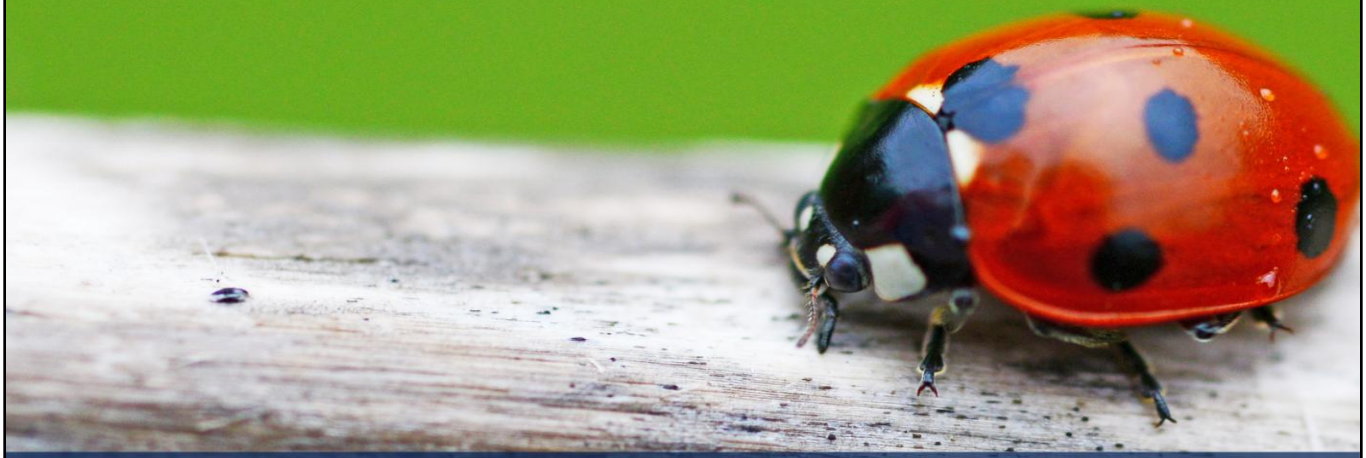
ISBN: 978-81-953600-3-1

Frontiers in Life Science

Volume III



Editor

Dr. Babita Rana

Dr. Ninad S. Dharkar

Dr. Swati N. Zodpe

Dr. Ganesh C. Nikalje

Bhumi Publishing

Bhumi Publishing

First Edition: 2021

Frontiers in Life Science (Volume III)

(ISBN: 978-81-953600-3-1)

Editors

Dr. Babita Rana

Department of Botany,

G. N. Khalsa College,

Matunga, Mumbai,

M.S., India

Dr. Ninad S. Dharkar

Department of Botany,

S. P. M. Science and

Gilani Arts, Commerce College,

Ghatanji, Dist. Yavatmal M.S., India

Dr. Swati N. Zodpe

Department of Microbiology,

Shri. Shivaji College of Arts,

Commerce and Science,

Akola, M.S., India

Dr. Ganesh C. Nikalje

PG Department of Botany,

Seva Sadan's R. K. Talreja College of Arts,

Science and Commerce,

Ulhasnagar, M.S., India

Bhumi Publishing

2021

First Edition: 2021

ISBN: 978-81-953600-3-1

Published by:

Bhumi Publishing,

Nigave Khalasa, Kolhapur 416207, Maharashtra, India

Website: www.bhumipublishing.com

E-mail: bhumipublishing@gmail.com

Book Available online at:

https://www.bhumipublishing.com/books/

PREFACE

"Science knows no country, because knowledge belongs to humanity, and is the torch which illuminates the world." – Louis Pasteur

Life Sciences is about seeking answers to fundamental questions in biology about the origin, diversity, organisms, ecology, evolution, environment and, seeing how such understanding can be put together into an applied perspective. This book entitled, Frontiers in life sciences (Volume lll) is a compilation of a wide range of topics from biodiversity to environment covering different disciplines of life sciences. Several aspects like composition of pteridophytes, gall midges of lndia, remote sensing, biotic and abiotic stress tolerance, tissue culture of medicinal plants, pest management, bioremediation, epidemic diseases, biotechnology, biofuel production, mushroom cultivation and bird diversity. The third volume of this book series, comprises of 17 chapters, covering Pteridophytic diversity in the Koppa Taluka of Central Western Ghats (South lndia), Cold stress induced biometric and biochemical changes in the fenugreek seeds, an overview on Plant quarantine- , Tissue culture techniques of medicinal plant Plumbago zeylanica, Taxonomic work of the gall midges in lndia, lmportance of Mushroom, Role of ladybird beetles (coccinellidae: coleoptera) for the management of sucking pests in different ecosystems. The range of topics also include L- carnitine as a mitochondria based nutraceutical against oxidative stress, mitochondrial dysfunction in neurodegenerative diseases, Acid tolerance marker of the probiotic bacteria at molecular level, Entomopathogenic microorganisms as biopesticides, Epidemiology, diagnosis and treatment of mucormycosis and Bird diversity of educational campus. The collection has some additional topics of relevance to energy and environment, which include, Bioremediation potential of nitrogen fixing bacteria, Remote sensing studies of the environment and biodiversity, White pollution of marine ecosystem: a global tragedy for our oceans and sea life, Biotechnology- for innovations in sustainable aquaculture and fishery, Comparative study of micro algae in production of biodiesel at laboratory condition.

l hope this book will be useful for the undergraduate, post graduate students and Ph.D. Scholars, researchers, and faculties of Botany, Zoology, Biotechnology, life science, Microbiology, Biochemistry department. l am thankful to all contributors for their valuable work. l thank all the contributors to this book volume 3 for sending their articles in time. My special thanks and appreciation to Bhumi Publications and process manager/author services manager, for their help in bringing out the book.

- Editorial Team

Frontiers in Life Science (Volume lll)

(lSBN: 978-81-953600-3-1)

CONTENTS

Frontiers in Life Science (Volume III)
ISBN: 978-81-953600-3-1

Pteridophytic Composition in Koppa Taluk, Central Western Ghats, South India

Parashurama T. R[1,2]*, J. Deepa[2], Girish K.G[2,3] and Prakash Kariyajjanavar[2,4]

[1]Department of Botany,
Kumadvathi First Grade College, Shivamogga
[2]Panchavati Research Academy for Nature, Kalamanji,
Linganamakki, Sagara, Shivamogga
[3]Department of Environmental Science,
L.B and S.B.S. College, Sagara, Shivamogga
[4]Department of P.G Studies and Research in Environmental Science,
Gulbarga University, Kalaburagi, Karnataka, India
*Corresponding authors E-mail: trparashurama@gmail.com

Abstract:

Pteridophytes are vascular cryptograms reside of transitional position between bryophytes and spermatophytes and were the first plants to widely colonize the terrestrial ecosystem. In the present research work, forty six species of pteridophytes belonging to nineteen families were documented through distribution study in the forests of Koppa taluk, Chikmagalur district of Central Western Ghats. *Adiantum philippense* L was observed as higher importance value index followed by *Thelypteris dentata* (Forsk.) E.P.St.John., *Pteris biaurita* L, *Adiantum raddianum* C.Presl, *Selaginella delicatula* (Desv.*ex* Poir) Alston and *Blechnum orientale* L. The Shannon's diversity index value (H^1) and Simpson's diversity (D) values for pteridophytic species were indicated high diversity and species richness. The extensive deforestation and habitat fragmentation in this region may pose serious threat to growth of pteridophytic communities. In this context, documentation and distribution studies of pteridophytic diversity are essential for conservation of threatened species.

Keywords: Diversity indices, conservation, pteridophytes, Western Ghats

Introduction:

India is diversified with rich pteridophytic flora due to its varied topographical climatic conditions and geographical position. About 9% world Pteridophytic species are found in India. In India, many detailed pteridophytic survey have been reported by Beddome (1883, 1892) to Fraser Jenkins (2010) filled the large lacuna in our knowledge relating to the pteridology. Dixit

(1984) has reported 1050 species and Chandra *et al.* (2008) put the number of pteridophytic species at 1150 from India. Later, Fraser Jenkins (2010) revised pteridophytic numbers to 1000 species in India including pteridophytes of Arunachal Pradesh (Fraser-Jenkins & Benniamin, 2010). Khullar (1994, 2000) listed 360 species of pteridphytes from Western Himalaya with 399 pteridophytes given by Fraser-Jenkins (2010). According to Manickam and Irudayaraj, (1992) Western Ghats supported 349 species out of 1100 to 1200 species of fern and fern allies in India. In Central Western Ghats, Karnataka region has richest pteridophytic diversity including Chikmagalur district (Sukumaran *et al.,* 2008, 2009; Deepa *et al*., 2011, 2013a, 2013b ; Nataraja *et al.*, 2011, Parashurama *et al.*, 2016, Fatima and Nandashree, 2019). The present study was under taken for fulfill the lacuna of information relating to the composition and distribution of different taxa of pteridophytes in forest region of Koppa taluk.

Materials and Methods:

Study area:

The study area lies between 13^{o} 32' 06 N latitude and between 75^{o} 22' 05" E longitude. The altitude is 763 meters above m.s.l. and the annual rainfall exceeds 2500 mm. It encompasses area of 572.4 km^{2} forest area.

Collection and identification:

A survey of pteridophytes in study area conducted during the period of 2015 to 2019. A total of 25 transects each measuring 50×2m were laid in forest of Koppa region. Different habitat forms of pteridophytes were recorded. In case of epiphytic form present on large tree considered as one colony and free floating hydrophytic form present in water sources also considered as one. Diagnostic features of all the specimens were studied and relevant field notes were made on fresh plant materials. Identification of specimens were made by referring to available literature and pteridophyte floras (Beddome, 1863, 1865, 1883; Clarke 1880, 1961; Blatter *et al.,* 1922; Tryon and Tryon, 1982; Bir, 1987; Khullar, 1994, 2000; Verma 2005 and 2008; Dixit, 1984; Chandra, 2008). Authentications of the species were done with the help of Indian Fern Society. All the collected specimens were properly processed and the herbariums have been deposited in the department of Applied Botany, Kuvempu University, Shankaraghatta. For nomenclature, Fraser-Jenkins (2008) has been followed.

Statistical analysis:

Data on various pteridophytic characters in different transect were collected and analyzed through statistical methods (Shukla, 2001; Tuomisto 2000; Deepa *et al.,* 2012). Density is an expression of the numerical strength of a pteridophytic species where the total number of

ISBN: 978-81-953600-3-1

individuals of each species in all transects are divided by the total number of transects studied. Frequency refers to the degree of dispersion of individual pteridophytic species in an area and usually expressed in terms of percentage occurrence. The samplings were made at different sampling units randomly in the study area and documented the species. Abundance is the study of the number of individuals of different species in the community per unit area. By transect method, samplings were made at random at several places and the number of individuals of each species was summed up for all the transects divided by the total number of transects in which the species occurred.

Relative density is the study of numerical strength of a species in relation to the total number of individuals of all the species. The degree of dispersion of individual species in an area in relation to the number of all the species occurred considered as Relative frequency. Importance Value Index is used to determine the overall importance of each species in the community structure. In calculating this index, the percentage values of the relative frequency, relative density and relative dominance are summed up together and this value is designated as the Importance Value Index or IVI of the species.

Based on the data of the occurrence of the species in the transects, Shannon's diversity index (H^1) was calculated which is represented by formula (H^1) = $-\Sigma p_i \ln p_i$, where $P_i = (n_i/N)$. Simpson's diversity index was calculated by formula (D) = $\Sigma [n_i (n_i\text{-}l)/ N (N\text{-}l)]$, Where, '$n_i$' is the Number of individuals of the i^{th} species and 'N' represented as total number of individuals.

Result and Disscussion:

In the present work, an attempt has made to study the distribution and species diversity of pteridophytes in Koppa taluk of Chikmagalur district**.** The phytosociological attributes such as abundance, density, frequency and their relative values (Table 1) and Importance Value Index were calculated in study area. The Shanon index is a measure of the average degree of "uncertainity", it is increases as the number of species increases and as the distribution of individuals among the species becomes even (John James, 1988). Simpson's index, which varies from 0 to 1, gives the probability that two individuals drawn at random from a population belong to the same species. If the probability is high and that both individuals belongs to the same species, then the diversity of the community sample is low (John James, 1988).

In Koppa taluk, 2,953 individuals were enumerated belonging to 19 families, 31 genera and 46 species. The pteridophytes of various habitats such as terrestrial, epiphytes, lithophytes and hydrophytes were found in this region.

Pteris is the largest genus having 5 species followed by Adiantum (4), Asplenium and Selaginella each of 3 species. There are17 out of 31 genera including single species in study area. *Adiantum philippense* L. is densely populated in study area followed by *Thelypteris dentata* (Forsk.) E.P.St.John, while, lowest for *Trichomanes companulatum* Roxb.. *Selaginella delicatula* (Desv.*ex* Poir) Alston found to be more abundant followed by *Selaginella tenera* (Hook. andGrev.) Spring, *Adiantum raddianum* C.Presl *Adiantum philippense* L, *Lepisorus nudus* (Hook.) Ching recorded as of less abundance. The maximum frequency found in *Pteris biaurita* L while *Trichomanes companulatum* Roxb. observed as of low frequency.

Adiantum philippense L. was reported by 342 individuals showing higher importance value index of 22.95 and followed by *Thelypteris dentata* (Forsk.) E.P.St.John (19.84), *P. biaurita* (18.3), *Adiantum raddianum* C.Presl (14.96) and *Selaginella delicatula* (Desv.*ex* Poir) Alston (14.36). The Shannon's diversity index value (H^1) 3.25 and Simpson's diversity (D) = 0.052 values for pteridophytic species in Koppa taluk (Fig. 1). Pteridaceae stands the dominant family of the study area with 12 species followed by Polyodiaceae with four species. *Pteris* and *Adiantum* were the largest genera with a maximum number of five and four species respectively.

Present study showing Pteridaceae was a dominant family followed by Polyodiaceae in contrast, the study made by Pinak *et al.* (2011) in Bark valley of Assam showed Polyodiaceae was largest family in followed by Thelypteridaceae, Aspidiaceae and Pteridaceae. Similar observation made by Sukumaran *et al.* (2009) also observed in Kanyakumari district of Tamilu Nadu. Present study indicated that heterogenous pteridophytic community, due to prediction of Raunkiaer (1934), the frequency class D is greater than A, B and C then the community is said to be heterogeneous. Mean rainfall positively correlated (p<0.01) with the species richness in the study area. Similarly, interpreting such bivariate relationships hampered by strong correlations between the climatic factors and ferns species richness (Jeremiah *et al.*, 1998). The extensive deforestation and habitat fragmentation activities would pose serious threat to growth of certain pteridophytic species. Henceforth documentation and distribution studies of pteridophytic diversity needs to be given top priority to help conservation resources and preservation of the threatened of species.

Anthropogenic activities are destructed and altered the habitat of different angiospermic species as well as pteridophytic species from the locality; besides this, it is also observed that encroachment of forest area for agriculture and developmental activities like expansion of roads and township extensions affecting these populations. In this context, present study provides fundamental information on a novel pteridophytic species composition and distribution in study area. This data could be used for designing conservation strategies in Koppa region of Central western Ghats.

ISBN: 978-81-953600-3-1

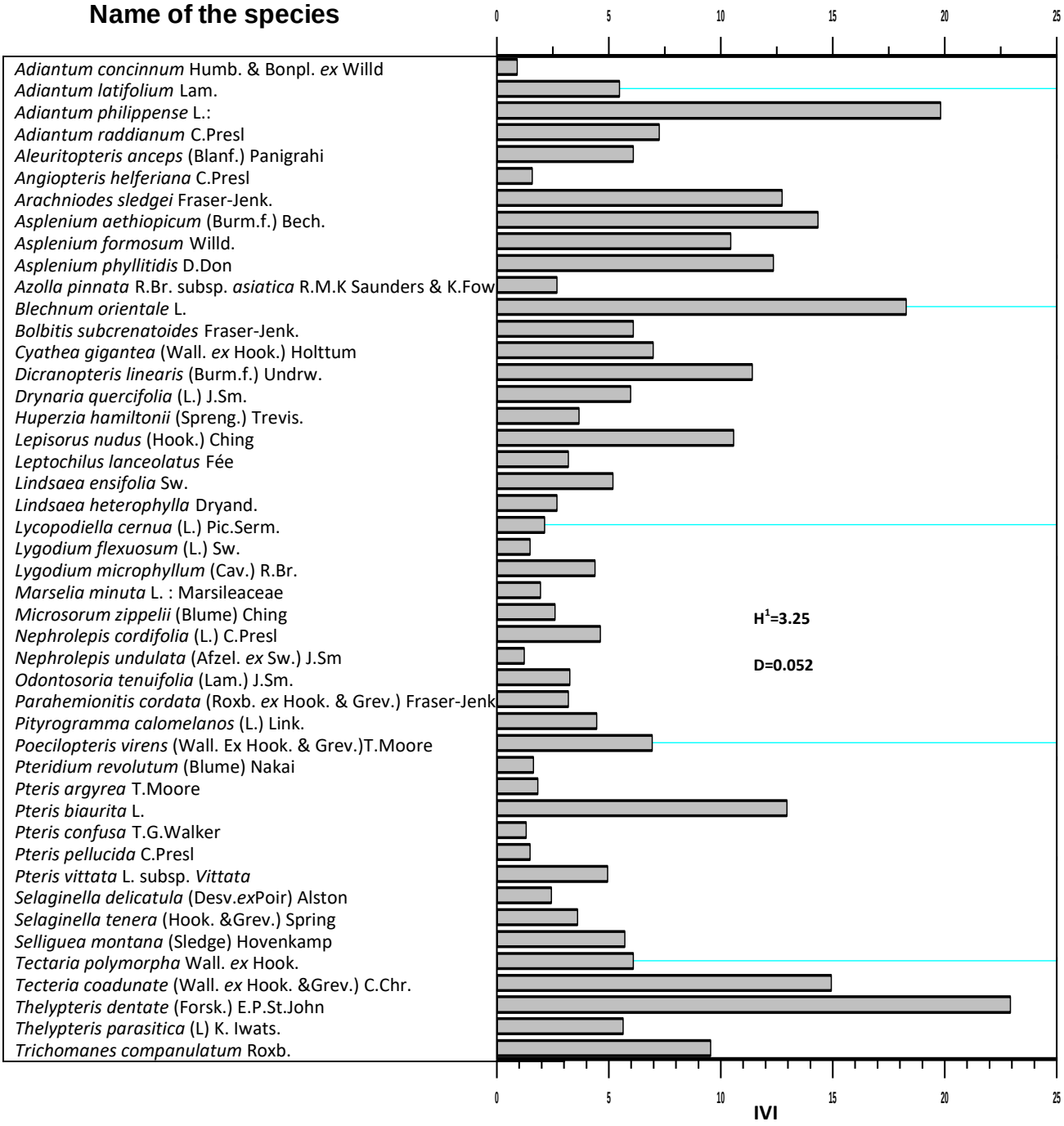
Table 1: Check list of Pteridophytes in Koppa taluk, Chikmagalur district, Karnataka

Sr. No.	Name of the Species and Family	Den	Fre	Abu	Re.Den	Re. Ab	Re. Fre
1	*Adiantum concinnum* Humb.and Bonpl. *ex* Willd : Adiantaceae	4.16	0.36	11.56	3.52	3.12	2.93
2	*Adiantum latifolium* Lam. Adiantaceae	1.88	0.24	7.83	1.59	2.12	1.95
3	*Adiantum philippense* L.: Adiantaceae	13.68	0.88	15.55	11.58	4.20	7.17
4	*Adiantum raddianum* C.Presl : Adiantaceae	7.84	0.48	16.33	6.64	4.41	3.91
5	*Aleuritopteris anceps* (Blanf.) Panigrahi: Pteridaceae	2.12	0.28	7.57	1.79	2.04	2.28
6	*Angiopteris helferiana* C.Presl: Marattiaceae	1.92	0.24	8.00	1.63	2.16	1.95
7	*Arachniodes sledgei* Fraser-Jenk.: Dryopteridaceae	0.92	0.16	5.75	0.78	1.55	1.30
8	*Asplenium aethiopicum* (Burm.f.) Bech.: Aspleniaceae	0.48	0.12	4.00	0.41	1.08	0.98
9	*Asplenium formosum* Willd. : Aspleniaceae	1.52	0.2	7.60	1.29	2.05	1.63
10	*Asplenium phyllitidis* D.Don : Aspleniaceae	0.2	0.08	2.50	0.17	0.68	0.65
11	*Azolla pinnata* R.Br. subsp. *asiatica* R.M.K Saunders and K.Fowler : Azollaceae	0.16	0.08	2.00	0.14	0.54	0.65
12	*Blechnum orientale* L.: Blechnaceae	6.24	0.6	10.40	5.28	2.81	4.89
13	*Bolbitis subcrenatoides* Fraser-Jenk. : Lomariopsidaceae	0.2	0.04	5.00	0.17	1.35	0.33
14	*Cyathea gigantea* (Wall. *ex* Hook.) Holttum: Cyatheaceae	0.24	0.08	3.00	0.20	0.81	0.65
15	*Dicranopteris linearis* (Burm.f.) Underw.: Gleichenaceae	2.52	0.36	7.00	2.13	1.89	2.93
16	*Drynaria quercifolia* (L.) J.Sm. : Polypodiaceae	1.28	0.24	5.33	1.08	1.44	1.95
17	*Huperzia hamiltonii* (Spreng.) Trevis. : Lycopodiaceae	0.72	0.12	6.00	0.61	1.62	0.98
18	*Lepisorus nudus* (Hook.) Ching: Polypodiaceae	0.4	0.32	1.25	0.34	0.34	2.61
19	*Leptochilus lanceolatus* Fée : Polypodiaceae	0.12	0.04	3.00	0.10	0.81	0.33
20	*Lindsaea ensifolia* Sw.: Lindsaeaceae	1.36	0.24	5.67	1.15	1.53	1.95
21	*Lindsaea heterophylla* Dryand. :Lindsaeaceae	0.52	0.16	3.25	0.44	0.88	1.30
22	*Lycopodiella cernua* (L.) Pic.Serm.: Lycopodiaceae	0.32	0.12	2.67	0.27	0.72	0.98
23	*Lygodium flexuosum* (L.) Sw.: Lygodaceae	1.24	0.24	5.17	1.05	1.40	1.95

24	*Lygodium microphyllum* (Cav.) R.Br. :Lygodiaceae	0.2	0.08	2.50	0.17	0.68	0.65
25	*Marselia minuta* L. : Marsileaceae	0.24	0.04	6.00	0.20	1.62	0.33
26	**Microsorum zippelii* (Blume) Ching: Polypodiaceae	0.56	0.12	4.67	0.47	1.26	0.98
27	*Nephrolepis cordifolia* (L.) C.Presl: Oleandraceae	1.08	0.08	13.50	0.91	3.65	0.65
28	*Nephrolepis undulata* (Afzel. *ex* Sw.) J.Sm : Oleandraceae	0.72	0.12	6.00	0.61	1.62	0.98
29	*Odontosoria tenuifolia* (Lam.) J.Sm.: Lindsaeaceae	4.8	0.36	13.33	4.06	3.60	2.93
30	*Parahemionitis cordata* (Roxb. *ex* Hook. and Grev.) Fraser-Jenk.: Pteridaceae	0.72	0.08	9.00	0.61	2.43	0.65
31	*Pityrogramma calomelanos* (L.) Link.: Pteridaceae	1.92	0.36	5.33	1.63	1.44	2.93
32	*Poecilopteris virens* (Wall. Ex Hook. and Grev.)T.Moore : Lomariopsidaceae	4.56	0.72	6.33	3.86	1.71	5.86
33	*Pteridium revolutum* (Blume) Nakai: Dennstaedtiaceae	2.56	0.24	10.67	2.17	2.88	1.95
34	*Pteris argyrea* T.Moore : Pteridaceae	2.12	0.28	7.57	1.79	2.04	2.28
35	*Pteris biaurita* L.: Pteridaceae	9.48	0.92	10.30	8.03	2.78	7.49
36	*Pteris confusa* T.G.Walker : Pteridaceae	0.56	0.12	4.67	0.47	1.26	0.98
37	*Pteris pellucida* C.Presl: Pteridaceae	5.64	0.64	8.81	4.77	2.38	5.21
38	*Pteris vittata* L. subsp. *Vittata*: Pteridaceae	4.72	0.36	13.11	4.00	3.54	2.93
39	*Selaginella delicatula* (Desv.*ex*Poir) Alston : Selaginellaceae	4.32	0.12	36.00	3.66	9.72	0.98
40	*Selaginella tenera* (Hook. andGrev.) Spring : Selaginellaceae	5.48	0.24	22.83	4.64	6.17	1.95
41	*Selliguea montana* (Sledge) Hovenkamp : Polypodiaceae	0.2	0.12	1.67	0.17	0.45	0.98
42	*Tectaria polymorpha* Wall. *ex* Hook.: Tectariaceae	2.12	0.28	7.57	1.79	2.04	2.28
43	*Tecteria coadunata* (Wall. *ex* Hook. andGrev.) C.Chr. : Dryopteridaceae	2.76	0.32	8.63	2.34	2.33	2.61
44	*Thelypteris dentata* (Forsk.) E.P.St.John :Thelypteridaceae	11.44	0.72	15.89	9.69	4.29	5.86
45	*Thelypteris parasitica* (L) K. Iwats.	1.8	0.24	7.50	1.52	2.03	1.95
46	*Trichomanes companulatum* Roxb.: Hymenophyllaceae	0.08	0.04	2.00	0.07	0.54	0.33

Note: Den-Density; Fre-Frequency; Abu-Abundance; Re.Den-Relative Density; Re.Fre-Relative Frequency; Re.Ab-Relative abundance*Near threatened

ISBN: 978-81-953600-3-1



Note: H^1: Shannon's diversity index value**; D:** Simpson's diversity**; IVI:** importance value index

Figure 1: Diversity of Pteridophytes with Importance value index in Koppa taluk of Chikmagalur district, Central western Ghats, South India

Acknowledgments:

The authors are grateful to Indian Fern Society and Department of Botany, Kuvempu University for help in identifications with valuable guidance and encouragement. Constant encouragement and motivation by Panchavati Research Academy for Nature (PRANA), Shivamogga, is greatly appreciated.

References:

Beddome, R. H. 1863-1865 The Ferns of Southern India, tt. 171. Gantz Bros., Madras

Beddome, R. H. 1865-1870 The Ferns of British India, tt. 345. Gantz Bros., Madras

Beddome, R. H. 1883 Handbook to the Ferns of British India, Ceylon and the Malay Peninsula, pp. 501. Thacker Spink and Co., Calcutta

Bir, S. S. 1987 Pteridophytic Flora of India: rare and endangered elements and their conservation, Indian Fern J. 4(1-2): 95-101

Blatter, E. and D'Almeida, J. E. 1922. The Ferns of Bombay, pp. 56-103. D.B. Taraporevala Sons and Co., Bombay

Chandra, S. 2000 The Ferns of India (Enumeration, Synonyms and Distribution), pp. 459. International Book Distributors, Dehra Dun

Chandra, S., Fraser-Jenkins, C. R., Kumari A and Srivastava A. 2008. A summary of the status of Threatened Pteridophytes of India Taiwania 53(2): 170-209

Clarke, C. B. 1880. A Review of the Ferns of Northern India. Trans Linn Soc London 2 Bot 1: 425-611

Deepa, J. Parashurama, T.R., Krishnappa, M. and Nataraja, S. 2011. Enumeration of Pteridophytes in Madhuguni Forest , Central Western Ghats, Karnataka, South India, Indian Fern J, 28: 112-119

Deepa J, T.R. Parashurama, M. Krishnappa and S. Nataraja 2013a. Pteridophytic Flora of Kemmangundi Forest, Karnataka, South India. Annals of Plant Sciences, pp 484 - 486.

Deepa, J., Parashurama, T.R., Krishnappa,M. and Nataraja, S. 2013b. Distribution of Pteridophytes in KiggaForest, Central Western Ghats,Karnataka, South India. Indian Fern Journal. 30: 18-24.

Dixit R, D. 1984 A Census of the Indian Pteridophytes, Flora of India ser. IV, pp. 177. Botanical Survey of India, Howrah

Dixit, R.D. and Vohra, J.N. 1984. A Dictionaryof the Pteridophytes of India (Flora of IndiaSeries 4) Botanical Survey of IndiaPublication, Department of Environment, Government of India, Botanical Garden,Howrah, pp. 1-177.

ISBN: 978-81-953600-3-1

Fatima- Tu-Zahora-Jabeen and Nandashree B. M. 2019. Diversity of Pteridophytes and their medicinal uses in Mullayyangiri region of Chikkamagaliru taluk, Karnataka-State, India. International Journal of Scientific Research and Review. 8:19-25

Fraser-Jenkins, C. R. and BENNIAMIN, A. 2010. ["2009"]. Fifty rarities and additions to the pteridophytic flora of Arunachal Pradesh, N.E. India, Panjab Univ. Res. J., Sci., 59: 1-38

Fraser-Jenkins, C. R. 2008 Endemics and Pseudo-Endemics in Relation to the Distribution Patterns of Indian Pteridophytes Taiwania,53(3):264-292

Fraser-Jenkins, C. R. 2008. Taxonomic Revision of Three Hundred Indian Subcontinental Pteridophytes With a Revised Census-List, pp. 685. Bishen Singh Mahendra Pal Singh, Dehra Dun

Fraser-Jenkins, C. R. 2010. Nepal's little known pteridophytes, the hidden work of David Don, and the geography and distribution of Ind-Himalayan ferns with State lists, pp. 208. Website version: www.groups.yahoo.com/group/Indian-Ferns

Jeremiah S.L., Andrew B., and Rose B. 1998. Assessing fern diversity:relative species richness and its environmental correlates in Uganda, Biodiversity and Conservation, 7:1387-1398.

John, A.L. and James, F.R. 1988. Statistical Ecology : A Primer on Methods and Computing. A Wiley-Interscience publication, p. 309.

Khullar, S. P. 1994 2000 An Illustrated Fern Flora of Western Himalaya. Vols.1 and 2. International Book Distributors, Dehra Dun

Manickam, V. S. and Irudayaraj, V. 1992. Pteridophytic Flora of the Western Ghats-South India, pp. 652. B I Publications Ltd. New Delhi

Nataraja, S., Deepa, J., Ramesh Babu, H. N. and Krishnappa, M. 2011. Pteridophytic survey in Agumbe forest of central Western Ghats, Karnataka Internat. J. Plant Sci., 6(2):345-347

Parashurama T.R., Deepa J. and Prakash Kariyajjanavar, 2016. Pteridophytic Diversity in Mudigere Taluk, Central Western Ghats, Karnataka, South India. International Journal of Current Research, Volume 8, pp. 40339 -40342.

Pinak, D., Aniruuddha, S., Mrinal, K.B. and Mostafa, K.P. 2011. A review of the ferns of Bark Valley, Assam, India. J. Taxon.Biodiv. Res., 5: 27-38.

Raunkiaer, C.1934.The Life forms of Plants and Statistical Plant Geography.Clarendon Press, Oxford.

Shukla, R. S and Chandel, P. S. 2001. Plant Ecology and soil Science, S.Chand and comp. Ltd. Ramnagar,New Delhi, pp:110-141

Sukumaran, S. Jeeva, S. Raj, A. D. S. Kannan, D. 2008. Floristic Diversity, Conservation Status and Economic Value of Miniature Sacred Groves in Kanyakumari District, Tamil Nadu, Southern Peninsular India TurkJ Bot 32: 185-199

Sukumaran, S. Jeeva, S. Raj, A. D. S. Kannan, D. 2009. Diversity of Pteridopytes In Miniature Sacred Forests Of Kanyakumari District, Southern Western Ghats Indian Journal of Forestry, Vol.32(3): 285-290

Tryon, R. M. and Tryon, A. F. 1982. Ferns and Allied Plants with Special Reference to Tropical America Springer-Verlag New York

Tuomisto, H. and Poulsen, Axel Dalberg. 2000. Pteridopyte diversity and species composition in four Amazonian rain forest, Journal of Vegetation Science. Vol.11(3):383-396

Verma, S. C. and Fraser-Jenkins, C. R. 2008. Adiantum philippense L., The correct name for A. lunulatum Burm.f. , and its subspecie In 'Verma SC, Khullar SP and Cheema HK (Eds) Perspectives in Pteridophytes, Bishen Singh Mahendra Pal Singh, Dehra Dun, India, pp 65-92.

ISBN: 978-81-953600-3-1

BIOMETRIC AND BIOCHEMICAL ANALYSES IN THE FENUGREEK SEEDS EXPOSED TO COLD STRESS

M. G. Ashwathi, P. Deepasri and M. Poonkothai*

Department of Zoology,

Avinashilingam Institute for Home Science and Higher Education for Women,

Coimbatore – 641 043, Tamilnadu, India

*Corresponding authors E-mail: poonkothaiadu@gmail.com

Abstract:

Plants struggle against extreme stress factors in the natural environment and they develop complex mechanism to overcome and adapt the stressful environment. The insight of the present study is to inspect the impact of cold stress on the growth and biochemical aspects of fenugreek (*Trigonellafoenum-graecum*). Seeds of the fenugreek was encompassed at refrigeration (4°C) and freezing (-18°C) temperaturein accordance with the varying time intervals (24, 48, 72,96 and 120 hrs). Biometric parameters such as germination percentage, shoot and root length and vigour index were assessed to insight the morphological growth of fenugreek seedlings. The biochemical constituents namely proteins, carbohydrates and lipids were assessed in the fenugreek seedlings for inspecting the metabolic activity under cold stress. Results revealed maximum germination for both temperatures (4°C and -18°C) with respect to short term chilling stress (24h and 48h) and gradual decrease in long term chilling stress (72, 96 and 120 hrs). Stunted growth, thinning of stem and curled leaves were perceived in fenugreek seedling exposed to more than 72hrs at both temperatures. Hence the results impart that short-term chilling stress left minimal effects when compared to long term stress and all these pernicious effects were due to the induction of cold stress.

Keywords: Biometric parameters**,** Biochemical constituents, Cold stress, *Trigonella foenum-graecum*

Introduction:

Abiotic factors such as drought, salinity and freezing temperature cause unfavorable effect in their physiological growth and development which directly or indirectly affect the series of morphological and biochemical characters of floral diversity. Cold stress is an adverse force of chilling, which inhibits the normal functioning and well being of plants. Sometimes plants

respond and adapt to the cold stress and survive under the conditions that depend on climatic regions (Koc *et al*., 2010). Low temperature resistance in plants is a very complex trait, involving many different metabolic pathways and cell compartments (Lie *et al*., 2011).

Exposure of plants to temperature stress leads to the modification of metabolism in two ways, viz., adjusting to cellular metabolism that altered due to rising or falling of temperatures and the modifications of metabolism in response to temperature stress that are mainly linked to enhance tolerance mechanisms. Many metabolites have important properties that could contribute to induce stress tolerance (Kolawole *et al*., 2011). Plants experience cold or chilling stress at temperatures from 0–15°C (Ahmad *et al*., 2009).

Fenugreek (*Trigonella foenum-graecum*) is an aromatic, cold seasonal plant which possesses culinary, nutritional, economic and medicinal uses. It is sensitive to chilling above 0°C but below 10°C they can cause damage to the seed. Also fenugreek has rapid growth rate, tolerance mechanism to physical stress and requires less accommodation for culturing. The impact of cold stress in fenugreek has an inhibiting effect on the growth, development and other physiological reactions. Hence the present study is an attempt to investigate the impact of cold stress on the growth of fenugreek.

Materials and Methods:

Collection and processing of fenugreek seeds:

The seeds were collected from the local market in Coimbatore, Tamilnadu. The seeds were surface sterilized with 0.1% $HgCl_2$ and washed with distilled water to remove the dust and impurities. Further the seeds were taken and exposed to 4°C and -18°C for 24hrs, 48hrs, 72hrs, 96hrs and 120hrs separately in order to induce cold stress. Fenugreek seeds which were not exposed to cold stress served as control. Further the seeds were subjected to pot culture experiment.

Pot culture experiment:

Red soil and sand in the ratio of 3:1 was filled in the cups selected for the study. The growing medium was kept moist by spraying water daily. The cold stressed seeds exposed to various temperatures, viz., 4°C and -18°C at different incubation period (24hrs, 48hrs, 72hrs, 96hrs and 120hrs) and the control plants were sown in the growing medium. The seeds were watered daily and the seedlings were uprooted on 7^{th} day to assess the biometric parameters such as germination percentage, vigour index, shoot length and root length in all the treatments. The leaves of the seedlings were also analysed for the biochemical constituents such as carbohydrates, proteins and lipids.

ISBN: 978-81-953600-3-1

Biometric parameters:

Biometric parameters were checked for the physiological and morphological development in plants after being cold stressed. The obtained parameters were compared with parameters of unstressed / control plant.

Germination Percentage:

After 7 days of sowing, germination percentage of the seedlings was calculated by using the formula.

$$Germination\ percentage = \frac{Numberofseeds\ grown}{Total\ number\ of\ seeds\ sown} \times 100$$

Plumule that was protruded was taken as the criterion for the germination.

Shoot and root length:

The plants were uprooted on 7^{th} day taking utmost care by not damaging the root tip and shoot. The length of the shoot and root was recorded and expressed in centimeter.

Vigour Index:

Vigour index was calculated as the product of germination percentage and plant height i.e., root length and shoot length. The vigour index was calculated using the formula

$$Vigour\ index = Germination\ Percentage \times (Root\ Length + Shoot\ Length)$$

Biochemical Measurements:

The leaf samples were subjected to biochemical constituents namely carbohydrates, proteins and lipids.

Carbohydrate Content:

The carbohydrate content present in the leaves of the test samples were estimated according to Hedge and Hofreiter (1962) using glucose as a standard. The samples were homogenized (100mg) with 0.9% sodium chloride solution (1ml) and 5% trichloroacetic acid (1ml) respectively. The homogenate was centrifuged at 8000 rpm for 20 minutes and the supernatant was analyzed for carbohydrate by treating with anthrone reagent (5ml) in water bath for about 10 to 15 minutes. The absorbance of reaction mixture was recorded at 630nm in a spectrophotometer. A standard graph was constructed using the standard and the amount of carbohydrate content present in the fenugreek leaves were determined.

Protein Content:

The protein present in the fenugreek leaves were estimated by Lowry *et al.* (1951) using Bovine Serum Albumin (BSA) as a standard. The sample leaves were homogenized with 0.9% sodium chloride solution (1ml) and 5% trichloroacetic acid (1ml). The homogenate was centrifuged at 8000 rpm for 20 minutes. The pellet was analyzed for protein by treating with

alkaline copper reagent (5ml) and allowed to incubate for 10 minutes at room temperature and then Folin- ciocalteau reagent (0.5ml) was added, and kept at room temperature for 30 minutes for the appearance of blue color. Absorbance of the reaction mixture was recorded at 660nm in a spectrophotometer. A standard graph was constructed using the standard (BSA) and the amount of protein content present in the fenugreek leaves were determined.

Lipid content:

The lipids present in the fenugreek seedlings was estimated by (Folch *et al.,* 1957). The plant sample was weighed and grounded well with chloroform methanol (3:1) mixture. The centrifuged homogenate was taken in a small beaker and placed inside a large beaker filled with water along the sides and kept overnight in hot air oven. In between, a white precipitate was formed. The methanol layer was removed and the chloroform was evaporated in oven at 60°C. The beaker was weighed and the difference between the final and initial weight of the beaker gives the lipid content of the sample.

Results:

Temperature had significant effect on both physiological and morphological growth of plants. Various measurements of shoot length, root length and germination rate in different temperature, viz., 4°C and -18°C shows that cold stress to seeds affects the morphological growth of fenugreek plant**.** The carbohydrate, protein and lipid content in the seedlings shows that, cold stress affects the physiological growth.

Biometric Parameters:

Table 1 depicts the biometric parameters of cold stressed fenugreek seedlings at varying time periods. The fenugreek seeds refrigerated at 4°C showed 100% germination at 24hrs and 48hrs, whereas the rate of germination declined to 93%, 85% and 60% at72hrs, 96hrs and 120hrs respectively. The shoot length was 7.6cm, 7.5cm, 7.1cm at 24hrs, 48hrs, 72hrs respectively and decreased to 6.9cm, 6.7cm at 96hrs and 120hrs. Similar trend was observed in the root length of fenugreek exposed to different incubation period at 4°C. The fenugreek seedlings responded to chilling conditions express decreased vigour index at varying time intervals. Short term chilling at 24hrs and 48hrs showed 1460 and 1360vigour index, eventually long-term chilling at 72hrs, 96hrs and 120hrs showed 1171, 1028 and 612. The unfavorable morphological growth has been seen, which is due to the induction of cold stress.

The fenugreek seeds freezed at -18°C showed 96% and 85% germination at 24hrs and 48hrs, whereas the rate of germination decreased to 73%, 67% and 53% at 72hrs, 96hrs and 120hrs respectively. The shoot length was recorded as 7.2cm, 7cm, 6.9cm, 6.3cm and 6.5cm at

ISBN: 978-81-953600-3-1

24hrs, 48hrs, 72hrs, 96hrs and 120hrs respectively. The root length was observed as 6.5cm, 6cm, 5.2cm, 5cm and 3cm at 24hrs, 48hrs, 72hrs, 96hrs and 120hrs respectively. Short term freezing at 24hrs and 48hrs showed 1315 and 1105, eventually long term freezing at 72hrs, 96hrs and 120hrs showed 883, 757 and 504 of vigour index.

The fenugreek seedlings that were grown in the ambient temperature (control) exhibited higher biometric parameters when compared to the cold stressed seeds. The control plants showed 100% germination percentage, the vigour index, shoot and root length of the control seedlings was 1510, 7.9 cm and 7.2cm respectively.

Table 1: Biometric parameters of cold stressed fenugreek seedlings

Biometric Parameters	**Temperature (°C)**										**Control**
	Refrigerator (4°C)					**Freezer (-18°C)**					
	24h	48h	72h	96h	120h	24h	48h	72h	96h	120h	
Germination Percentage	100	100	93	85	60	96	85	73	67	53	100
Shoot Length(cm)	7.6	7.5	7.1	6.9	6.7	7.2	7	6.9	6.3	6.5	7.9
Root Length(cm)	7	6.1	5.5	5.2	3.5	6.5	6	5.2	5	3	7.2
Vigour Index	1460	1360	1171	1028	612	1315	1105	883	757	504	1510

*Values are mean of triplicates

Biochemical constituent measurements:

Table 2: Biochemical constituents of cold stressed fenugreek seedlings

Biochemical constituents (mg/g)	**Temperature (°C)**										**Control**
	Refrigerator (4°C)					**Freezer (-18°C)**					
	24h	48h	72h	96h	120h	24h	48h	72h	96h	120h	
Carbohydrates	10.1	9.5	8.5	7.9	7.3	8.2	7.6	7.2	6.5	6.3	12.5
Proteins	6.8	5.2	4.5	4.3	3.8	5.2	4.1	3.6	3.1	2.7	7.3
Lipids	4.7	4.1	3.5	3.1	2.7	3.5	3.1	2.8	2.3	1.7	5.2

*Values are mean of triplicates

Table 2 represents the biochemical constituents of fenugreek exposed to 4°C and -18°C at different time intervals. The amount of carbohydrate present in the leaves at 4°C was 10.1mg/g, 9.5mg/g, 8.5mg/g, 7.9mg/g and7.3mg/g respectively whereas it decreased gradually at -18°Cwith respect to increased time intervals as shown in Table 2. Similar trend was observed in protein and lipid content of fenugreek leaves exposed to different temperature at varying time intervals (Table 2).

Discussion:

Plant responses to stress depending on different factors such as duration and degree of stress, growth stage and time of stress exposure (Gupta and Sheoran, 1983). Low temperature is a stress that affects practically every aspect of plant growth and metabolism and also acts as a limiting factor during germination (Brigg and Aytenfisu, 1979). Seed germination and vigor are the prerequisites for the accomplishment of crop plants. In many crop plants, seed germination and early seedling growth are the stages that are most sensitive to environmental stresses (Cook, 1997). Germination proved to be an important stage in the growth of plant. Germination is sensitive to cold temperature because the absorption of seeds needs water by enzymatic activity or breathing. In the present study, chilling temperature at 4°C and -18°C showed a significant delay on the onset of germination and also reduced the rate of germination at higher hours of incubation. Biochemical constituents also play a vital role in metabolic regulation and germination of seeds. The overall effect of chilling stress, in particular long-term stress of incubation period above 72 hours of fenugreek seedlings clearly reduced the growth of the plant, speed of germination, percentage of germinating seeds, root length, shoot length and also showed variation in the plant morphology such as stunted growth, thinning of stem, poor germination, dehydration, plasma membrane damage, yellowing of leaves, withering and curled leaves. Similar such effect was percieved in the plants exposed to chilling temperatures in the range of 0 to 12°C (low temperature stress) where the germination significantly delayed the onset, reduced the rate and increased the dispersion of seed germination events (Jones, 1986; Foolad and Lin, 1997). Borowski and Michalek (2014) reported that low temperature, has an inhibiting effect on the growth, development and other physiological processes of cold-sensitive plants such as soybean. Borowski and Blamowski (2009) reported that chilling stress causes leakage of intracellular electrolytes from tissues as a result of the loss of cytoplasmic membrane integrity. Chilling also induces the production of hydrogen peroxide and other reactive oxygen species (ROS) in plants. Under chilling conditions, plants activate the enzymatic system that prevents ROS accumulation. On the other hand, reactive oxygen species induce lipid peroxidation in cell membranes, the loss of their integrity and, as a result of that, also a partial

ISBN: 978-81-953600-3-1

loss of selective permeability. Increased synthesis of proline and other substances serving as osmoprotectants and antioxidants under the influence of chilling requires large energy inputs from plants, which causes the inhibition of growth and development under stress conditions. During chilling period imbibition leads to decrease in the speed of germination and percentage of seed germination. It also slower the diffusion of water molecules into the interior of seeds due to higher water viscosity. A much larger reduction in the speed of germination at higher temperature clearly corresponding to length of the radicle, probably resulted from the decreased activity of numerous enzymes involved in the deterioration of seed storage reserves. Even from the germination, degraded products are transported, and the usual metabolism that happens in the embryonic roots is degraded.

Rigidity in the cells are increased and a great reduction in the fluidic content in the cellular membranes, when exposed to cold stress. Exposing seeds right before germination not only results in the cellular and molecular changes but also in the metabolic activities like membrane fluidity, nucleic acid and protein conformation changes and the changes are even witnessed in the metabolite concentration (Yadav, 2010).

Conclusion:

Cold stress affects all aspects of cellular function in plants. Such changes caused by cold stress adversely affect the growth and development of plants. The cold stress imparts its adverse effect on the biochemical components of the fenugreek seedlings such as carbohydrate, protein and lipid, on higher incubation period. Short-term chilling stress (24 hours and 48 hours) left only minimal negative effects on the fenugreek seedlings comparatively to the long-term stress.

Acknowledgement:

The authors acknowledge the authorities of Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore for providing the facilities to conduct this study.

References:

Ahmad, S., Ahmad, M., Ashraf, M.Y., Ashradf, M and Waraichi, E.A. (2009): Sunflower (*Helianthus annus* L.) response to drought stress at germination and seedling growth stages, Pak. J. Bot., 41 (2): 647-654.

Borowski, E. and Michalek, S. (2014): The effect of chilling temperature on germination and early growth of domestic and Canadian soybean (Glycine max (L.) Merr.) cultivars, Acta Sci. Pol. Hortorum. Cultus, *13*:31-43.

Borowski, E. and Blamowski, Z.K. (2009): The effects of triacontanol 'TRIA' and Asahi SL on the development and metabolic activity of sweet basil (*Ocimum basilicum* L.) plants treated with chilling, Folia Hort, 21 (1): 39-48

Brigg, K.G. and Aytenfisu, A. (1979): The effect of seedling rate, seeding date and location on grain yield, maturity, protein percentage and protein yield of some spring wheats in central Alberia. Can. J. Plant Sci., 59: 1129-1146.

Cook, R.E. (1997): Patterns of juvenile morbidity and recruitment in plants. In: Sorbig OT, Jain S, Johnson GB and Raven PH (Eds), Topics in Plant Population Biology, Columbia Univ. Press, Los Angeles: 207-301.

Folch, J., Lees, M. and Stanley, S.G.H. (1957): A simple method for the isolation and purification of total lipids from animal tissues, J Biol Chem., 226(1):497-509.

Fooland, M.R., and Lin, G.Y. (1997): Genetic potential for salt tolerance during germination in *Lycopersicon* species, Hort. Sci., 32: 296-300.

Gupta, P., and Shearon, I.S., (1983): Response of some enzymes of nitrogen metabolism to water stress in two species of Brassica. Plant. Phsiol. Biochem., 10: 5-13.

Hedge, J.E. and Hofreiter, B.T. (1962): Carbohydrate chemistry 17. Whistler, R.L. and Be Miller, J. N., Eds., Academic Press, New York.

Jones, R.A. (1986): High salt- tolerance potential in *Lycopersicon* species during germination. *Euphytica*, 35: 576-582.

Koc, E., Dslek, C., Ustun, A.S. (2010): Effect of cold on protein,proline, phenolic compounds and chlorophyll content of two pepper (*Capsicum annum* L.), G.U. Journal of Science, 23(1): 1-6.

Kolawole, A., Oladele, O.I., Wakatsuki, T. (2011): Profitability of different saw- ah rice production models within lowlands in Nigeria, Jounal of Food, Agriculture and Environment, 9 (3&4): 183-188

Lei, L.F., Wu, J., Ping, Z.Y., Lin, X.H., Qing, L.L. and Fei, L.H. (2011): Influences of temperature regime on germination of seed of wild soya bean (*Glycine soja*), Agric. Sci. Technol., 12 (4): 480-483.

Lowry, O.H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951): Protein measurement with the Folin phenol reagent, J Biol Chem., 193(1):265-75.

Yadav, S.K. (2010): Cold stress tolerance mechanisms in plants. A review, Agron. Sustain. Dev. 30: 515–527.

Frontiers in Life Science (Volume III)
ISBN: 978-81-953600-3-1

PLANT QUARANTINE - AN OVERVIEW

R. S. Deshmukh
Department of Botany,
B. Raghunath Arts, Commerce and Science College,
Parbhani – 431401 M.S., India
*Corresponding authors E-mail: rsdeshmukh19@gmail.com

Introduction:

Over the few decades, international agricultural and food markets have observed a number of changes due to which domestic and international markets come closer and the trade related to agricultural commodities has been increasing day by day internationally and becoming 'global'. A large number of plant pathogens have spread over the world through this global trade. Thus a disease or pathogen which was endemic to a particular region or a country has become pandemic and spread in other countries where it was not present earlier. One of the best examples of this is the late blight of potato which was introduced to Europe in 1840s and caused the devastating loss of potato crop which was a staple food of Irish people resulting in famous Irish famine and subsequent diaspora (Goss, 2014). Such diseases and their spread or introduction in to a new region or country is a permanent threat to agriculture. Taking this into consideration that a disease present in a particular area in serious form and likely to be transmitted by plant material, Government passes necessary regulations to stop entry of such plant material from the infected area, these are known as quarantine regulations.

Thus plant quarantine is defined as rules and regulations framed by a government to restrict the introduction of plants, planting materials, plant products, soil, cultures of living organisms, packing materials and various agricultural commodities as well as their containers to protect agriculture and the environment from damage caused by hazardous organisms. The term 'exclusion' relates to keeping organisms out; plant quarantine relates to keeping plants out.

The terms frequently used in plant quarantine are *hazard, risk* and *safeguards*. *Hazard* is the danger that a specified pathogen is known to present to the agriculture of the importing country and the pathogen gain entry on imported items and subsequently established. *Risk* is the chance that a hazardous organism will enter and become established. *Safeguards* are actions taken to reduce the risk of introducing hazardous organism (Agarwal and Sinclair, 2012).

The term Quarantine is originated from the Latin word *quarantum*, meaning 40 d. It refers to the 40 days period of detention of ships arriving from countries with bubonic plague and cholera in the middle ages. The first such quarantine was imposed in Venice in 1374 (Mathys& Baker, 1980). Present quarantine laws now include plants. Plant quarantines are regulated by government or group of governments which restricts entry of various agricultural commodities. They keep out dangerous organisms and permits plants and plant products to enter (Kahn, 1988).

Historical account:

Plant quarantine laws were first enacted in France in 1660 to suppress and prevent the spread of barberry plants, which was even then believed to be related with rust disease of wheat. In 1903 Denmark initiated plant quarantine laws followed by USA. The Federal Plant Quarantine Act was passed in USA in 1912. In India plant quarantine rules and regulations were framed under Destructive Insects and Pests Act i.e. DIPA in 1914 and subsequently supplemented by other provisions.

Presently more than 150 countries have passed certain quarantine regulations and established organizations to enforce them.For the implementation of plant quarantine regulations, proper check is maintained at air pots, sea ports and land frontier stations.

Table 1: Points of Entry for Import of plants/ plant materials in India

Sea ports	Airports	Land Frontier Stations
1. Alleppey (Kerala)	1. Amritsar (Punjab)	1. Agartala (Tripura)
2. Bhavnagar (Gujarat)	2. Bangalore (Karnataka)	2. Amritsar Rly. Stn. (Punjab)
3. Kolkata (West Bengal)	3. Kolkata (West Bengal)	3. Attari Rly. Stn. (Punjab)
4. Calicut (Kerala)	4. Chennai (Tamil Nadu)	4. Attari Wagha Border (Punjab)
5. Chennai (Tamil Nadu)	5. Hyderabad (Andhra Pradesh)	5. Bongaon (West Bengal)
6. Cochin (Kerala)	6. Mumbai (Maharashtra)	6. Gede Road Rly. Stn. (West Bengal)
7. Cuddalore (Tamil Nadu)	7. New Delhi (Delhi)	7. Jogbani (Bihar)
8. Goa (Goa)	8. Patna (Bihar)	8. Moreh (Manipur)
9. Gopalpur (Orissa)	9. Tiruchirapalli (Tamil Nadu)	9. Panitanki (West Bengal)

ISBN: 978-81-953600-3-1

10. Haldia (West Bengal)	10. Trivananthapuram (Kerala)	10. Raxual (Bihar)
11. Jamnagar (Gujarat)	11. Varanasi (Uttar Pradesh)	11. Rupadiha (Uttar Pradesh)
12. Beypore (Kerala)	12. Guwahati (Assam)	12. Sonauli (Uttar Pradesh)
13. Kakinada (Andhra Pradesh)		13. Banbasa (Uttar Pradesh)
14. Kandla (Gujarat)		14. Zokhwathar (Mizoram)
15. Karwar (Karnataka)		
16. Krishnapattinam (Andhra Pradesh)		
17. Machlipatnam (Andhra Pradesh)		
18. Mandvi (Gujarat)		
19. Mangalore (Karnataka)		
20. Mumbai(Maharashtra)		
21. Mundra (Gujarat)		
22. Nagapattinam (Tamil Nadu)		
23. Nova Shiva (Maharashtra)		
24. Navlakhi (Gujarat)		
25. Okha (Gujarat)		
26. Paradeep (Orissa)		
27. Pondicherry		
28. Porbander (Gujarat)		
29. Rameswaram (Tamil Nadu)		
30. Trivananthapuram (Kerala)		
31. Tuticorin (Tamil Nadu)		
32. Veraval (Gujarat)		
33. Visakhapatnam (Andhra Pradesh)		
34. Vizhinjam (Kerala)		

Source: https://plantquarantineindia.nic.in/pqispub/docfiles/Schedule-I.html

Basic principles of plant quarantine

The basic principle of plant quarantine is to check the entry and spread of potentially dangerous plant pathogens and insects imported along with the germ plasm. In spite of quarantine regulations, plant pathogens have been introduced in different countries. Plant quarantine regulations have certain prerequisites (Neergaard, 1980; Chock, 1979; Morrison, 1977). They must be -

1. Based on sound biological grounds. Only pests that pose a threat to major crops or forest should be taken into consideration.
2. Formulated to control or prevent the entry of pests and not to hinder trade or attainment of other objectives. Quarantine measures are for crop and not trade protection.
3. Derived from adequate legislation and operated solely under the law.
4. Modified as conditions change or further facts become available.

Categories of quarantine objects:

According to Neergaard (1984) quarantine objects are classified into three categories based on following criteria-

Category A:

Dangerous pathogens that are not present in a region of introduction and have a high or considerable epidemic potential. Pathogens belonging to this category generally occur only trace amounts in seeds. Quarantine measures should prohibit introduction of such seeds from infected areas or valuable seed material from infested areas must be filtered through post entry control measures by growing plants in special glasshouses under closed quarantine. It includes viruses and viroids like arabis mosaic, barley stripe mosaic, peanut stunt, etc.

Category B:

Dangerous plant pathogens that are not present in the region of introduction or present in restricted areas under effective control. Such pathogens have a moderate epidemic value. Examples are bean common mosaic virus, lettuce mosaic virus and pea early browning virus.

Category C:

These pathogens are not strict quarantine objects but may be important to the field-planning value of seeds. However, there may be a potential risk of introducing new virulent strains into the region. The seed material is tested to prevent introduction of new strains. For example, the alfalfa mosaic virus, soybean mosaic virus and cucumber mosaic virus.

ISBN: 978-81-953600-3-1

Plant quarantine measures:

The objectives of regulatory actions are-

1. To prevent or delay entry of the pathogen
2. If pathogen succeeds to enter,prevent infection
3. If infection succeeds, prevent establishment of pathogen
4. If pathogen gets established, minimize its spread

Plant quarantine measures are of three types such as-

1. Domestic quarantine:

It includes rules and regulations which prohibit the movement of insects and diseases and their hosts from one state to another state in India. It exists for two pests namely Rooted scale and Sanjose scale and three diseases viz. bunchy top of banana, banana mosaic and wart disease of potato.

Bunchy top of banana is present in Kerala, Assam, West Bengal, