals contributed 5 percent of the total catch, Ambassidae with 15 individuals contributed 4 percent, Gobiidae with 8 individuals contributed 2 percent of the total catch and Aplocheilidae with 6 individuals contributed one per cent of the total catch. The dominant family Cyprinidae with 9 species classified under 4 genera. The *Puntius* was the largest genus with 5 species. The genus Gara with 2 species and all other genera, Salmostoma, and Rasbora recorded only one species. All other families listed only one species each (Table –1).

The downstream of Bedti River recorded 18 species under 10 families (Table-1). Among the families Cyprinidae dominated with 9 species. All other families listed only one species each. The downstream of Bedti documented a total of 765 individuals. The family Cyprinidae with 621 individuals contributed 81 per cent of the total fish catch. The high value was due to the abundance of *Puntius amphibeus*, Rasbora rasbora (Hamilton-Buchanan), *Puntius filamentosus*, Gara gotyla stenorhynhos, Salmostoma boopis (Day) and *Puntius jerdoni* (Day). The genus *Puntius* appeared to be more abundant compared to other two zones with 396 individuals, which was estimated to be about 55 per cent of the total catch. The *Puntius amphibeus* was dominating among the *Puntius* genus which was estimated about 37 percent of the total catch. The family Mugilidae with 84 individuals contributed 11 per cent of the total catch. The family Gobiidae with 18 individuals contributed 2 percent of the total catch, Balitridae with 9 individuals, Aplocheilida with 8 individuals, Syngnathidae with 8 individuals, Claridae with 5 individuals, Bagridae, Belonidae with 4 individuals and Cichlidae with 4 individuals contributed one percent each of the total catch.

The dominant family Cyprinidae with 9 species classified under 4 genera. The *Puntius* was the largest genus with 5 species. The genus Gara with 2 species and all other genera, Salmostoma, and Rasbora recorded only one species. All other families listed only one species each (Table1). The predominant fish fauna in south Asia belongs to the carp family (Cyprinidae) (Bhat and Anurahda, 2000; Jayaram et al., 1976). The carp family alone in the river was prominent with *Puntius* as major genus.

In summary the Bedti river, the upstream recorded a total 19 species classified under 6 families with 476 individuals, while in the middle stream of the same river was noted that 6 families with 14 species and 440 individuals. The downstream was documented 18 species under 10 families and 765 individuals. The middle stream is less diversified with less species richness, but abundance was very high. The upstream stream showed more species richness but the abundance was poor and diversity of families was at par with middle stream. The fish abundance was less in downstream, but more than middle stream and upstream. The diversity of family was more than up and middle stream. It has been further argued that the increase in the number of species indicates less anthropogenic pressure Ajit and Mittal, 1993; Jayaram, 1981; Jayaram 1999). The diversity of family Cyprinidae was same in middle stream and downstream, while it was slight more in upstream. Family Cyprinidae and aplocheilidae were represented in all the streams. Ambassidae species were not found in downstream.

Balitridae, Syngnathidae and Mugilidae families were exclusively found in downstream, while Siluridae family was unique to up stream. Claridae and Bagridae families were not found in middle stream. Gobidae, Belonidae and Cichlidae families were not recorded in upstream. There is slight variation in the number of families among the different streams of Bedti river, while species richness showed ascending order from middle stream to downstream to up stream. The upstream recorded the maximum species diversity with 19 species among the other two streams.
Table 1: Distribution of fishes in three streams of Bedti River

<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>Order and Family</th>
<th>Species</th>
<th>Upstream</th>
<th>Middle stream</th>
<th>Down stream</th>
<th>Total</th>
</tr>
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<tr>
<td></td>
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<tr>
<td>1</td>
<td></td>
<td>Rasbora rasbora (Hamilton-Buchanan)</td>
<td>15</td>
<td>68</td>
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<td>223</td>
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</tr>
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<td>5</td>
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<td>280</td>
<td>332</td>
</tr>
<tr>
<td>6</td>
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<td>7</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>Puntius ticto ticto (Hamilton-Buchanan)</td>
<td>16</td>
<td>8</td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>Salmostoma boopis (Day)</td>
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<td>95</td>
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<td>152</td>
</tr>
<tr>
<td>9</td>
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<td>10</td>
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<td>Tor tor (Hamilton-Buchanan)</td>
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<td>11</td>
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<td>Garra mulya</td>
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<td>Garra garra</td>
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<td>28</td>
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<td>28</td>
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<tr>
<td>14</td>
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<td>Gara gotyla stenorrhynchos</td>
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<td>49</td>
<td>235</td>
</tr>
<tr>
<td>15</td>
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<td>Devario regina (Fowler)</td>
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</tr>
<tr>
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<tr>
<td>16</td>
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<tr>
<td>17</td>
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|  | 476 | 440 | 765 | 1681 |

Figure 1- Distribution of fish families Percent in three streams of Bedti
Species Richness in three streams of Bedti River:

The main streams of River Bedti of Uttara Kannada district recorded a total of 12 families and 28 species and 1681 individuals (Table-1 and Figure 1). Here also family Cyprinidae contributed major share to species richness and abundance with 15 species and 1246 individuals, which was almost 53 per cent of species richness and 74 per cent of total individuals. The family Bagridae was the next major family with 2 species and 47 individuals which accounted about 7 per cent of the species richness and 3 per cent of the abundance. In Bedti river the Siluridae family was also recorded 2 species and 51 individuals which was about 7 per cent of the species richness, but contributed only 3 per cent to the abundance.

The families Mugilidae, Claridae and Balitridae were recorded one species each with 84, 37, and 9 individuals respectively. The species richness of all families was 3 percent. But the percentages of abundance of the above families were 5 percent, 2 percent and 1 percent respectively.

The families Ambassidae, Belonidae, Cichlidae, Gobiidae, Aplocheilidae and Syngnathidae were recorded 77, 53, 27, 26, 16 and 8 individuals respectively. The species richness of all families was 4 percent. But the percentage of abundance of the above families was 5 percent, 3 percent, 2 percent, 2 percent, 1 percent and less than 1 percent respectively. All families except Cyprinidae, Bagridae and Siluridae were recorded only one species. Therefore the studies were reflected that the family Cyprinidae was dominating followed by the families Bagridae and Siluridae (Figure-2 and 3).

Figure 2: Histogram showing abundance of various families of fishes & their species in three streams of Bedti.

Figure 3: Family wise species richness in three different streams of Bedti River.
Study of diversity indices of fishes:

It is necessary to mention in this context that Shannon and wiener’s diversity value is high in upstream compared to middle and downstream streams. The middle and downstream have almost same value. The Simpson’s diversity value is also high in upstream and middle stream as compared to the downstream where as the Simpson’s dominance value is high in downstream than the up and middle streams. These are depicted in figure 4.

**Figure 4**: Histogram showing diversity indices & species richness of fishes of 3 different streams of Bedti River

The Bedthi River has different ecological characteristics, which has abundantly influenced the fish population. It has natural course of water without any dams and pollution. However in recent times Bedti River has been reported as polluted through urban sewage water flow. Moreover, the fishes have proved that they have the evolutionary flexibility to produce species to fill the spectrum of niches presented. They can be very big or very small, inhabit open waters or stay close to the bottom and they are present at every consumer trophic level in both the grazing and decomposer chains. For example *Garra species* is very well adapted to torrential water flow which has a suction cup on the ventral region, just below the mouth, can adhere to rocks, thus protects itself from torrential flow of water.

Diversity of fish species is determined generally by several physical factors, size, depth, quality of stream and biotic conditions such as food, vegetation and substratum (Bhat and Anuradha, 2000; Arunachalam, 2000). Habitat destruction due to deforestation results in increased erosion and suspended matter and deposition of fine sediments resulting in habitat loss and destruction of spawning grounds and species extermination (Jayaram, 1981; Jayaram 1977). Different river systems are known to harbor some species exclusive to the system. As per the present study family richness was more in downstream as compared to other two streams

**CONCLUSION**

This study was carried out in the animal rearing houses; it clearly revealed the concentration of different fungal species in the environment. The data of fungal spore content in indoor environment helped us to prepare the fungal spore calendar on this region and prediction model will be helpful to forecast the allergenic fungal spore
load in the air of Hessaraghatta village. Respiratory allergic problems and hospital admission with relevant diseases of that zone are related to the presence of airborne allergenic fungal spores. The results of the present study could be incorporated while taking suitable measures to prevent health hazards of animals and workers, living or working in such infectious environments.

ACKNOWLEDGEMENT

We would like to acknowledge University Grants Commission New Delhi and SWRO Bangalore for funding this research work. We also thank to The CDC, Karnataka University Dharwad and Principal of our college for providing the infra-structure facilities to conduct this study.

REFERENCES

**WOLBACHIA ASSOCIATION AND ITS PHYLOGENETIC AFFILIATION OF BRUGIA MALAYI PARASITES FROM INDIA**

Ravikumar H, Surendra N S, Prakash B M and Puttaraju H P.
Department of Biological Sciences, School of Natural Science, Jnanabharathi campus, Bangalore University, Bangalore- 560056, India.

**ABSTRACT:**

Wolbachia have established a mutualistic association with filarial nematodes and has a phenomenal implication in its normal development, reproduction and survival. Elimination of Wolbachia by tetracycline class of antibiotic compounds have been suggested and successfully implemented for the treatment of lymphatic filarial parasites. Thereby, is necessary to assess the prevalence of the Wolbachia in B. malayi before such new strategies are employed, across the world. In the present communication, the presence of Wolbachia and phylogenetic affiliation in B. malayi collected from Sevagram, Maharashtra, India, has been addressed.

This article is to be cited as:

**INTRODUCTION**

Filarial nematodes like Brugia malayi, Wuchereria bancrofti and Onchocerca volvulus cause several important human diseases across tropics and sub tropics. They belong to the order Spirurida and family Onchocercidae, have been reported to harbour the Wolbachia endosymbionts (Casiraghi et al., 2001). These bacteria have been implicated not only in establishing a mutualistic association with filarial nematodes but also in the pathogenesis of filarial disease (Bandi et al., 2001). Wolbachia acts on the host immune system and accelerates the rate of inflammation. During pathogenesis the β cell proliferation of the host is directed specifically towards Wolbachia surface antigens which strengthen the possible role of Wolbachia in filarial pathogenesis (Lamb et al., 2004). After the death of the nematodes, the host respond to Wolbachia by releasing stimulatory and modulatory factors from neutrophils and monocytes (Hise et al., 2004).

The typical immunological response of the host in producing mimics of lipopolysaccharide and activation of Toll like receptor-4 (TLR4) are known to be induced by Wolbachia (Tylor et al., 2000a; Andre et al., 2002). The regulation of Th1 and Th2 cytokines, which are the potential targets for filarial pathogenesis, is known to be governed by TLR4 (Tylor et al., 2000b). The strategies of combating filarial nematodes through Wolbachia by tetracycline class of antibiotic compounds directly hinder their proliferation and manage their pathogenesis (Hoti et al., 2003). In view of the importance of Wolbachia for the survival, developmental stages, reproduction and pathogenesis, it is necessary to assess the prevalence of Wolbachia in B. malayi from different geographical locations in India. However, earlier research by Hoti, et al., (2003) and Gayen, et al., (2010) identifies the occurrence of Wolbachia only in W. bancrofti collected from various geographical locations in India. The present study is an attempt to screen for the presence of Wolbachia in B. malayi (Bm-I) collected from the Jamnalal Bajaj Tropical Disease Research Centre at Mahatma Gandhi Institute of Medical Sciences, Sevagram, Maharashtra, India and to fill this impending gap.
MATERIALS AND METHODS

Genomic DNA was extracted from microfilariae (approximately 1500 in number) by Column based Animal tissue kit (Chromous Biotech Pvt, Ltd, Bangalore, India) with manufacturer’s protocol. DNA was quantified through Bio-photometer (Eppendorf AG, Hamburg, Germany). A polymerase chain reaction (PCR) assay was done through thermocycler (Eppendorf AG, Hamburg, Germany), with the reaction mixture containing 10 μl 10X buffer (5 Prime Eppendorf), 3 μl 25 mM MgCl2, 1.25 μl dNTPs (10 mM each), 1 μl 10 pmoles of both forward and reverse primers, 1.5 unit of Taq DNA polymerase (5 Prime Eppendorf) and template DNA. The PCR conditions followed for each step included 3 min at 95°C for the initial denaturation step followed by 35 cycles of 45 s at 94°C (denaturation), 1 min at 51°C (annealing), 1 min at 72°C (primer extension) and 7 min at 72°C for the final extension. For amplification filarial-specific 28 S rRNA, Wolbachia 16 S rRNA and Wolbachia surface protein (wsp) gene primers (Gayen et al., 2010; Smith and Rajan, 2000; Bazzocchi et al., 2000). PCR products were resolved in 1.2% agarose gel and stained with green view dye (Chromous Biotech Pvt, Ltd, Bangalore, India). The amplicons were observed and recorded in a gel documentation unit (Alpha Imager (R) EP, Canada). The size of the PCR product was determined using a 1-kb ladder (GeNei, Bangalore, India).

