

INTERNATIONAL JOURNAL OF PURE & APPLIED BIOSCIENCE

Isolation and a comparative study of Phylloplane mycoflora of Muga host plants Som and Sualu from Goalpara district of Assam

Manjit Kumar Ray^{1*}, Piyush Kumar Mishra², Pradip Kumar Baruah³ and Dipika Choudhury⁴

¹SRF, DBT-Biotech Hub, B.N College, Dhubri, University of Science & Technology, Meghalaya--793101

² Assistant Professor, Dept. of Botany & PI , Biotech Hub, B.N College, Dhubri, Assam, India-783324

³ Associate Professor & HOD, Dept. of Botany Cotton College, Guwahati, Assam-781001

⁴M.A Economics, Department of Economics, Vishwa-Bharti University, West Bengal-731235

*Corresponding Author E-mail: manjit_ray2002@yahoo.com

ABSTRACT

Phyllosphere is one of the major microbial habitat on the earth that provides shelter to diverse and complex microbial communities . Phylloplane microorganisms influence the growth of their host plants , either negatively as pathogens or positively by increasing the stress tolerance and disease resistance. A comparative study was conducted on the Muga silk worm (Antheraea assamensis Helfer) , host plants Som (Persea bombycina King ex Hook. f. Kosterm) and Sualu (Listea polyantha Juss.) from Goalpara district , Assam during the winter season. A total of 10 species of fungi were isolated and identified on the basis of colony morphology, mycelia, sporangiophore and spore structure from different groups. Among all the species the dominant fungal genera on both the P. bombycina and L. polyantha phylloplane were the Rhizopus species which were present in mature leaves. The other genera includes Mucor, Alternaria, Cladosporium, Trichoderma, Penicillium, Cercospora, Curvularia, Aspergillus and Verticillium. The results clearly indicates that fungal species belongs to class Zygomycetes and Deuteromycetes actively colonize on P. bombycina and L. polyantha phylloplane.

Keywords: *Phylloplane, Antheraea assamensis, Persea bombycina , Listea polyantha, Goalpara district.*

INTRODUCTION

Muga silkworm (*Antheraea assamensis* Helfer) is endemic to Northeast India which produces golden silk. They are polyphagous, multivoltine and semi-domesticated in nature but thrive primarily on two host plants. *Persea bombycina* King ex Hook. f. (Kosterm) commonly known as “Som” and *Listea polyantha* Juss. commonly known as “Sualu”. Other food plants include “Dighloti” (*Litsea salcifolia*) and Mejankari (*Litsea citrata*). In Assam muga silk culture is practiced in the districts of upper Assam and certain parts of lower Assam. In lower Assam eastern part of the Goalpara district produces some quantities of muga cocoon. Goalpara district is located between latitudes 25.53° and 26.30° North and 90.07° & 91.05° east. Sericulture in Goalpara district existed almost as a practice among the people since a long time. Goalpara district has been given the geographical identification mark because its climate is suitable for silkworm rearing¹².

Phylloplane microorganisms influence the growth of their host plants, either negatively as pathogens or positively by increasing the stress tolerance and disease resistance^{7,9}. Phyllosphere is one of the major microbial habitat on the earth, that provides shelter to diverse and complex microbial communities like bacteria, yeast, fungi, actinomycetes, algae, protozoa etc.^{7,17,18}. The leaf surface contains different types of stimulatory & inhibitory substances that regulate the microbial colonization on phyllosphere^{7,14}. The filamentous fungi are present predominantly as spores whereas rapidly sporulating species, bacteria and yeast colonize this habitat more actively. The nature & types of microbial population of the leaf surface particularly the economic crops with leaves have received considerable attention^{10,13,16}.

Interactions of surface microflora with leaf pathogens and its impact in disease development has also been studied with reference to some cereal crops^{5,8,15}. The influence of surface microflora has been further enhanced by recent studies showing the existence of cyclic pattern of appearance of air phylloplane litter soil microflora¹.

MATERIALS AND METHODS

Different age leaves viz. tender, semi mature and mature of *P.bombycina* and *L. polyantha* were randomly collected during rearing (outdoor) season Winter in sterile polybags and taken back to the laboratory from 3 different places of Goalpara district namely Govt. Sericulture farm, Agia; Dorapara CMG, Agia and Muga seed multiplication centre, Madang, Rangjuli. Serial washing technique as described by Aneja³ and leaf sectioning and plating method described by Preece & Dickinson (1971) were employed. Leaf discs were cut for each leaf categories with the help of sterilized borer. Pieces from leaf categories were placed separately in 20 ml of sterile distilled water in 250 ml of Erlenmeyer flask shaken for 20 minutes at 120 rpm. The extract of the detachable fungal propagules from the leaf surface was determined by plating 1ml solution from washing to the petriplates containing PDA media. The cut out leaf discs of upper and lower surface were imprinted on the surface of PDA media containing petridishes. The petri dishes were inoculated at $23^{\circ} \pm 2^{\circ}$ C for 5 days followed by the examination of plates for the development of fungal colonies. The isolated fungi were identified. The mycelia and spore characters of fungi were studied under trinocular research microscope (Labomed, Germany) using Lactophenol cotton blue staining and with the help of “A manual of soil fungi by Gilman¹¹ and illustrated genera of imperfect fungi by H.L. Baranatt⁶.”

RESULT AND DISCUSSION

A total of 10 species of fungi were isolated and identified on the basis of colony morphology, mycelia, sporangiophore and spore structure in winter season from different groups. Among all the species the dominant fungal genera on both the *P. bombycina* and *L. polyantha* phylloplane were the *Rhizopus* species which were present in mature leaves. The other genera includes *Mucor*, *Alternaria*, *Cladosporium*, *Trichoderma*, *Penicillium*, *Cercospora*, *Curvularia*, *Aspergillus* and *Verticillium*. The cultural, morphological and microscopic studies revealed the characteristics of vegetative and reproductive structure of the fungal isolates and it was found that fungal species belongs to Class Zygomycetes and Deuteromycetes actively colonize on *P. bombycina* and *L. polyantha* phylloplane. Observation of fungal isolates from phylloplane of muga food plants Som and Sualu during winter season is presented in the Table 1. Where as Percentage of occurrence of Phylloplane mycoflora on both the dorsal and ventral surfaces of the leaves of both Som and Sualu are shown in Fig 1. And Fig 2. respectively.

Fig.1: Map of Goalpara district, Assam, India

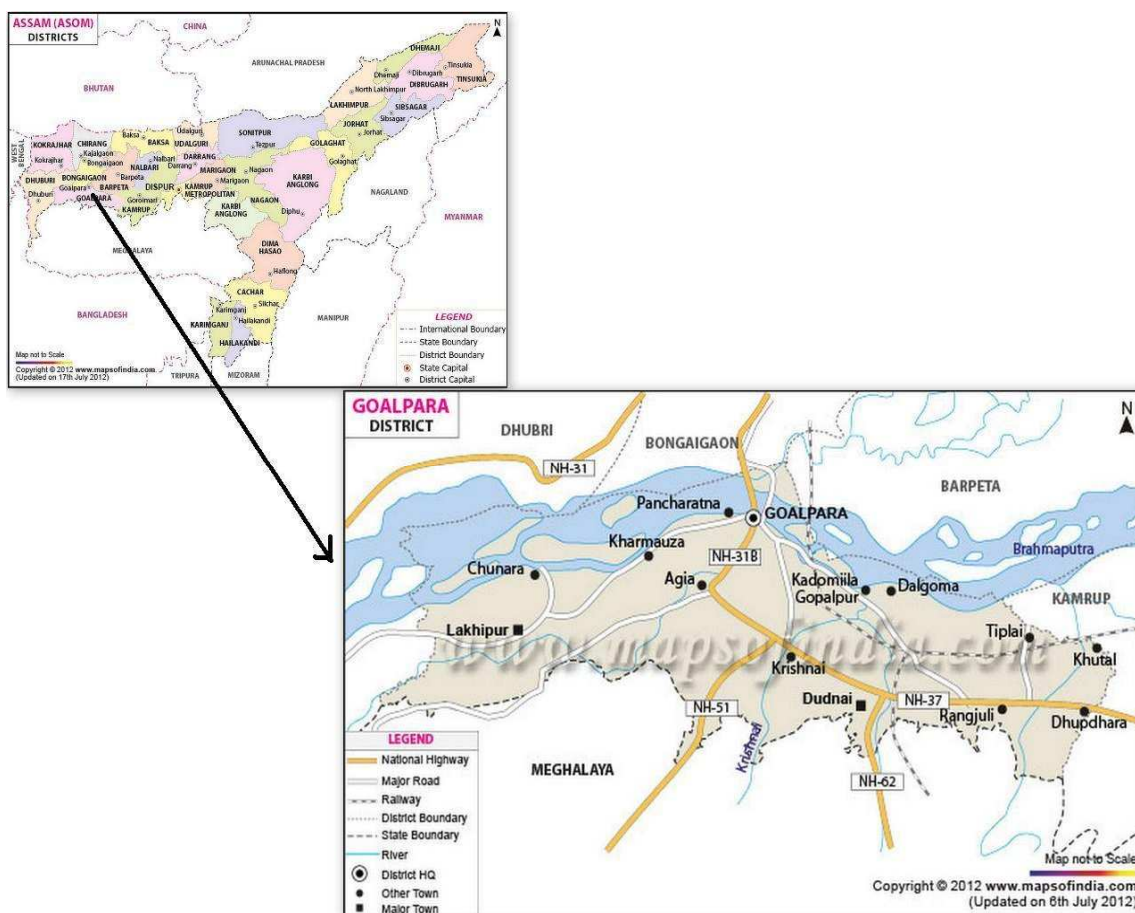


Table1. Observation of fungal isolates from phylloplane of Som and Sualu during winter season

| Fungal isolates | Types of leaves | Types of surface | % of occurrence (Som) | % of occurrence (Sualu) |
|--------------------------|-----------------|------------------|-----------------------|-------------------------|
| <i>Rhizopus spp.</i> | Tender | Dorsal | 40.5 | 70.0 |
| | | Ventral | 45.5 | 65.5 |
| | Semi mature | Dorsal | 50.5 | 56.5 |
| | | Ventral | 60.0 | 68.0 |
| <i>Mucor spp.</i> | Tender | Dorsal | 20.5 | 15.5 |
| | | Ventral | 25.5 | 15.0 |
| | Semi mature | Dorsal | 0 | 24.5 |
| | | Ventral | 0 | 12.5 |
| <i>Alternaria spp.</i> | Tender | Dorsal | 15.5 | 0 |
| | | Ventral | 13.5 | 9.0 |
| | Semi mature | Dorsal | 0 | 14.0 |
| | | Ventral | 0 | 0 |
| <i>Cladosporium spp.</i> | Tender | Dorsal | 13.5 | 0 |
| | | Ventral | 10.5 | 0 |
| | Semi mature | Dorsal | 15.0 | 0 |
| | | Ventral | 15.0 | 0 |
| | Mature | Dorsal | 10.5 | 0 |
| | | Ventral | 20.0 | 0 |

| | | | | |
|--------------------------|-------------|---------------------|--------|----------|
| <i>Trichoderma spp.</i> | Tender | Dorsal | 10.0 | 0 |
| | | Ventral | 5.0 | 0 |
| | Semi mature | Dorsal | 0 | 0 |
| | | Ventral | 0 | 0 |
| | Mature | Dorsal | 0 | 0 |
| Ventral | | 0 | 0 | |
| <i>Aspergillus spp.</i> | Tender | Dorsal | 0 | 4.0 |
| | | Ventral | 0 | 0 |
| | Semi mature | Dorsal | 24.5 | 0 |
| | | Ventral | 20.0 | 11.0 |
| | Mature | Dorsal | 0 | 0 |
| Ventral | | 0 | 4.5 | |
| <i>Penicillium spp.</i> | Tender | Dorsal | 0 | 0 |
| | | Ventral | 0 | 0 |
| | Semi mature | Dorsal | 10.0 | 0 |
| | | Ventral | 5.0 | 8.5 |
| | Mature | Dorsal | 0 | 0 |
| Ventral | | 0 | 0 | |
| <i>Cercospora spp.</i> | Tender | Dorsal | 0 | 0 |
| | | Ventral | 0 | 0 |
| | Semi mature | Dorsal | 0 | 5.0 |
| | | Ventral | 0 | 0 |
| | Mature | Dorsal | 15.5 | 0 |
| Ventral | | 15.5 | 0 | |
| <i>Curvularia spp.</i> | Tender | Dorsal | 0 | 10.5 |
| | | Ventral | 0 | 10.5 |
| | Semi mature | Dorsal | 0 | 0 |
| | | Ventral | 0 | 0 |
| | Mature | Dorsal | 13.0 | 0 |
| Ventral | | 10.0 | 0 | |
| <i>Verticillium spp.</i> | Tender | Dorsal | 0 | 0 |
| | | Ventral | 0 | 0 |
| | Semi mature | Dorsal | 0 | 0 |
| | | Ventral | 0 | 0 |
| | Mature | Dorsal - (Ventral) | 0- (0) | 9.0- (0) |

Fig. 1: Percentage of occurrence of Phylloplane mycoflora on Dorsal surface of the leaves of both Som and Sualu

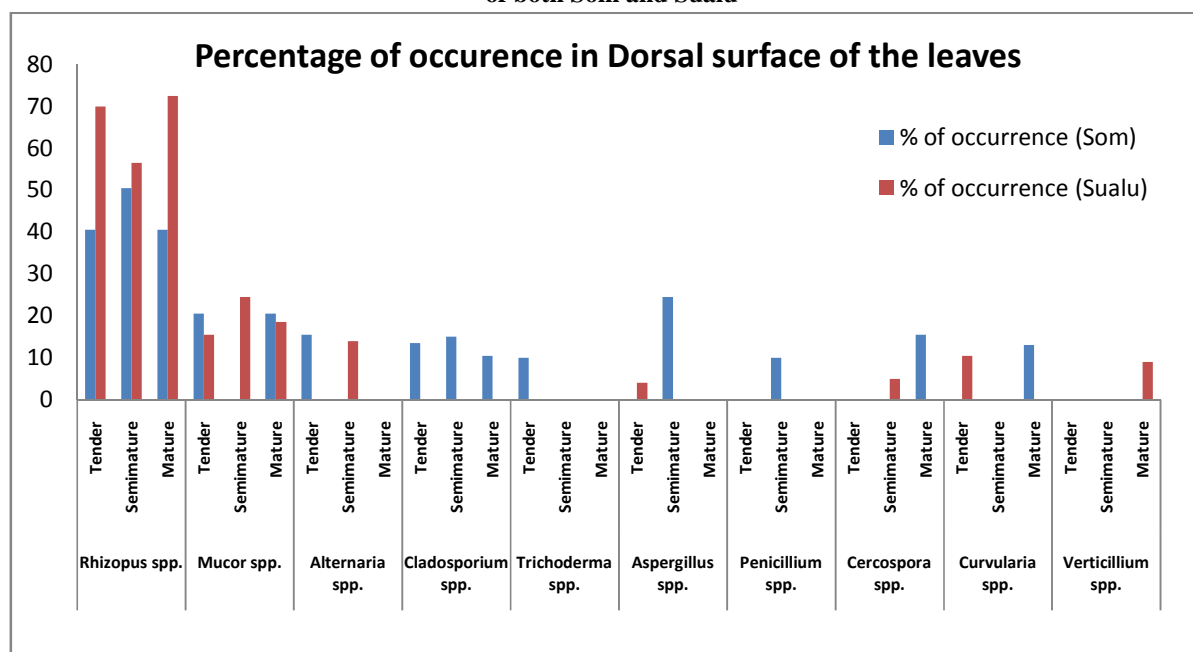
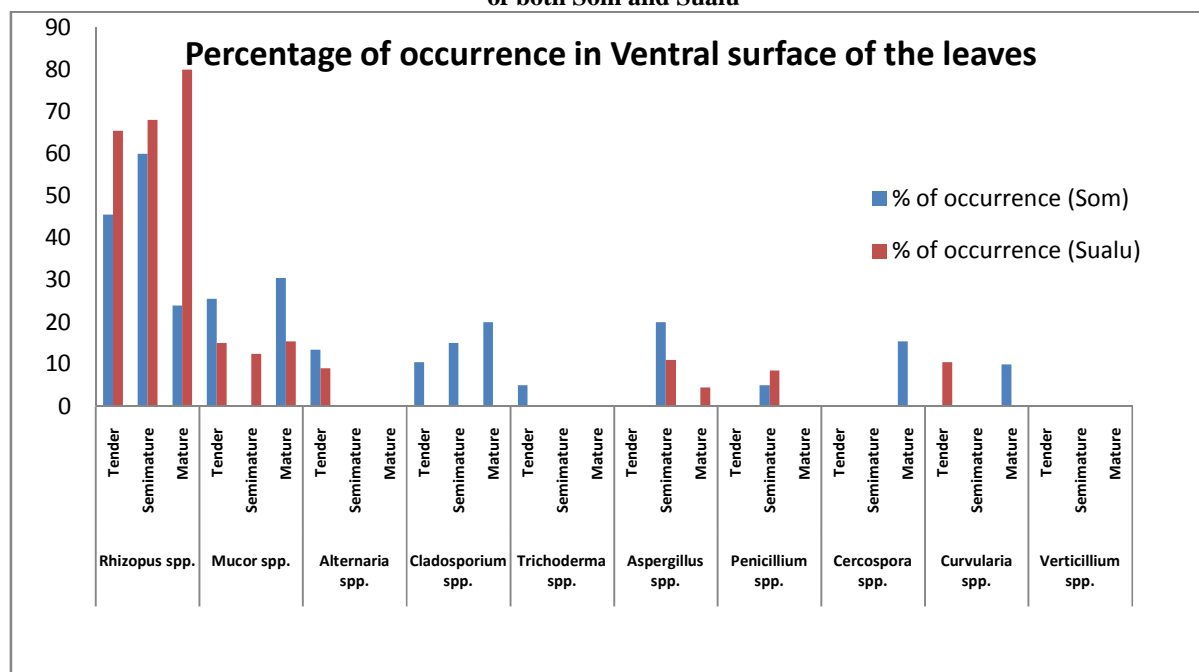


Fig. 2: Percentage of occurrence of Phylloplane mycoflora on Ventral surface of the leaves of both Som and Sualu



CONCLUSION

The initial studies gave the qualitative data on the som and sualu phylloplane mycoflora during Winter season of Goalpara district, Assam, India. More works will be carried out and will be communicated for seasonal variation on phylloplane mycoflora of both Som and Sualu in due course of time.

Acknowledgement

Financial assistance received from the Department of Biotechnology, Government of India is gratefully acknowledged.

REFERENCES

- Adhikari, R.S. and Tiwari, A. Some experimental studies of phylloplane and litter fungi of *Quercus semicarrufolia*. *J.Ind.Bot. Soc.* **70**: 129-134 (1991)
- Atlas R.M. and Parks L.C. Handbook of microbial media. 2nd ed. C press; Boca Raton. (1997)
- Aneja, K.R. Experiments in microbiology, Plant pathology and biotechnology, 4th ed, *New age International publisher*. 176-177 (2003)
- Bakshi, B.K. Dying of Sal II. Role of Hypoxylon. NS Bisht, R.K. Khatri, D. Khanna, A., A Siddiqui. *Indian Forester* **89**: 265 -68 (1963)
- Barnes, G. Inhibition of Erysiphe polygoni on clover leaf surface by saprophytic spores in "Ecology of leaf surface microorganisms" edited by Preece and Dickinson (A.P.London) (1971)
- Baranatt H.L. Illustrated Genera of Imperfect Fungi . 2nd Ed, Published by Burgess Publishing Co. (1960)
- Bhuyan, P.M. Sandilya, S.P. and Gogoi, D.K., Phyllosphere Microflora of Muga Silkworm Host Plant *Persea bombycina* Kost (Som) Leaves in Jorhat District of Assam, India. *International Research Journal of Biological Sciences*, **2(12)**: 60-65 (2013)

8. Blakeman, J.P. and Brodie, I.D.S., Inhibition of pathogens by epiphytic bacteria on aerial plant surface in “Microbiology of Aerial plant surface” edited by Preece and Dickinson. (1976)
9. Cordier, T. Robin, C. Capdevielle, X. Desprez Loustau, M.L. and Vachor, C., Spatial variability of phyllosphere fungal assemblages: genetic distance predominates over geographic distance in a European beech stand (*Fagus sylvatica*), *Funghi Ecol.*, **5(5)**: 509-520 (2012)
10. Dickinson, C.H and Preece, T.F., “Microbiology of Aerial plant surface” A.P. London. (1976)
11. Gilman Joseph Charles. A manual of soil fungi. Published by Printwell. (1995)
12. Goswami Chandrama and Manisha Bhattacharya. Contribution of Sericulture to Women’s Income in Assam -A Case Study in Goalpara District of Assam, India. *International Journal of Scientific and Research Publications*, **3(3)**: (2013)
13. Leben, C. Epiphytic microorganisms in relationship to plant diseases. *Annl. Rev. Phytopathology*, **3**: 209-230 (1965)
14. Leveau, J., Life on leaves, *Nature*, 461, 741 (2009)
15. Sarkar, S.K. and Samaddar, K.R., Occurrence and interactions between aerial plant surface microorganisms. *Ind. Sci. Cong. Ari. Sect.* **65**: B (Abstract). (1978)
16. Sinha, S., Microbiological complex of the phyllosphere and disease control. *Indian Phytopathology*, **18**: 1-20 (1965)
17. Kim, M. Singh, D. Lai-Hoe, A. Go, R. Rahim, R. A. Ainuddin, A.N. Chun, J. and Adams, J.M., Distinctive Phyllosphere Bacterial Communities in Tropical Trees, *Microb Ecol.*, **63 (3)**: 674-681 (2012)
18. Morris, C. and Kinkel, L., Fifty years of phyllosphere microbiology: significant contributions to research in related fields. *Phyllosphere Microbiology*, Lindow, S., E. Hecht-Poinar and V. Elliott, (Eds.). APS Press, St. Paul, MN, USA, 365-375 (2002)