The PCR product of wsp gene were purified using Chromous PCR Clean-up kit (Chromous Biotech Pvt, Bangalore, India) and directly sequenced with respective primers using an automated sequencer (3130 Genetic Analyzer, ABI, Foster city, California, USA). The sequences obtained have been deposited in GenBank under the accession number JX506736. Phylogenetic analysis of the wsp gene sequence was done at BLAST-x program at NCBI. Multiple sequence alignment was done by using CLUSTAL W program. The phylogenetic trees were constructed using Kimura-2-distances and the Neighbor-Joining algorithm was computed using MEGA 4 program (Tamura et al., 2007).

RESULTS AND DISCUSSION

PCR amplification was carried out with Wolbachia- specific 16S rRNA and wsp gene primers to confirm the presence of Wolbachia infection in B. malayi. 16S rRNA gene amplified at 207 bp and wsp gene amplified around 590 bp fragments, whereas filarial-specific 28S rRNA primers was used confirming the quality of template DNA and authenticity of the experimental protocol yielded distinct band at 150 bp as shown in the figure-1.

The evolutionary history of Wolbachia lineages in the B. malayi of Indian populations was investigated by phylogenetic analysis performed with Neighbour-Joining algorithm using Kimura-2-distance. Direct sequencing of the PCR products gave only one sequence without double peaks, indicating the presence of only one strain in the Bm-I. These sequences have been submitted to the Genbank database and phylogenetic tree based wsp gene was constructed. B. malayi grouped in D supergroup as shown in the figure 2. The wsp gene sequences of Wolbachia from Bm-I strain showed highly homology with the previously reported B. malayi strain Bm-1, proving they belonged to the same strain.
The study investigated the presence and phylogenetic affiliation of *Wolbachia* in *B. malayi* (Bm-I) from India. The presence of *Wolbachia* in *W. Bancrofti* has been well documented in India by Hoti et al (2003) and Gayen et al (2010). *B. malayi* is considered to be high endemic to several states of India since decades. But there is a lack of information on Indian strain Bm-I. Thus, the current preliminary study has shown that *Wolbachia* is present in *B. malayi* (Bm-I) and the phylogenetic analysis shows the sequence similarity with members of Bm-I strain of D super group and correlated well with the earlier works of Bazzocchi et al (2000) and Casiraghi et al (2001). *Wolbachia* has displayed a mutualistic relationship with nematodes, and therefore elimination of *Wolbachia* by tetracycline class of antibiotic compounds decreases the host fitness and ultimately leads to its mortality (Smith and Rajan, 2000). The prevalence of *Wolbachia* in *B. malayi* from various geographical areas across India, and the extent of mutualism, its application in filarial management programmes is an interesting proposition for future studies.

**Figure 1:** PCR amplification of *B. malayi* (Bm-I) using filarial 28S rRNA-specific primers (Lane 1), *Wolbachia*-specific 16S rRNA primers (Lane 2), *Wolbachia* surface protein-specific primers (Lane 3) and Lane M is the migration pattern of the 1 kd DNA ladder.

**Figure 2:** Phylogenetic tree of *Wolbachia* based on the wsp sequences, constructed from Kimura-2-distance and the Neighbour-Joining algorithm. The numbers near the node indicate percentage of 1000 bootstrap replicates. Names correspond to host species. The GenBank accession numbers are also mentioned.
ACKNOWLEDGEMENT

Authors would like to thank DR. M. V. R. Reddy Dept. of Biochemistry, Jamnalal Bajaj Tropical Disease Research Centre at Mahatma Gandhi Institute of Medical Sciences, Sevagram, Maharashtra, India for providing B. malayi sample and highly thankful to the ICMR (No.5/8-7(309)V-2011/ECD-II, Vector Science Forum) for Research Associate position.

REFERENCES


IN-VITRO STUDIES IN CALAMUS PRASINUS, C. THWAITESSI AND C. VATTAYILA

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¹ Department of Botany, Jnanabharathi, Bangalore University, Bangalore – 560 056, India.
² Principal Secretary (Retd.), Forest and Environment Govt. of Karnataka, Bangalore – 560 001, India.

ABSTRACT:

*Calamus prasinus*, *C. vattayila* and *C. thwaitessi* are economically important rattans in furniture industry. The need for propagation of rattans is considered as most urgent on priority basis since over exploitation and deforestation have resulted in the depletion of the germplasms of *Calamus* species. *In vitro* micropropagation is an alternative strategy to mass propagate such threatened taxa. Shoot tips of *C. prasinus*, *C. thwaitessi* and *C. vattayila* were inoculated on Murashige and Skoog’s and Phillips and Collins media supplemented with various hormones to study their morphogenetic potential. Multiple shoots were obtained from the cultures of all the selected taxa on Phillips and Collins medium supplemented with auxins and cytokinins. Among the combinations tried, NAA and BAP combinations favoured the induction of maximum multiple shoots. Thus obtained shoots were rooted on auxin supplemented media. The plantlets were acclimatized on vermiculate + perlite before transferring them to pots containing soil:sand:manure in 1:1:1 ratio. About 30% of survival of plantlets was recorded.


INTRODUCTION

*Calamus* species commonly known as rattans are mostly trailing or climbing spiny palms with characteristic scaly fruits, classified under the sub family Calamoideae of the family Arecaceae. They constitute one of the most important non timber forest products particularly in South East Asia. They are known for their strength, durability, elasticity, lightness, lustrous brown colour and bending strength which confer them the title of “Green Gold” (Mohan Ram and Tandon, 1997). The rattan stem is extensively used for cane furniture industry and play an important role in the socio-economy of the people of South East Asian Countries (Dransfield, 1979, Dransfield and Manokaran, 1993 and Singh et al., 2004). There is an extensive demand for both raw and processed canes. Rattans generate employment for more than half a million people of Southeast Asia. According to Phillipines Business report (2004) the world demand for rattan furniture is about US $6.5 billion per year. The mass scale harvest of rattans before flowering and fruiting has resulted in the depletion of the germplasms. Vegetative propagation through suckers also has certain limitations as the survival rate of sucker is not significant. At this juncture, plant tissue culture plays an important role in mass multiplication of threatened species. This technology has established as an alternate strategy for production of large quantities of planting material of genetically uniform stock.
*Calamus prasinus* Lakshmana and Renuka, commonly known as ‘Onte betta’, is a solitary and high climbing cane (Fig.1A). It is endemic to Western ghat regions of Karnataka. It is one of the most sought after cane for furniture making. It can be grown as an intercrop in rubber plantations. Except its distribution and taxonomic studies, it is totally underexploited.

*Calamus thwaitessi* Becc & HK.f., a robust high climbing clustered rattan is commonly known as “Handibetta”and extensively used for furniture making (Fig.1B). As a result of continuous deforestation, most of the populations are fragmented and also is rapidly decreasing due to overexploitation by cane industries (Sreekumar and Renuka, 2006). Though *in vitro* studies were initiated earlier in this taxon, micropropagation studies are needed to develop an efficient protocol for mass multiplication (Valsala and Muralidharan, 1999; Hemanthakumar et al.,2013, 2014).

*Calamus vattayila* Renuka, an high climbing solitary cane, is commonly known as “Devarubetta” and considered as good cane for furniture making (Fig.1C). It is an endemic and endangered rattan of the Western ghats where a development of a protocol for their ex-situ conservation is needed (Jacob and Decruse, 2015). Except seed germination studies, cryopreservation and its taxonomic description, no other studies have been carried out in this taxon (Renuka, 1992 ,Tejavathi et al., 2013 & Jacob&Decruse,2015). With this background, the present study is an attempt to mass propagate the above selected taxa by employing shoot tip cultures.

**MATERIALS AND METHODS**

Frequent visits were made to Western ghats to collect the seeds and seedlings. Seedlings are maintained in the departmental garden, Bangalore University, Bangalore – 560 056.

a. Surface sterilization of the explants

Shoot tips (0.5 cms) were excised from one year old healthy seedlings. They were thoroughly washed with tween-20 in running water for 30 min and then surface sterilized with Bevastin, a fungicide (0.1%) for 30 min. Explants were then washed in tap water to remove the traces of Bevastin for 30 min followed by treatment with mercuric chloride (1.0%) for not more than 2 min. After the treatment, the explants were thoroughly washed with sterile double distilled water for five times to remove the traces of sterilients.

b. Culture medium and conditions

Murashige and Skoog’s (MS, 1962) and Phillips and Collins (L2, 1979) were supplemented with auxins like 2, 4-D, IAA, IBA and NAA and cytokinins such as BAP, Kin, 2-ip, AS and TDZ either alone or in combinations at various concentrations. Sucrose (3%) and Bacteriological grade agar agar (0.8%) were used as carbon source and gelling agent respectively. PH of the medium was adjusted to 5.6 – 5.8 and autoclaved for 15 min at 108 kpa. The cultures were incubated at 25±2°C under florescent tube lights with 16:8h light and dark regime at the intensity of 25µ mol m⁻²s⁻¹.

c. Rooting and acclimatization of regenerated plants.

The multiple shoots obtained thorough *in vitro* culture were transferred to rooting medium containing either IAA or NAA at various concentrations. After 45 to 50 days the rooted plantlets were transferred to plastic cups containing vermiculate and kept in polyhouse for acclimatization. Thus hardened plantlets were transferred to pots containing soil: sand: manure in 1:1:1 ratio. Three month old seedlings were transferred to field.

d. Data analysis

The results were recorded once in 30 days and represented as ± standard error based on ten replications. The data was analysed by One way ANOVA and significant ‘F’ ratios between the groups were further subjected to DMRT using SPSS version 1.5. Probability values <0.05 were considered as significant.
RESULTS

Among the two media tried, L2 medium supplemented with growth regulators was found to be more suitable for the cultures to respond. Hence L2 medium was used for further studies. The responses that are mentioned below are common to all the three species studied. Specific response from a particular species is mentioned wherever it is needed. The explants fail to show any response on the basal medium, hence the basal medium was supplemented with auxins, cytokinins and their combinations to study their effect on morphogenesis.

**Effect of auxins**

Shoot tip explants were inoculated onto L2 medium supplemented with various auxins like 2, 4-D, IAA, IBA and NAA at different concentrations to study their morphogenetic effect. When the explants were inoculated on 2, 4-D at 9.05-18.1 µM, a friable white callus was initiated from the explants of Calamus vattayila after two weeks of culture. Even after several subcultures to same hormone or different hormones, the callus remained non-morphogenetic. However, proliferation of the callus was observed when it was sub-cultured to 2, 4-D supplemented medium. NAA, IAA and IBA supplemented media have no effect on the explants of Calamus vattayila, explants remained green even after 8 weeks of culture. However, explants of Calamus prasinus and Calamus thwaitessi have grown into single shoot without the formation of multiple shoots on all the auxin supplemented media (Fig.2A).

**Effect of cytokinins**

Shoot tip explants were inoculated onto L2 media supplemented with various cytokinins like Kin, BAP, AS, TDZ and 2-ip at different concentration either alone or in combinations. Single shoot is formed when the explants were cultured on all cytokinin supplemented media (Fig.2B). Then they were sub-cultured to combinations of cytokinins for multiple shoot induction. However, multiple shoots were not formed though the growth of the single shoot was observed on all the combinations of cytokinins.
Effect of auxins and cytokinins

Shoot tips of the three taxa selected for the present study were inoculated onto L2 medium supplemented with various combinations of auxins and cytokinins (Table 1). Kin, BAP and 2-ip were supplemented with 2, 4-D, IAA, IBA and NAA at various concentrations to study their synergistic effect on morphogenesis. Among the combinations, NAA with BAP favoured the induction of multiple shoots from the cultures. The explants of C.prasinus produced white friable callus on L2+IAA (5.71µM) + BAP (8.87µM) after 2 weeks of culture. Rhizogenesis was recorded from callus after 6 weeks of culture. However, NAA +BAP promoted the formation of brownish nodulated callus from the explants after 4 weeks of culture. Thick whitish roots and green shoot buds started initiating from the callus after 8 weeks of culture on L2+NAA (10.74µM) + BAP (17.74µM) (Fig.2C). Lower concentrations however favoured the proliferation of the callus without any morphogenesis. The explants of C. thwaitessi responded better on the media supplemented with higher concentration of cytokinins. On L2+ NAA (5.37µM) + BAP (8.87µM), the explant has produced only 2-3 shoots per culture, while on L2+ NAA (10.74µM) + BAP (17.74µM), the shoot has proliferated into 10-12 shoots (Fig.2D) with basal callusing. The explants have failed to show any morphogenetic effect on the medium supplemented with 2-ip, Kin with auxins. When the explants of C. vattayila were inoculated on L2 medium supplemented with NAA (2.69µM) + BAP (8.87µM), the shoot apex proliferated into 4-5 shoots per culture with basal callusing. Whereas on L2 + NAA (5.37µM) + BAP (17.74µM), 9-11 shoots were formed from the explant which were healthy and elongated (Fig.2E).

Figure 2: Effect of auxins and cytokinins on morphogenesis

2A: Single shoot regeneration from the shoot tip cultures of C.thwaitessi on L2+NAA (10.74µM)
2B: Single shoot regeneration from the shoot tip culture of C. prasinus on L2+BAP (8.87µM)
2C: Induction of nodulated callus and shoot buds from the shoot tip cultures of C.prasinus on L2+ NAA (10.74µM) + BAP (17.74µM)
2D: Multiple shoot induction from the shoot tip culture of C. thwaitessi on L2+ NAA (10.74µM) + BAP (17.74µM)
2E: Multiple shoot regeneration from the shoot tip cultures of C.vattayila on L2+NAA (5.37µM) + BAP (17.74µM)
2F: Acclimatization of regenerated plantlet on vermiculate
**Rooting and acclimatization of regenerated plantlets**

Thus obtained shoots from both direct and indirect organogenesis were transferred to L2 media supplemented with auxins for induction of roots. NAA at 10.74µM and IAA at 28.54µM were able to induce 4-5 healthy roots from the basal part of the shoots without any callus formation. Then the young plantlets were removed from the culture bottle and washed thoroughly to remove the adherent medium. They were transferred to plastic cups containing vermiculate and perlite (Fig.1F). After one month of hardening they were transferred to the pots containing soil:sand:manure in 1:1:1 ratio. Three months old seedlings were planted in the field. Nearly 30% of survival was recorded.

**Table 1:** Effect of Plant growth regulators on multiple shoot induction from shoot tip cultures of *Calamus prasinus*, *C. thwaitessi* and *C. vattayila*.

<table>
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<th>Media + PGRs</th>
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<th><em>C. vattayila</em></th>
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<tr>
<td>L2+ NAA(2.69 µg/l)+ BAP (4.44 µg/l)</td>
<td>2.00±0.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.80±0.79&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.40±0.52&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td>L2+ NAA(2.69 µg/l)+ BAP (8.87 µg/l)</td>
<td>2.20±0.42&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.50±0.53&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>5.10±1.73&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>L2+ NAA(5.37 µg/l)+ BAP (8.87 µg/l)</td>
<td>5.00±1.72&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.60±0.52&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.80±1.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>L2+ NAA(5.37 µg/l)+ BAP (17.74 µg/l)</td>
<td>3.90±0.74&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.70±0.82&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.00±1.76&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>L2+ NAA(10.74 µg/l)+ BAP (8.87 µg/l)</td>
<td>2.70±0.82&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>3.90±0.56&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.00±0.82&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>L2+ NAA(10.74 µg/l)+ BAP (17.74 µg/l)</td>
<td>12.00±2.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.70±1.57&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>2.70±0.82&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are Mean ± SD, each with 10 replicates. Mean followed by the same letter are not significantly different at P= 0.05 according to Duncan’s Multiple Range Tests

**DISCUSSION**

Though *Calamus* species produce abundant fruits during the season, there is a difficulty in supplying high quality of seeds of desired species to the cultivators. The mass scale harvest of rattans has led to a situation of inadequate number of mature plants to produce flowers and fruits. Further inaccessibility of the mature plants in thick forest is another reason for non availability of the required seeds. In this context, tissue culture technology plays an important role in supplying large quantities of seedlings to raise the plantations that is the need of the hour. The most favoured explant in tissue culture of Rattans is embryo (Barba et al., 1985, Dekkers and Rao, 1989, Yusoff and Manokaran 1985, Goh et al., 2001, Hemanthakumar et al., 2013). However, shoot tips are considered as best explants to get genetically identical clones. Based on the two level polymorphism between the genotypes of *Calamus* *thwaitessi* using ISSR markers, Hemanth Kumar et al., (2014) have concluded that the plantlets regenerated from sucker derived shoot tip cultures are likely to be genetically true to their mother plants.

Extensive research has been carried out in respect of taxonomy and conservation of genetic resources of Rattans (Griffith, 1844, Cook, 1901-1908, Gamble, 1915-1936, Dransfield, 1979, Basu, 1985, 1995, Bhat et al., 1989, Renuka, 1992, 1993a, 1993b, 1997, 1999, Lakshmana, 1993, Mohan Ram & Tandon, 1997, Singh et al., 2004, & Lalnuntluanga et.al., 2010) since the publication of floras during late 19 & 20<sup>th</sup> century. Keeping in view of gradual depletion of genetic resources of rattans, tissue culture studies were initiated during 1980s. Umali-Garcia (1985) cultured the shoot apices of 11 species of Rattans and two of *Daemonorops* on MS medium supplemented with various hormones. Multiple shoots were initiated from the callus on BA and 2, 4-D
supplemented medium in three species of *Calamus*. *Calamus thwaitessi* and *C. vatayila* are most seriously affected economically important species in Western ghats inspite of their wide distribution by deforestation and indiscrimination exploitation (Hemathakumar et al., 2013). In the present study, the multiple shoots have proliferated from shoot tip explants on L2 supplemented with NAA and BAP. In *C. flagellum*, however shoot tip callus derived from 2, 4-D medium has produced multiple shoots on NAA + BAP combinations (Kundu and Sett, 1999). Tejavathi et al., (2013, 2015) have reported multiple shoot induction from the shoot tip callus of *C. nagabettaei* and *C. huegilianus* on NAA + BAP combinations. However Krishna Kumar et al., (2012a, b) have obtained multiple shoots from the shoot tip cultures of *C. travancoricus* and *C. nagabettaei* on MS supplemented with BAP alone. The efficiency of BAP in inducing shoot buds in *in vitro* cultures is well established (Reinert & Bajaj, 1979 & Bhojwani & Razdan, 1996). It has been suggested that efficacy of BAP in promoting organogenesis is due to its ability to induce the production of natural hormones such as Zeatin. Embryoids were obtained from the embryo derived callus of *C. thwaitessi* on BA and NAA supplemented medium (Hemathakumar et al., 2013). However multiple shoots were obtained from the shoot tip cultures of *C. thwaitessi* in presence of BAP and NAA along with TDZ (Hemathakumar et al. 2014).

Hormones play an important role in controlling the morphogenetic response of the cultures. Endogenous growth regulator content is known to differ from one meristematic centre to another (Norton, 1986). Balance between the endogenous and exogenously supplied hormones determine the morphogenetic response. In the present study, explants produced only single shoot in presence of single hormones in the medium. Multiple shoots were formed on the media supplemented with auxins and cytokinins. Both auxins and cytokinins readily form conjugates in plants. Thus the conjugation may be a way of prescribing the biological activity of the plant growth regulators (Skoog & Miller, 1957). Kin along with 2, 4-D induced multiple shoot induction from embryo explants of *C. latifolius* on onWPM (Meitram & Sharma,2006). Shoot tip of *C. vattayila*, a solitary cane, had proliferated into 10-12 multiple shoots on L2 medium supplemented with auxins and cytokinins. Whereas multiple shoots were formed from nodulated callus derived from shoot tip cultures of *C. prasinus* which is also a solitary cane, on NAA+BAP supplemented media. Goh et al., (1999) were able to induce somatic embryos from the root callus of *C. manan*, a solitary cane, on MS medium supplemented with picloram. Yusoff (1989) was succeeded in inducing multiple shoots from the collar region of *in vitro* raised seedlings of *C. manan* on MS supplemented with BAP/Kin. Thus solitary canes in nature can be made to produce shoots in *in vitro* by manipulation of hormones in the culture media. Thus obtained shoots from direct and indirect organogenesis were transferred to media containing IAA/IBA/NAA at various concentrations for root induction. IBA is the most favoured auxin for induction of roots in many species (Arya et al., 1999). However, in the present study, IBA was not efficient in inducing roots from the shoots. NAA and IAA had promoted the formation of roots from the basal parts of the shoots. In *C.flagellum* NAA had promoted maximal number of roots than IAA and IBA (Kundu and Sett, 1999). The rooted plantlets were removed carefully from the medium and washed thoroughly to remove the traces of media and transferred to plastic cups containing vermiculate and perlite. After 30 days of hardening, plantlets were transferred to pots containing soil: sand: manure at 1:1:1 ratio before planting them in field. Nearly 30% of survival was recorded.

**MS medium** was used in the earlier studies to raise the cultures of Rattans. However, in the present study, L2 medium was found to be suitable to raise the cultures and to obtain multiple shoots. L2 medium was first formulated by Phillips and Collins (1979) to initiate callus and suspension cultures of red clovers. Subsequently, it is being used for other species also. L2 medium contains lower levels of ammonium salts and higher levels of Ca and Mg compared to MS medium.
CONCLUSION

It can be concluded from the aforesaid data that shoot tip cultures can be employed to micropropagate these taxa to meet the demand to raise the plantations either for in situ or ex situ conservation programmes.

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In vitro studies in *Calamus* Tejavathi et al., 27


CLIFF SWALLOWS ARE GOOD MANSIONS FOR THEIR OWN PLAN
AND ARCHITECTURE OF NEST
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ABSTRACT:

Cliff swallows (Petrochelidon fluvicola) construct a gourd shaped mud nests below the cliff or extensions of the buildings. For constructions this bird draws a blue print of the nest designed to be completed. The bird uses the beak for construction. The material of the construction includes mud with consistency and organic materials. The mud is converted into pellets before placing into organised shape. The entire process of arrangement is documented as well discussed using JPEG image process. These birds act as good mansion for their own design of nest.

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INTRODUCTION

Cliff swallows have a highly developed phenomenon of nesting. These birds are migratory usually build mud nest on cliffs, rock over hangs, beneath the bridges, sloping edges of manmade construction. Four basic criteria are found to play a key role to establish colonial nests by swallows such as a open habitat for foraging, suitable surface for nest attachment, mud of proper consistency to build nests and water body to supplement drinking water to these birds (Elmen 1954). Cliff swallow nests are gourd shaped enclosed structure with an entrance tunnel that opens downward. The tunnel is generally present but in a few cases it may be missing. The mud pellets used to build the nest consists of sand, smaller amount of slit and clay. The nest chamber is lined with grass, hair and feather (Kilgore Jr and Knudsen 1977, Chaya H C etal 2013). Among cliff swallows nest building is a social activity. Construction and mud gathering is even initiated and assisted by unmated swallows. Mated swallows may build more than one nest and all the nest may not be used for breeding there by count of nest under construction will not describe the number of pairs in a colony. Both male and female cliff swallows construct the nest (Brown C R etal 2000). The building activity proceeds slowly to allow the mud dry. Depending upon the availability of mud and weather conditions the construction may take 1 to 2 weeks (Gorenzel and Salmon 1994, Chaya H C etal 2014)
MATERIALS AND METHODS

Material of the study comprises the colony of Cliff Swallows Petrochelidon fluvicola. The cliff swallows nest building activity is recorded by digital photography using “Sony Cyber-Shot DSC-HX7V” camera. Each step of the nest building is recorded on each day of nesting activity, starting from its blueprint marking of the nest till the complete guard shape with a tunnel is produced. Movement of swallows of both sex while building the nest is digitally recorded. The use of beak and its artistry is well documented both by still and videography. Girth measurement of pellets, girth of the nest, its length is recorded by either using scale or by measuring tape. The size of the cliff, the cliff forming area the vegetation around, the distance of the water body from the above cliff are recorded by using measuring tape.

Graphical analysis of nest construction – procedure

Tool Used for the design: Adobe Photoshop 7.0 (About the tool) : Adobe Photoshop is a graphics editing application popular for its extensive amount of features. Photoshop is also, currently, the leading graphics editing application. Photoshop is also image creation software as well as an editor. Photoshop can create any effect or style needed in a drawing or painting or layout. There are graphic software that can do specialized work faster and more efficient than Photoshop (such as painter for realistic paint effects), but Photo shop can do it all in one program. Photoshop works by altering individual pixels in an image as opposed to a vector drawing program that draws with points, lines and objects mathematically. Photoshop is best with images that have complex textures, blends and photo realism, but Photoshop is also very good at vector drawing as long as the image doesn't need to be scaled and you do not need specialized CAD drawing tools.

Design process

The JPEG (24 bits/pixel - 16 million colors) image “CurveShapedSphere.jpeg” which is showed in the fig.01 is used to represent the pellet as the basic component of the design in the canvas area, the area where the complete design takes place. The image was resized by selecting “Image Size” from the “Image” menu to almost 35%-40% of the original size and the resultant image was subjected to Stroke Layer Style effect by selecting “Add a layer style” button under “Image” menu on the highlighting that Layers of the image “CurveShapedSphere.jpeg” in Layers window with the color “red”. A single layer was made to hold a single pellet in it which was designed with above procedure i.e. a pellet in each layer. The words pellet and layer signifies same in our discussion, sometimes the name can be used interchangeably. By using the “MOVE” tool from the “Tool” window we can position the pellet at any position in the canvas. Using the oblique -“Marquee” tool from the “Tool” window we draw a dotted circle as to signify the base mark, which is used to guide the shape of nest construction. Now multiple the pellet by the duplication method by clicking the “Duplicate” button by right-clicking on the layer in the “Layer” Window. Once the multiple pellets have been created using the duplication process, place the pellet according to the design pattern using the “MOVE” tool for “Tool” window to design the complete nest. Once the complete nest is designed or partially nest design is done if you need to record/save the JPEG images go to “Settings,” select “JPEG” from the drop-down menu. Go to the dropdown menu with “JPEG High” displayed. There you can determine the quality of the image. Naturally, higher quality images will have a larger file size. It is not necessary that images be extremely high quality. Usually, “Medium” is a good setting to choose. To refine the quality of the image, use the “Quality” setting. Here, you can save the JPEG as a percentage of the original image’s quality. For example, 50% would be half the quality of the original image. When you are ready, click “Save” and save the image by giving it a file name.
OBSERVATIONS AND DISCUSSION

Indian Cliff Swallows are also known as Streak Throated Swallows – Petrochelidon fluvicola are highly colonial. Indian Cliff Swallows are also known as Streak Throated Swallows – Petrochelidon fluvicola are highly colonial; weight about 10.6-15.0gm, migrant passerine birds. Cliff swallows exhibit no obvious sexual dimorphism and are sexually monochromatic with males and females essentially identical in all plumages. Within pairs the male may have larger patch on the head. They are diurnal, aerial insectivores, feeding exclusively on flying insects and can forage only when the weather conditions allow flying insects to be active. They build enclosed mud nests, gourd shaped that are approximately 15-20cm in diameter and have entrances that are 5-7cm wide. Individual nests in cliff swallow colonies are often densely packed and nesting is highly synchronous within colonies. Nest entrances are, an average approximately 25-30cm apart and nests often share walls. Most nests contain clutches ranging in size from 1-4 eggs clutches with more than 4 eggs are cases of intraspecific brood parasitism or egg transfer or extra pair fertilization occurs frequently noticed only in one case. Brood sizes generally range from 1-4 eggs nestlings per nest.

The Nest

The nest is gourd like (Fig-15b) constructed using mud of suitable quality, the mud often mixed with grass for preparing the mud pellet for construction each of the steps observations are as below. The metric profile of the nest revealed that the girth of the nest 43.5±1.3cms with a length of 17.8±0.5cms. The width of the entrance 3.8cms. The depth of the nest ranged 9.5 to 12.3cms, with a diameter of 10.2cms, the girth of the mud wall of the nest was 1.2cms at the base 1.4cms in the middle and 1.5cms at the entrance (Chaya H C etal 2014). Each pair of birds construct the nest according to the blue print mark drawn earlier on the nesting surface. For construction the bird uses its beak (Fig-15a). Both the sexes are involved in construction. Interestingly a single pellet of soil is first placed at the exact half of the lower hemisphere of the nest marking (blue print) subsequently the birds alternatively carry mud pellets mix it well before aligning and the pellets are arranged on both the sides of this centrally placed pellet to form a platform. After partial completion of the lower basal lining the pellets are allowed to dry for 2-3 days. Consequence of this a hard basal lining of pellets is produced above this additional 2-3 layer arranged the sides the basal line were also extended by the arrangement of pellets. After this the days of rest ranged between 2-8 days to construct additional layers. Whenever the nest layers were constructed the proceedings were recorded both by still & video graphics. After completion of the lower half of the nest is the form of a semi lunar cup, the upper half was initiated. The initiation as usual started with the laying of a single pellet and subsequent arrangement on arch upper line marked earlier layer by layer and pellet by pellet arrangement done as the mason workers do, by each of the birds. This construction continued till a gourd shape in alignment with the lower half is produced. After the entrance construction was initiated here also same of alignment is followed. Around 1000 pellets are arranged to construct the nest.

Process of construction

The construction material majorly consists of mud from a selected site. During this process the mud is chewed well by the bird to mix with its saliva; and filled into the mouth carried to the nest site. The other partner which was on the nest waits each time for the arrival of the other with the contingent mud to be placed for construction. Even though the pellet alignment is completed by the bird compulsorily waited arrival of the partner as an act of safeguard to protect the nesting material that could be stolen by the neighbour in the colony.
Often the bird washes off its beak by dipping into the water below the cliff may be to clear off the mud struck or it may be an act of drinking water.

**Figure 1: CurveShapedSphere.jpeg**

**Figure 2: Approximated marking on the cliff**

**Figure 3: First pellet placed at the base of the marking**

**Figure 4: Initial pellet and left or right or hybrid pellet pattern summarized flow.**

**Figure 5: Flow diagram based on possible design pattern for the formation of Base Layer-1.**
Figure 6: Front view Design of Layer1-3 with the left dominant pattern with pellet numbers

Figure 7: Side view Design of Layer1-3 with the left dominant pattern with pellet numbers

Figure 8: Front view Design pattern summarized for Layers1-3 with the hybrid dominant pattern.

Figure 9: Side view Design pattern summarized for Layers1-3 with the hybrid dominant pattern.

Figure 10: The pellet placed at layer above the base layer as the rightmost pellet and leftmost pellet

Figure 11: The base upper layer pellets placed to form the complete layer.
Graphical analysis of nest construction

The design of the nest construction at the initial stage starts from the making on the cliff as shown in the Fig.02, the marking is not exactly in the shape of circle or elliptical as shown but it is rough surfaced circle. The first pellet is placed on the marking approximately at the base, as the direct contact with the cliff with enough physical and chemical property in the sand to withstand the gravitational force as shown in the Fig.03. Then the preceding pellets can be placed as the neighbour pellets to the first pellet in any of the pattern shown in the Fig.05. The Fig.04 gives a graphical/pictorial representation of how the different flow of pattern can be observed in the sense of formation of first layer (B1 in Fig.06) of the base construction by different birds. Once the initial pellet (1: Initial) is placed at random position at the base of the marking the preceding pellets can be either to the left (2:1L) of the initial pellet or to the right (3:1R) of the initial pellet. Once the preceding second pellet is placed as the neighbour of initial pellet the pattern may be as 2:1L or 3:1R in the Fig.05. The other preceding pellets from the current 2-pellet pattern (P2) to the 3-pellet pattern (P3) can take a formation of the pattern 4:2L or 5:2R/3L or 6:3R depending upon the left dominant pattern or the right dominant pattern or the hybrid (combination of left & right) dominant pattern. The same design procedure is followed for the 4-pellet (P4), 5-pellet (P5) and 6-pellet (P6) pattern. In any pattern the design flows at the final stage of the base layer-1 formation the common pattern will be as formed in 16:Base Layer-1 in Fig.05 as the 7-pellet (P7) pattern. Here in our analysis we have taken 7-pellet pattern, i.e 1 as the initial pellet & 3 each pellet to the left or right of the initial pellet. But this is an example pellet pattern we have taken for the discussion; but in reality it may be the same 7-pellet pattern what we are discussing or it may 9-pellet pattern to 15-pellet pattern depending upon the architectural technique of the bird building the nest. The complete layer-1 design pattern with Initial pellet and left or right or hybrid pellet pattern can be summarized as shown in Fig.04.

The same procedure will be followed for the formation of Base layer-2 and Base layer-3, but here we follow the left dominant pattern for the analysis and explanation of the nest design pattern. The front view of the nest once the three layers of the base are formed is shown in Fig.06 and this is main strategy of the architectural technique to withstand the complete nest against the gravitational force. Hence the base formation is of the nest is stressed and considered as the main part of our discussion. As mentioned we follow the left dominant pattern for the explanation and analysis as to support it and for the understanding purpose we have numbered each pellet in the design from 1 to 26. Here the 1 indicates the first pellet to be used for the design strategy, the preceding numbers after 1 again which indicates the preceding pellets after the first/main pellet respectively by what we follow the left dominant pattern. And a observation should be made while referring the number assigned to the pellets that there are few numbers missed out at 13 to 22 the reason with this is explained now. Once the B1 is formed by using 7-pellet design formation with the numbers representing 1 to 7, the layer B2 will be the next step of design strategy to act as the supportive withstand force to and with B1 for the gravitational force issues. The B2 as per our design is the next step as soon as the formation of B1 is done; hence it takes the next number from 8 to 12. Now the concern is that why the representation of number 13 to 22 is missing, the reason for this is that once the B2 is formed the next step of design is not B3 as we think but it is in the B1 as the second course i.e U2 in Fig.08 and in this graphical view we have named B1 even as U1, hence both indicates the same layer and can be used interchangeably. The pellets form 13 to 19 will be used to forms the B1-U2 layer and pellets numbered with 20 to 22 will be used to form the B2-U2 layer and this pellets 13 to 22 is not shown as the limitation of the 2-dimension plane. The same design issue will be related with the formation of horizontal layers (U2 & U3) formation as we have graphically represented in the Fig.08 with the Base Thick-2 and Base Thick-3, which represents the thickness of the base horizontally. The design pattern of
the front and the side view of the base formation of the nest construction which is shown in Fig.06 and Fig.07 can be summarized as shown in the Fig.08 and Fig.09 respectively, where the arrow indicates the flow strategy of the nest construction in the process of main pellet or the first pellet positioned and the phases to follow with the positioning of neighbour pellets. The dashed arrow is used in to represent the tracing back to the upper layer for the design pattern. Once the complete base formation is as per the architectural design of the bird. It has enough belief that the base will act and react to withhold the weight which will be formed as the construction of the nest moves towards the completion and the base is able to withstand the gravitational force. It is observed that layer B1 is has the enough strength to withstand the pressure of 25% of nest construction, but the further nesting build may be subjected to unhandled pressure and may collapse completely, hence for this reason with the intelligent move from the bird it has a design plan to build a three layer horizontal & vertical thickness to hold and handle the 100% pressure of the nest with combinational physical property of the base layers to withstanding the all the phases and stages of the construction.

![Figure 12: Approximate Centre convergence of upper layers for ~25% completion.](image)

![Figure 13: The Approximate centre of convergence for the complication for upper layers](image)

![Figure 14: The Nest construction design with Circular Pattern (a) at 90% and (b) at extension of entrance](image)

![Figure 15-a: The bird in nest construction](image) ![Figure 15-b: Nest](image)
Now let us move to the layer which will be formed on the base construction. From this phase the pellet will be placed above the base layer-1 (B1) on the marking as the leftmost pellet in the layer or the rightmost pellet in the same layer as shown in Fig.10. The preceding pellets will be placed in the layer from leftmost pellet or the rightmost pellet which may converge approximately at the centre pellet or it may convergence approximately at the leftmost pellet if the design flow is flown from the rightmost pellet towards the right most pellet and vice versa for the completion of the layer as shown in the Fig.11. In our discussion we take the convergence of centre approximation. The process explained and the pattern shown is followed with the same procedure for the formation of next stages of the construction, and we end up with the view of the nest as shown in the Fig.12. The further construction process will be carried out in the same fashion. The design pattern what we have discussed till the 80% can be summarized with the graphical view as shown in the Fig.13. Till the construction of around 80% the pattern follows as explained with the approximated centre convergence pattern and once this state is reached the bird goes with the circular pattern in an anticlockwise or clockwise direction or even the random circular point pattern. As shown in the Fig.14 (a) and extending its entrance as a narrow piped pattern as shown in Fig.14 (b). To conclude the nest construction process which initiates at the stage of placing a single pellet as the main pellet through the base construction follows to make the base strong enough to play a vital role to play against the gravitational force and withstand it with the holding the complete weight of the nest and evenly distributed over the leftmost and rightmost pellets in each upper layer formed on the base layer till the formation of entrance extended. The three basic patterns will be followed by the bird during the construction process of Base, Mid and final stages. At the base stage Left-, Right- or Hybrid-dominant stretch pattern will be followed, at the mid stage Leftmost, Rightmost or Random convergence pattern will be followed and the final stage the Clockwise or Anti-clockwise convergence pattern will be followed to form the entrance and the entrance extended. All these predictive analysis reveals the avian engineering skill imprinted through evolution in these birds.

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ABSTRACT:

Air is vital for the survival of human life as well as other organisms. Air pollution seems to be continuously increasing both in an industrialized and in developing parts of the world. There are several identified reasons for this upsurge in air pollution, the main influencing factors being an increasing number of vehicles, factories, power stations, etc., that emit various pollutants typical of urban and industrial sources. The air that we breathe not only comprises nitrogen, oxygen and carbon dioxide but also traces of other gases, inorganic particles and particles of biological origin. Adversative health effects of exposure to airborne particles have been described in many epidemiological studies. The present research on outdoor airborne fungal spores of Bangalore city and correlation with meteorological data was done. Samples were collected on each month fortnightly in duplicates from May 2013 to August 2013 by using Andersen two stage air sampler. Petri plates containing Malt Extract Agar media are used as sampling medium and exposed for 5 minutes. Altogether, 17 species were recorded from outdoor; the dominant fungal species identified were Cladosporium (78.60%), Rhizopus (3.83%), A. niger (3.82%), Mucor (2.47%) and Rhizopus (2.63%). An attempt has been made to forecast outdoor airborne fungal spores of Bangalore city.

INTRODUCTION

Airborne particles are present all through the environment. Despite the fact that atmospheric air does not favour growth of microorganisms due to lack of nutrients, the microorganisms are present in aerosol form or suspended in the air (Eduard, 2009). The basic sources of microbes are soil, water, animals and humans and they originate in many different forms and affect visibility, climate, human health and the quality of life (Ruizer and Harley, 2005). Airborne microbial quantity and quality vary with time of day, year and location (Lighthart, 2000). Airborne particles may consist of pathogenic or non-pathogenic live or dead bacteria, fungi, viruses, high molecular weight allergens, bacterial endotoxins, mycotoxins, peptidoglycans, pollen, plant fibres, etc. Airborne particles derived from microbial, plant or animal origin that is often used synonymously with organic dust is known as bioaerosols (Douwes et al., 2003).
Airborne fungi have much attention from medical researchers as well as environmentalists (Zhao and Yang, 2007 & O’Gorman, 2011). Several investigations have shown that exposure to fungi and their materials may be connected with acute toxic effects, allergies and asthma (Lee et al., 2006.). In addition, concentrations of fungal spores in the air was linked to seasonal variations and meteorological parameters such as temperature, wind speed, humidity and rainfall, that affects numbers and types of airborne fungi (Di Giorgio et al., 1996). Various factors, such as the type of collection medium, identification process, period of the day in which the collection of fungi takes place, sampling frequency and duration, influence the airborne fungi monitoring (Takahashi, 1997). The present work is confined to quantitative and qualitative determination of culturable airborne fungi present in air from the outdoor environments of Bangalore city.

**MATERIALS AND METHODS**

**Sampling Site:**

The airborne fungal sampling sites were selected at Bangalore city as indicated. The ten different types of areas selected were Banasawadi and Khaji Sonnanahalli (Residential area), Victoria Road and Central Silk Board (Commercial area), Indira Gandhi Institute of child health (Sensitive area), Jnana Bharathi campus and RV College (Silent area), International machine tools of Peenya, Graphite India Limited of Whitefield and KHB industrial area of Yelahanka (Industrial area).

**Sampling Period:**

This study was conducted from May 2013 to August 2013; outdoor airborne fungal samples were collected fortnightly in duplicates.

**Sampling Instrument:**

Andersen two stage viable air sampler was used (Andersen, 1958). Standard 90 mm petridish with Malt Extract Agar media are used as collecting surfaces on each stage and sampling time was limited to 5 minutes.

**Treatment of Samples:**

The air sampled plates were incubated for 5 to 7 days at room temperature between 25°C to 30°C and colony morphological characteristics were observed microscopically with strain determination by using manuals and reference slides (Lacey et al., 2006 & Gherbawy et al., 2010). The results for each stage of the sampler were expressed as Colony Forming Units per cubic meter of air (CFU/m³).

**Meteorological Data:**

Meteorological data recorded from May 2013 to August 2013 was collected from the Indian Meteorological Department, Bangalore.

**RESULTS**

**Jnana Bharathi campus:**

In this location major fungal species identified were *Cladosporium* (83.82%), *Rhizopus* (2.9%), *A. niger* (2.3%), *Alternaria* (2.2%), *Pencillium* (2.2%) and *Fusarium* (2.06%). Monthly variation of maximum fungal count was recorded in June (1526) followed by August (1431), May (756) and least in July (700). In the month of May, *Cladosporium* showed maximum average monthly distribution (71%) followed by *A. niger* (6%), *Alternaria* (6%), *Rhizopus* (3%) and *Pencillium* (3%). In the month of June, *Cladosporium* showed maximum avg. monthly distribution (91%) followed by *Rhizopus* (2%), *Fusarium* (1%), *Pencillium* (1%), *A. niger* (1%) and *Alternaria* (1%). In the month of July, *Cladosporium* showed maximum average monthly distribution 78%
followed by Pencillium (6%), Rhizopus (4%), Mucor (4%) and Alternaria (3%). In the month of August, Cladosporium showed maximum avg. monthly distribution 87% followed by Fusarium (3%), Rhizopus (3%), A. niger (2%) and Mucor (1%).

**International machine tools, Peenya:**

In this location Cladosporium was the major fungi with (79.3%) followed by A. niger (5.5%), Rhizopus (3.36%), Fusarium (1.95%) and Mucor (1.77%). Fungal count of June has recorded the highest count (1256), followed by July (1230), August (1050) and May (420). In the month of May, Cladosporium was the dominant fungi with avg. monthly distribution of (43%), A. niger (21%), Rhizopus (7%), A. fumigatus (5%) and Fusarium (4%). In the month of June Cladosporium was the dominant with avg. monthly distribution of (87%), A. niger (4%), Rhizopus (2%), Alternaria (2%) and Pencillium (1%). In the month of July, Cladosporium was the dominant fungi with average monthly distribution of (80%), A. niger (5%), Fusarium (3%), Rhizopus (3%) and Trichoderma (3%). In the month of August, Cladosporium was the dominant fungi with avg. monthly distribution of (82%), Rhizopus (4%), Mucor (3%), Alternaria (2%) and A. niger (2%).

**RV College:**

Dominant fungal species in this area were Cladosporium (75.7%), A. niger (4.5%), Rhizopus (3.4%), Alternaria (2.7%) and Pencillium (2.3%). Fungal count was high in July (964) followed by August (938), June (794) and least in May (639). In the month of May, Cladosporium was dominant with avg. monthly distribution (63%) followed by A. niger (14%), Pencillium (5%), A. ochracious (4%) and Rhizopus (3%). In the month of June, Cladosporium was dominant with avg. monthly distribution (75%) followed by Rhizopus (4%), Alternaria (4%), A. niger (3%) and A. flavus (2%). In the month of July, Cladosporium was dominant with avg. monthly distribution (82%) followed by A. fumigatus (4%), A. niger (3%), Pencillium (2%) and Alternaria (2%). In the month of August, Cladosporium was dominant with avg. monthly distribution (78%) followed by Rhizopus (6%), Alternaria (3%), Fusarium (2%) and Curvularia (1%).

**Victoria Road:**

Dominant fungal species in this area were Cladosporium (72.6%) followed by Rhizopus (5.65%), A. niger (5.17%), Pencillium (3%) and Alternaria (2.4%). Fungal count was high in August (815) followed by June (812), July (767) and least in May (518). In the month of May, Cladosporium was dominant with avg. monthly distribution (46%) followed by A. niger (18%), Alternaria (7%), Pencillium (6%) and A. flavus (6%). In the month of June, Cladosporium was dominant with avg. monthly distribution (79%) followed by Rhizopus (4%), A. niger (4%), Fusarium (2%) and A. fumigatus (2%). In the month of July, Cladosporium was dominant with avg. monthly distribution (79%) followed by Rhizopus (6%), A. niger (3%), A. fumigatus (3%) and Pencillium (2%). In the month of August, Cladosporium was dominant with avg. monthly distribution (76%) followed by Rhizopus (97%), Pencillium (4%), Mucor (4%) and A. fumigatus (2%).

**Indira Gandhi Institute of Child Health:**

In this location major fungal species identified were Cladosporium (78.44%) followed by A. niger (6%), Mucor (3.08%), Rhizopus (2.76%) and A. ochraceous (1.7%). Fungal count was high in August (1204) followed by June (1144), July (1034) and least in May (935). In the month of May, Cladosporium was dominant with avg. monthly distribution (63%) followed by A. niger (16%), A. ochracious (4%), Mucor (3%) and Fusarium (3%). In the month of June, Cladosporium was dominant with avg. monthly distribution (84%) followed by A. niger (4%), Mucor (2%), Alternaria (2%) and Pencillium (1%). In the month of July, Cladosporium was dominant with avg. monthly distribution (79%) followed by A. niger (4%), Rhizopus (3%), A. fumigatus (3%)
and Mucor (2%). In the month of August, Cladosporium was dominant with avg. monthly distribution (85%) followed by Rhizopus (3%), A. niger (2%), Trichoderma (2%) and Fusarium (1%).

**Graphite India Limited, Whitefield:**

In this location Cladosporium was the major fungi with (75.30%) followed by Rhizopus (4.99%), A. niger (4.3%), Fusarium (3.96%) and Penicillium (3.11%). Fungal count was high in June (1260) followed by July (1029), August (941) and least in May (479). In the month of May, Cladosporium was dominant with avg. monthly distribution 64% followed by A. niger (9%), Fusarium (7%), Rhizopus (5%) and Penicillium (5%). In the month of June, Cladosporium was dominant with avg. monthly distribution (78%) followed by Rhizopus (5%), A. niger (5%), Fusarium (3%) and Mucor (2%). In the month of July, Cladosporium was dominant with avg. monthly distribution (84%) followed by Rhizopus (6%), Mucor (4%), Fusarium (2%) and A. fumigatus (1%). In the month of August, Cladosporium was dominant with avg. monthly distribution (69%) followed by Penicillium (6%), A. niger (6%), Rhizopus (4%) and Alternaria (3%).

**KHB Industrial Area, Yelahanka:**

In this location Cladosporium was the major fungi with (81.69%) followed by Mucor (3.4%), Fusarium (3.39%), Rhizopus (3%) and Penicillium (2.1%). Fungal count was high in June (1449) followed by August (858), July (778) and least in May (532). In the month of May, Cladosporium was dominant with avg. monthly distribution (77%) followed by Rhizopus (6%), Mucor (4%), Penicillium (2%) and A. niger (2%). In the month of June, Cladosporium was dominant with avg. monthly distribution (85%) followed by Rhizopus (3%), Mucor (2%), Penicillium (2%) and Alternaria (1%). In the month of July, Cladosporium was dominant with avg. monthly distribution (81%) followed by Mucor (5%), Fusarium (5%), Penicillium (3%) and A. niger (1%). In the month of August, Cladosporium was dominant with avg. monthly distribution (81%) followed by Fusarium (4%), Mucor (3%), Rhizopus (3%) and Alternaria (1%).

**Banasawadi Police Station:**

In this location major fungal species were Cladosporium (78.63%), Rhizopus (4.54%), A. niger (3.23%), Mucor (3.02%) Penicillium (3.02%) and Fusarium (2.42%). Maximum fungal count recorded in June (1131), followed by August (938), May (609), and least in July (795). In the month of May, Cladosporium showed maximum avg. monthly distribution (72%), followed by Rhizopus (8%), Penicillium (5%), A. niger (4%) and Alternaria (3%). In the month of June, Cladosporium showed maximum avg. monthly distribution (74%) followed by Rhizopus (5%), Mucor (4%) and A. niger (3%). In the month of August, Cladosporium showed maximum avg. monthly distribution (83%) followed by A. niger (3%), Mucor (3%), Fusarium (2%) and Penicillium (2%).

**Khaji Sonnanahalli, Hoskote Road:**

Dominant fungal species in this area were Cladosporium (78.38%), Rhizopus (5.82%), Mucor (2.85%), A. niger (2.74%) and Penicillium (2.63%). Fungal count was high in June (1012) followed by May (781), August (739) and least in July (658). In the month of May, Cladosporium was dominant with avg. monthly distribution (77%) followed by Rhizopus (6%), A. niger (4%), Penicillium (4%) and Fusarium (3%). In the month of June, Cladosporium was dominant with avg. monthly distribution (84%) followed by Rhizopus (4%), Alternaria (3%), Mucor (3%) and Penicillium (2%). In the month of July, Cladosporium was dominant with avg. monthly distribution (78%) followed by Mucor (7%), Rhizopus (3%), Fusarium (3%) and A. fumigatus (2%). In the month of August, Cladosporium was dominant with avg. monthly distribution (73%) followed by Rhizopus (10%), A. niger (6%), Penicillium (2%) and Alternaria (1%).
Central Silk Board, Hosur Road:

Dominant fungal species in this area were *Cladosporium* (78.81%), *Penicillium* (3.30%), *Mucor* (3.02%), *A. niger* (2.83%) and *Rhizopus* (2.63%). Fungal count was high in June (1054) followed by August (1050), July (942) and least in May (665). In the month of May, *Cladosporium* was dominant with avg. monthly distribution (72%) followed by *Rhizopus* (6%), *A. niger* (5%), *Penicillium* (4%) and *Mucor* (3%). In the month of June, *Cladosporium* was dominant with avg. monthly distribution (85%) followed by *Mucor* (4%), *Rhizopus* (2%), *Penicillium* (2%) and *Fusarium* (2%). In the month of July, *Cladosporium* was dominant with avg. monthly distribution (81%) followed by *Rhizopus* (4%), *Fusarium* (3%), *Mucor* (3%) and *Alternaria* (2%). In the month of August, *Cladosporium* was dominant with avg. monthly distribution (79%) followed by *Penicillium* (6%), *A. niger* (4%), *Fusarium* (93%) and *Rhizopus* (1%).

**DISCUSSION**

In the present study, *Alternaria, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus ochraceus, Aspergillus terreus, Cladosporium, Curvularia, Fusarium, Helminthosporium, Mucor, Neurospora, Nigrospora, Pencillium, Rhizopus, Sporulariosis Candida and Trichoderma* were observed from outdoor environment of Bangalore. Fungal entities are common in outdoor environment, and approximately 10% of global persons have fungal allergy (Burge, 2001). Report also advocates that >6.5 million people have severe asthma with fungal sensitizations (SAFS), nearly 50% of mature asthmatics appearing secondary care have fungal sensitization, and an estimated 4.8 million adults have allergic bronchopulmonary aspergillosis. Researcher believed that, more than 80 genera of fungi are associated with symptoms of respiratory tract allergies (Horner et al., 1995).

Among the sampling stations (Table 1), Jnana Bharathi Campus showed highest fungal count (8828), following Indira Gandhi Institute of Child Health (8638), International machine tools (7914), Graphite India ltd, Whitefield (7421), Central silk board (7420), KHB Industrial area, Yelahanka (7232), Banasawadi Police station (6944), RV College (6672), Khaji Sonnanahalli (6377) and Victoria road (5826). The important atmospheric airborne fungal spore counts in outdoor and indoor air vary and depend on various environmental and other factors.

The main reason for variation of the fungal counts was due to the strength and availability of organic matter, as Jnana Bharathi campus is an open area and covered by plants and trees. In such large amount of decay and decomposition of leaves which induces the survival of fungi and their dispersal in to the air. In Indira Gandhi Institute of child health the main source was the improper disposal of waste generated by hospital. Victoria road had less count due to less availability of organic matter.

In all the locations dominant fungal species were *Cladosporium* (78.60%), *Rhizopus* (3.83%), *A. niger* (3.82%), *Mucor* (2.47%) and *Rhizopus* (2.63%). It was clear that *Cladosporium, Pencillium, Mucor, Fusarium, A.niger, Rhizopus, Alternaria and A. fumigatus* were ubiquitous and their dominance in other parts of the world has been well documented. Numerous studies have shown that exposure to fungi may be associated with acute toxic effects, allergies and asthma (Bush and Portnoy, 2001). Over 100 species of fungi were involved with serious human and animal infections, whereas many other species caused serious plant diseases (Cvetnic and Pepelnjak, 1997).

The result of correlation analysis, reveals that there is a strong negative correlation between fungi and daily average temperature i.e. with decrease in temperature there is increase in fungal count (Table 2). There is a positive correlation between fungal count and relative humidity i.e. with increase in humidity the fungal count increases and there is a positive correlation between fungal count and rainfall i.e. with increase in rainfall the fungal count increases due to increased moisture content in air.
**Table 1:** Airborne fungal spores of all site samples collected from May 2013 to August 2013

<table>
<thead>
<tr>
<th>Genera and Species</th>
<th>JB Campus</th>
<th>IICR</th>
<th>Graphite Indus Ind</th>
<th>Central Silk Board</th>
<th>Victoria road</th>
<th>Preynya Industiral area</th>
<th>KHB Industrial area</th>
<th>RV College</th>
<th>Bannawadi</th>
<th>Bhujia Sumamathl</th>
<th>Total CFU/m³</th>
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<td>119</td>
<td>105</td>
<td>91</td>
<td>140</td>
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<td>72</td>
<td>182</td>
<td>70</td>
<td>126</td>
<td>1239</td>
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<td>21</td>
<td>77</td>
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<td>7</td>
<td>42</td>
<td>14</td>
<td>14</td>
<td>252</td>
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<td>133</td>
<td>64</td>
<td>122</td>
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<td>105</td>
<td>301</td>
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<td>245</td>
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<td>154</td>
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<td>217</td>
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**Table 2:** Karl Pearson Correlation coefficient for meteorological data and total fungal count

<table>
<thead>
<tr>
<th>Fungal Species</th>
<th>Average Temperature</th>
<th>Relative Humidity</th>
<th>Rainfall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation coefficient</td>
<td>-0.605</td>
<td>0.799*</td>
<td>0.257</td>
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</tbody>
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*Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed)

The Correlation between individual fungal species and meteorological parameters, it is clear that not all the fungal species showed definite relationship with meteorological parameters only few of them showed. *A. fumigatus, Cladosporium, Fusarium, Mucor, Neurospora, Nigrospora, Rhizopus* and *Trichoderma* showed negative relationship with daily average temperature (Table 3). Whereas, *Alternaria, A. flavus, A. niger* and *Penicillium* showed positive relationship with daily average temperature. *Alternaria, A. fumigatus, A. flavus* and *A. niger* showed negative relationship with daily average relative humidity. Whereas, the *Cladosporium, Mucor, Neurospora, Nigrospora, Penicillium* and *Rhizopus* showed positive relationship with daily average relative humidity. *A. flavus, Mucor, Penicillium, and Rhizopus* showed negative relationship with daily average Rainfall. *Alternaria, A. fumigatus, A. niger, Cladosporium* and *Fusarium* showed positive relationship with daily average Rainfall.

At present there are numerous studies highlighting seasonal variations in both outdoor and indoor environments, especially in buildings frequented by a large number of people, who may be exposed to this type of aeroallergen (Black *et al.*, 2000; Meriggi *et al.*, 1996 & Lim *et al.*, 1998) and many authors indicated that the dominant fungi were *Cladosporium, Alternaria, Penicillium* and *Aspergillus* in the atmosphere and their concentration differed from place to place, because of local environmental variables, fungal substrates and human activities.
Table 3: Karl Pearson Correlation coefficient for meteorological data and fungal species

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Genera and Species</th>
<th>Average Temperature</th>
<th>Relative Humidity</th>
<th>Rainfall</th>
</tr>
</thead>
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<td>A. niger</td>
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<td>Cladosporium</td>
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<td>8</td>
<td>Curvularia</td>
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</tbody>
</table>

*Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed)

Reponen et al., (1996) reported that the deposition of fungal spores in lungs and their effects on human healthy not only depended on their composition and concentration but also size. The manifestation of a fungal allergy ranges from the common conjunctivitis, rhinitis and rhinoconjunctivitis to the more detrimental in ascending order of severity, i.e., sinusitis, asthma, bronchopulmonary mycoses, hypersensitivity pneumonitis and allergic alveolitis (Fink, 1998). From the studies it is clear that all the major fungi observed were potential allergens and some even pathogenic to human, plants and animals. The fungi spores can be main reason for allergy and bronchial problems in urban environments.

CONCLUSION

Aeromycological characteristics of outdoor are the largest in quantity, variety and importance to the urban environment. The present study was carried out using cultural method, which is dependent on the cultivability of the spores trapped and collection efficiency of the instrument used also the present method employed was discontinuous. Potential allergens include active spores, broken mycelial fragments and metabolites, which could be a valuable data for successful treatment of allergic diseases. Hence it is recommended to carryout continuous sampling method and evolves a method which can enumerate both culturable and nonculturable microorganisms with microbial fragments that could mimic the human respiratory system to maximum extent.

ACKNOWLEDGEMENT

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IMPACT OF MUD PUDDLING ON REPRODUCTIVE SUCCESS OF TALICADA NYSEUS L. (LEPIDOPTERA: LYCAENIDAE) HARBORING WOLBACHIA INFECTION

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Department of Life Science Bangalore University, Bangalore-56, India.

ABSTRACT:

Puddling is an essential phenomenon exhibited by butterflies (imago) and have a significant role in their reproductive life. Puddling on various sources enable butterflies to gain mineral nutrition, which further helps them in their flight, oviposition and egg production. Till date, the importance of puddling is thoroughly studied in several butterfly species however limited information is available regarding the same with respect to small non migratory butterflies. In the present study we investigated the impact of mud puddling on the reproductive success of Talicada nyseus which harbors Wolbachia and shows female biased sex ratio. The outcomes revealed that the reproductive success of Talicada nyseus is independent of their puddling activity with unaffected fecundity and longevity. This is in favor of Talicada nyseus infected with Wolbachia. However direct correlation of Wolbachia with mud puddling behavior of Talicada nyseus needs further investigation.

This article is to be cited as:

INTRODUCTION

The reproductive success of butterflies is regulated by two important parameters viz. adult feeding and mud puddling. Mud puddling is an important and unique behavior observed in butterflies and moths. Lepidopteran gather around wet clay or sands, edges of streams, carrion and excreta to suck the dissolved nutrients present them (Adler, 1982; Beck, et al., 1999; Boggs & Jackson, 1991). Mud puddling is mainly carried out to gain essential minerals such as sodium (Beck, et al., 1999) or calcium phosphate (Lai-Fook, 1991) and proteins (Boggs & Dau, 2004). During mating, the mineral nutrients acquired by males are transferred to females through spermatophores (Sculley & Boggs, 1996; Molleman, et al., 2005; Boggs & Jackson, 1991). This further facilitate the reproductive success of the females in several means (Boggs & Jackson, 1991; Pivnick & McNeil, 1987; Adler & Pearson, 1982). Moreover, sodium supports the neuromuscular events in both males and females during their flight (Molleman, et al., 2005).

The mud puddling activity in imago is the response to the limited availability of sodium during their developmental stages. The presence of sodium and other minerals is limited in both foliage of host plants as well as in nectar. Generally, puddling is done by both male and female imago (Boggs & Jackson, 1991), however it is much more predominant in young males than old males and female (Adler, 1982; Adler & Pearson, 1982; Boggs & Jackson, 1991; Sculley & Boggs, 1996).
Talicada nysaeus G. (Lepidoptera:Lycaenidae) is a common butterfly found in India and Sri Lanka (Karunaratne, et al., 2002). Like any other butterflies, *Talicada nysaeus* feeds on nectar but also known to feed on lichens (Karunaratne, et al., 2002). Recent studies revealed that the butterfly harboring an endosymbiotic α-proteobactreia, *Wolbachia* (Ankola, et al., 2011; Salunke, et al., 2012) and shows female biased sex ratio (Ankola, et al., 2011). The biased sex ratio is expected to be due to *Wolbachia* induced feminization (Rousset, et al., 1992; Hiroki, et al., 2002) or male-killing (Fialho & Stevens, 1997; Fialho & Stevens, 2000; Hurst, et al., 1999; Hurst & Jiggins, 2000). In both the cases, the biased sex ratio influencing the reproductive success of this butterfly by reducing the male population. On the contrary, *Talicada nysaeus* is a small butterfly with less flying ability and hence always found near their host plant. It is obvious that the butterfly will experience the scarcity of nutrition which will further influence on their reproductive success. In addition, a very limited information is available on the puddling activity and its role in the life history of this butterfly. On this rationale, the present study was conducted to investigate the role of mud puddling on reproductive success of *Wolbachia* infected population of *Talicada nysaeus*.

**MATERIALS AND METHODS**

The imagos were collected from the Butterfly Park, Bannerughatta Biological Park Bangalore India and transferred to the Insectaria, Division of Biological Sciences, Bangalore University. Adult butterflies were transferred to a butterfly rearing cage which further maintained as stock population cage. The butterflies were allowed to reproduce in the stock population cage and the eggs were separately reared in the aerated box. The young larvae were fed with fresh leaves of their host plants and maintained in the aerated boxes till it metamorphoses into pupae to adult.

*Experimental setup*:

Two experimental setup viz. HPEX01 and HPEX02 were created to study the influence of mud puddling on the reproductive success of *Talicada nysaeus*. Newly eclosed imago were used for the experiments. The butterflies in both HPEX01 and HPEX02 were allowed to reproduce and compete their lifecycle with and without mud puddling respectively. Both the experimental setups were maintained at 27.14 ± 1.36°C and provided with a host plant and 10% sucrose solution in the cotton pad. The data regarding the fecundity, hatchability and adult longevity were collected and were analyzed through one way ANOVA 7.5.

**RESULTS AND DISCUSSION**

Although, being one of the essential phenomenon in butterflies, *Talicada nysaeus* shows less affinity towards puddling activity. In butterflies, mud puddling is evolved as a family and species specific behavior (Boggs & Dau, 2004). The necessity and availability of adult nutrition directly influence on the performance of mud puddling. During puddling, the priority is often given to inorganic mineral ions however there are few instances where butterflies prefer protein (Boggs & Dau, 2004; Beck, et al., 1999). The mineral ions (especially sodium) plays a vital role in the neuromuscular events and hence assist the butterflies in their flight. Since *Talicada nysaeus* is a small butterfly with less flying ability, they involve less in the puddling activity.

Both male and female *Talicada nysaeus* are actively involved in the puddling activity. In most of the families, puddling behavior is predominantly observed in males than in females (Molleman, et al., 2005). The mineral nutrients gained by males during puddling are transported to female through spermatophores as a “nuptial gift” (Molleman, et al., 2005). Females further utilize these mineral nutrients for several activities associated with reproduction like ovipositioning.
The fecundity (Table 1a), hatchability (Table 1b) and adult longevity (Figure 1) of *Talicada nyseus* is independent of their puddling activity. The minerals and other nutrition gained through mud puddling (or as nuptial gift) by females helps them in the production of eggs (Boggs & Jackson, 1991; Pivnick & McNeil, 1987; Adler & Pearson, 1982). The unaffected fecundity of *Talicada nyseus* in both the experimental setup eliminate the possibility of interference of puddling in egg production.

**Table 1**: Influence of mud puddling on reproductive success of the butterfly *Talicada nyseus*: The mud puddling has no influence on the (a) fecundity and (b) hatchability of *Talicada nyseus*. The mating pairs of experimental setup HPEX01 were given mud puddling source whereas HPEX02 were devoid of mud puddling.

(a)

<table>
<thead>
<tr>
<th>Experimental Setups</th>
<th>HPEX01</th>
<th>HPEX02</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mating pairs</td>
<td>07</td>
<td>06</td>
</tr>
<tr>
<td><em>Wolbachia</em> infection Status</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Fecundity</td>
<td>72.714 ± 10.90</td>
<td>71.166 ± 11.51</td>
</tr>
<tr>
<td>F value</td>
<td>0.057*</td>
<td>0.057*</td>
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<tr>
<td>Df</td>
<td>1</td>
<td>1</td>
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<tr>
<td>Within groups</td>
<td>11</td>
<td>11</td>
</tr>
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(b)

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<thead>
<tr>
<th>Experimental Setups</th>
<th>HPEX01</th>
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<tr>
<td>Mating pairs</td>
<td>07</td>
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</tr>
<tr>
<td><em>Wolbachia</em> infection Status</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hatchability</td>
<td>69.571± 9.09</td>
<td>67.333± 10.40</td>
</tr>
<tr>
<td>F value</td>
<td>0.157*</td>
<td>0.157*</td>
</tr>
<tr>
<td>Df</td>
<td>1</td>
<td>1</td>
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<tr>
<td>Within groups</td>
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*. The mean differences are not significant at 0.05 (*P*>0.05).

**Figure 1**: No significant variation has been observed in the longevity of both male and female T. nyseus with and without mud puddling. The male longevity is less than the female in the respective experimental setups.
It is also worth notice that the members of family Lycaenidae prefers protein resources more during puddling (Beck, et al., 1999). They use olfactory signals released by the decaying organic matters to search nitrogenous resources. Like sodium, these nitrogenous sources also help to increase the reproductive success of the butterflies (Beck, et al., 1999). The negligible impact of puddling on *Talicada nyseus* also reveals poor influence of nitrogenous source on the reproductive success of this butterfly. However, the response of *Talicada nyseus* towards puddling source contain specific nitrogen compound is not verified in the present study. Nevertheless, the nitrogenous source might act as supplementary entity which increases the reproductive performance in this butterfly, but not necessarily.

Further, the unaffected fecundity and hatchability of *Talicada nyseus* is may be due to *Wolbachia* infection. *Wolbachia* is an endosymbiotic bacteria, known to enhance fecundity in their insect hosts (Dedeine, et al., 2001). This is to compensate the effect of reproductive anomalies caused by *Wolbachia*. Enhancing the fecundity is beneficial for both host and its endosymbiont to survive in their natural habitat.

The adult longevity of *Talicada nyseus* is also found to be independent of their puddling activity. The adult longevity is another factor which determines the reproductive success of the butterflies. The life time fecundity of females and mating success of males are regulated by their longevity. The male longevity plays a crucial role in those butterflies where *Wolbachia* induced female biased sex ratio is predominant. *Talicada nyseus* infected with *Wolbachia* (super group B) and shows female biased sex ratio (Ankola, et al., 2011). The sex ratio distortion in this butterfly is might be due to *Wolbachia* infection. However, it is still unclear that the biased sex ratio is whether because of male-killing or feminization. However, the fecundity and hatchability data provide few clues which strengthen the probability of feminization.

The presence of female biased sex ratio reduces the male population and hence increase the competition among the females to mate with existing males. This facilitate the existing males to mate with females several times and hence fertilize more number of eggs. This interns become a real challenge to the mating success of individual males and is depend on their longevity. Conversely nitrogenous sources like amino acids can increase the longevity and reproductive success of the butterflies (Gilbert, 1972). Generally, the amino acid is the dietary entity but is also supplemented by puddling in butterflies. Sucrose was the only food source used in the present investigation and hence the only probability of getting amino acid was through mud puddling. However, it is very unlikely that the puddling source used for the present experiment could have any amino acid content in them. These outcomes clear that the reproductive biology of *Talicada nyseus* is independent of mud puddling. However, the association of *Wolbachia* induced anomalies with mud puddling in this butterfly needs further investigation.

REFERENCES


EVALUATION OF ENDOPHYTIC FUNGAL SPP. FOR BIODIESEL PRODUCTION

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ABSTRACT:
Due to the environmental concern and limited resources of fossil fuel, the demand for biodiesel has increased. Production of biodiesel using microorganisms has been considered as a promising alternative for biodiesel production. In the present study, Aspergillus spp. Penicillium spp. Alternaria spp. Colletotrichum spp. Cladosporium spp. Bipolaris spp. Fusarium spp. Mycelia sterilia were isolated from Jatropha plant parts. About 20 percent of Aspergillus species were noticed among the fungal population. The isolated fungal lipids were screened qualitatively by Sudan black B staining and quantitatively by various lipid extraction methods such as Folch method, Bligh and Dyer method, Isopropanol: hexane extraction, Soxhlet extraction. The efficient lipid producing fungal isolate JGK – 12 was identified as Aspergillus niger based on the 18s rDNA sequencing, with sequence similarity of 100% in phylogeny as compared with other Aspergillus counterparts, yielding 257.8±9.59 mg of lipid per gram of dry fungal biomass by Folch method.

INTRODUCTION
Continued use of petroleum sourced fuels is now widely recognized as unsustainable because of depleting supplies and the contribution of these fuels to the green house effect. Due to population growth and industrialization, the demand for energy has increased rapidly in recent years, and the world energy consumption is projected to increase by 49% from 2007 to 2035 (http://www.eia.doe.gov/oiaf/ieo/highlights.html). Renewable, carbon neutral, transport fuels are necessary for environmental and economic sustainability. Biodiesel derived from oil seeds is a potential renewable and carbon neutral alternative to petroleum fuels. Unfortunately, biodiesel from oil crops, waste cooking oil and animal fat cannot realistically satisfy even a small fraction of the existing demand for transport fuels.

Oil obtained from microorganisms has been considered as single cell oil (SCO) because it synthesizes the oils with high purification and is less expensive than agricultural and animal sources (Certik and Shimizu, 1999; Cohen and Ratledge, 2005). Lipid producing organisms have been known for many years. Oleaginous microorganisms are defined as organisms that contain more than 20 to 25% of their dry biomass in the form of lipids (Ratledge, 1988, Dyal and Narine, 2005). When organisms are grown in N2 limitation medium, beyond 70% oil accumulation is observed in oleaginous fungus whereas non-oleaginous fungus do not accumulate lipid. An Active lipid synthesizing apparatus makes oleaginous microorganism attractive oil source with high growth rate on wide varieties of substrates including various waste substrates.

This article is to be cited as:
The ability to accumulate high amounts of lipid depend mostly on the regulation of the biosynthetic pathway, supply of the precursors (Acetyl co A, MalonylcoA and Glycerol -3-phosphate) and the cofactor (NADPH). Yeasts and fungi (especially molds) are considered as favorable oleaginous microorganisms since 1980s (Abraham and Srinivasan, 1984). A filamentous fungus – *Mortierella alliacea* Strain YN-15, accumulated arachidonic acid (AA, C20:4n-6) mainly in the form of triglyceride in its mycelia. Use of Sudan Black B for staining and direct observation under the microscope enables the rapid observation of qualitative status of lipid production in the cells, change in color of stain from dark to light blue observed during the course of fermentation has been found to be related to a change in extent of unsaturation of lipids (Thakur et al., 1988).

**MATERIALS AND METHODS**

*Collection of plant samples*

*Jatropha curcas*, non-edible oil seed plant was collected from Gandhi Krishi Vignana Kendra, University of Agricultural Science, Bangalore for isolating oleaginous fungi.

*Isolation of fungal spp.*

Different aerial parts of fresh healthy plant samples were cut into small pieces (5mm × 2mm) using a sterile blade, the smaller pieces are surface sterilized by exposing them to 4 % sodium hypochlorite solution for 90s followed by 70% ethanol for 5s and thoroughly washed with distilled water (Suryanarayanan and Thennarasan, 2004). The surface sterilized samples are placed on Potato Dextrose Agar. After incubation at 30°C for 7 to 14 days, purity of the cultures was determined by colony morphology (Suthepwiyakrutta *et al.*, 2004). These isolates were used for further research work and pure cultures of *Mortierella alpina* (MTCC no.6344) and *Mortierella hyalina* (MTCC no.6301) were procured from Microbial Type Culture Centre (MTCC) Chandigarh for comparative studies.

*Identification of fungal spp.*

The filamentous fungal isolates were identified in the laboratory by morphological studies. Identification was based on macroscopic observation of the colonies and examination of the micro structural characteristics using universal identification keys for fungi (Klich, 2002; Gilman, 1998; Barnet and Hunter,1988). Identified isolates were maintained on PDA slants and stored at 4°C, sub-culturing was carried out every fortnight throughout this study.

*Screening for lipid production by Sudan black B staining method*

Selected fungal strains were stained with Sudan black B as described by Burdon *et al.*, (1946) and Thakur *et al.*, (1989). Presence of blue or grayish oil globules within the mycelium were observed under oil immersion microscope.

*Extraction of total lipids*

Four different methods were followed, (1) Folch method (2) Bligh and Dyer method (3) Isopropanol: hexane extraction (4) Soxhlet extraction. One gram of dried fungal mycelial mat was taken for lipid extraction in each method.

- **Folch method**
  Fungal lipid was extracted with 3 ml of chloroform: methanol (2:1,v/v) (Folch *et al.*, 1957) by vortexing (1 min) and centrifugation at 2000rpm for 15 min at room temperature, supernatants were collected and
residues re extracted thrice. The lower organic phases were collected and evaporated to dryness under nitrogen and total lipid content was determined gravimetrically.

- **Bligh and Dyer method**
  Lipids were extracted with 3ml of chloroform: methanol (1:2, v/v) (Bligh and Dyer, 1959) by vortexing and centrifugation as described for previous method. Lower organic phases were collected after centrifugation at 2000rpm for 5 min. at room temperature, evaporated to dryness under nitrogen and total lipid contents were determined gravimetrically.

- **Isopropanol: hexane extraction**
  The dry biomass was extracted for total lipids in Isopropanol: hexane (3:1) mixture (Somashekar et al., 2002).

- **Soxhlet extraction**
  Hexane was used as the solvent for the extraction of total lipid; the dried fungal biomass was fixed in soxhlet apparatus, run for six hours at 65°C. The extracted lipid in the organic solvent was dried in rotary evaporator at 600°C (Somashekar et al., 2002).

**Characterization of oleaginous fungus**

Genomic DNA of fungal isolate was extracted according to the method of Moller et al., (1992). Partial region of small subunit (SSU) of rDNA was amplified by PCR using universal fungal primers, ITS1: 5’- TCCGTAGGTGAACCTGCGG-3’, ITS4: 5’- TCCTCCGCTTATTGATATGC3’ (White et al., 1990). The 18s sequence obtained was aligned using BLAST algorithm to find matches within the non-redundant database at NCBI (National Centre for Biotechnology Information; http://blast.ncbi.nlm.nih.gov/Blast.cgi) (Zheng et al., 2000). Sequence data were submitted to Gen Bank through submission tool BankIt of NCBI.

**Scanning Electron Microscopy**

Scanning electron microscopy analysis was carried out at the central instrumentation facility of Indian Institute of Science, Bengaluru to study the microscopic characteristics under Stereoscan 440, LEO/Leica, Cambridge, UK as per Partha and Natarajan, 2008.

**Statistical analysis**

Data obtained from three independent analysis was expressed as mean Standard deviation. Experimental data was subjected to analysis of variance and Duncan’s multiple range test (p <0.05) using the Statistical Analysis System (Duncan’s, 1965).

**RESULTS**

**Enumeration of fungal spp.**

In the present study, a total of 46 different endophytic fungal species associated with stem, leaf and seeds parts of *Jatropha curcas* were isolated (Figure 1.1). The isolated fungi were identified as *Aspergillus* spp., *Penicillum* spp., *Alternaria* spp., *Colletotrichum* spp., *Cladosporium* spp., *Bipolaris* spp., *Fusarium* spp. and *Mycelia sterilia* isolates. Whereas, Ihejirika et al., (2014) have isolated *Fusarium oxysporum*, *Septoria apii*, *Rhizoctonia* spp. and *Aspergillus* spp. from seeds and leaves of *J. curcas*, Susheel and Nutan, (2013) have also reported endophytic fungi in the leaves of *Jatropha curcas*, which were identified as *Colletotrichum truncatum*, *Nigrospora oryzae*, *Fusarium proliferatum*, *Guignardia cammilla*, *Alternaria destruens* and *Chaetomium* spp.
In our study the isolates were coded as JGK (J: Jatropha, GK: GKVK, Bangalore) and numbered from 1 to 46 respectively.

**Figure 1.1:** Endophytic fungal isolates from *Jatropha curcas*

Qualitative screening of fungal spp. by Sudan black B staining method

The lipids were found to be accumulated in cell membrane and intracellularly as well. Among the 46 fungal isolates, 19 fungal isolates were found to be accumulating significantly higher lipids.

The results revealed that, the isolates JGK – 1, 2, 5, 7, 11,12, 13, 14, 15,17, 23, 24, 29,30, 39, 40, 46, MTCC 6344 and MTCC 6301 were deeply stained, which indicates the occurrence of more number of lipid globules, followed by JGK - 6, 16 and 22 which were moderately stained corresponding to lesser lipid globules. The remaining isolates, JGK – 3, 4, 8, 9, 10, 18, 19, 20, 21, 25, 26, 27, 28, 31, 32, 33, 34, 35, 36, 37, 38, 41, 42, 43, 44 and 45 were found to be poorly stained, depicting their lesser accumulation of lipid globules. The staining results are well in agreement with Thakur *et al.*, 1989 and Mamatha, (2009), having applied similar methodology to screen oleaginous fungi (Mucor spp.) where similar results were also reported by Pan *et al.*, (2009) in marine yeasts and fungi. Finally, only 19 deeply stained isolates were carried for further studies.

Total lipid extraction by various methods

Among the different extraction methods, Folch method (chloroform: methanol, 2:1), gave the best results as compared to other 3 methods and confirmed as the ideal solvent system for extracting total lipids from dry fungal biomass which are well in agreement with Somashekar *et al.*, 2002 and Certic *et al.*, 1996. The Folch method yields 257.8±9.59 mg of lipid per gram of dry fungal biomass, followed by Bligh and Dyer method (Chloroform: methanol, 1:2), resulting 222.9±7.93 mg/g of total lipids, in Hexane: Isopropanol (3:2) 198.83±6.53 mg/g of lipid yield and in Soxhlet method, it was 172.93±1.13 mg/g of lipid yield which is less among all the solvent systems (Figure 1.2). So, throughout the research work Folch *et al.*, (1957) method was followed for further lipid extractions.

Quantitative screening of oleaginous fungi by gravimetric method

The results indicated (Fig 1.3) that, biomass (on dry weight basis) varied from a minimum of 1.85 ±0.17 g/L to maximum 7.93±0.05 g/L. Lowest biomass was observed in JGK - 29, *Alternaria* sp. (1.85 ± 0.17 g/L) and highest in JGK – 12, *Aspergillus* sp. (7.93 ± 0.05 g/L) cultures Total Lipid yield varied from 2.04±0.009 g/L in *Aspergillus* sp. JGK - 12 to 0.24 ± 0.009 g/L in *Aspergillus* sp. JGK – 14 and total lipid content was varied from a maximum in *Aspergillus* sp. JGK – 12 i.e., 25.63 ±0.04 % and minimum in *Aspergillus* sp. JGK – 14 i.e., 9.12 ±0.34 %. The standard cultures were found to have 1.4±0.002 g/L with 23.26±0.30% of lipid yield.
in Mortierella alpina (MTCC no.6344) and 0.96±0.007g/L with 19.36±0.17% of lipid yield in Mortierella hyalina (MTCC no.6301).

According to the earlier reports, the fungi isolated from soil accounted to 17% of lipid accumulation, which are explored for the production of special kind of lipids such as Docosahexaenoic acid, Gamma linolenic acid and Eicosapentaenoic acid (Ma, 2006; Du Preez et al., 1997). Hence, among the 19 screened fungal isolates, the highest dry biomass, total lipid yield and lipid content giving Aspergillus sp. JGK – 12 strain was selected for further studies. The ability of microorganisms for lipid production in nitrogen limited media had been supported by Gema et al., 2002.

Figure 1.2: Extraction of lipids with different organic solvents

(Data are expressed as mean ± SD of three replicates)

Figure 1.3: Gravimetric estimation of total lipids in Aspergillus isolates and MTCC cultures

(Values represent mean ± SD of three parallel experiments)
**Morphological features of isolated oleaginous fungus**

The genus *Aspergillus* has upright conidiophores, simple, terminating in a globose of clavate, welling, bearing phialides at the apex and radiating from the entire surface. The conidia are one celled, circular often varying in colors and mass. The observations were in confirmation with the findings of Barnette and Hunter, 1998 where the conidia were smooth or rough walled, basipetal arranged in chains, forming long dry chains which may be divergent (radiate) or aggregated in compact columns (Figure 1.4 and Figure 1.5).

**Figure 1.4 & 1.5: Aspergillus sp.**

**Pure culture of Aspergillus sp. JGK- 12**

**Mycelia with sporangiospores**

**Molecular identification of oleaginous fungus**

On the basis of ITS rDNA sequence and phylogenetic analysis, the culture was identified as *Aspergillus niger* and has been deposited in the NCBI GenBank database with the accession number KP201497. Six hundred and fifty seven (657) bases of *Aspergillus sp*. JGK – 12 was found to be 96% similar in sequence homology with *Aspergillus niger*. Various molecular approaches that target the 18s rDNA gene, mitochondrial DNA, the intergenic spacer region and the internal transcribed spacer (ITS) regions have been used previously for rapid detection of *Aspergillus* from environmental and clinical samples (Henry et al., 2000). In a recent study, species of *Aspergillus* were identified by comparing partial 18s rDNA sequences of other different fungal isolates, with the available ribosomal sequences using BLAST search (Oktay et al., 2011). Automated molecular techniques that would combine extraction of microbial DNA from clinical materials, DNA amplification and amplicon detection are currently under commercial development for identification of fungal pathogens (Loefler et al., 1997). Based on the Partial ITS rDNA sequence and phylogenetic analysis, the culture JGK – 12 was identified as *Aspergillus niger* strain (Figure 1.6).

**Scanning Electron Microscopy**

In SEM analysis, morphology and structural studies of *Aspergillus niger* JGK - 12 have been observed from fungal pellet. The pellets showed superficial hyphae (Figure 1.7), which have unclear holes representing lesser portion of total area, since there were no factual channels. The images depict large number of spores which are scattered around major section (Figure 1.8., 1.9 and 1.10). But, in normal culture systems, *Aspergillus niger* JGK – 12 cells consist of an outer shell of growing hyphae and an inner mass of non-growing mycelium. Researchers have suggested that the formation of pellets originated from the adherence of germinated spores to solid particles in medium (Troung et al., 2004). As per Nitin verma et al., (2011), the nutrient deficiency rate will determine the thickness of the outer growing layer of pellets.
Figure 1.6: Phylogenetic tree of *Aspergillus niger* (JGK 12)

Figure 1.7, 1.8, 1.9, 1.10: SEM photographs of Endophytic *Aspergillus niger* (JGK - 12) from *Jatropha curcas*
CONCLUSION

Biodiesel (fatty acid methyl- or ethyl-esters) is considered a promising alternative fuel and it can be defined as the mono-alkylesters of long chain fatty acids. Fatty acid methyl esters (FAMEs) are the most-common constituents used for biodiesel preparation, which are derived from triacylglycerols (TAGs). Currently vegetable oils and animal fats are used as most common sources for the TAG, however biodiesel production from these sources has its limitations (availability of oil-seed, competition for food). On the contrary biodiesel production from microbial systems is receiving more attention due to lack of these limitations. Hence, Microbial lipids (single cell oils, SCOs) accumulated by oleaginous microorganisms viz., bacteria, algae and fungi are being considered as a source for sustainable/renewable fuel. High oil yielding plant *Jatropha curcas* known as biodiesel plant was selected for the isolation of endophytic fungi associated with it for screening in nutrient rich medium. Based on the external morphology and microscopic observations, the isolates were identified. These isolates were screened for their oleagenicity both qualitatively and quantitatively, staining with Sudan Black – B and gravimetric method respectively. The screened fungal isolates were cultivated on fat production medium to study the growth characteristics, lipid contents. The results indicated that the highest biomass, total lipid yield and lipid content were observed in *Aspergillus niger* JGK – 12 and can be exploited for biodiesel synthesis.

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REFERENCES


INSTRUCTIONS TO THE AUTHORS

Editorial policy

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The cover letter should indicate the corresponding author's full address and telephone/fax numbers and should be in an e-mail message sent to the Editor, with the file, whose name should begin with the first author's surname, as an attachment. The authors may also suggest two to four reviewers for the manuscript.

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3. Key words,
4. Text,
5. Acknowledgements,
6. References,
7. Tables,
8. Figures,
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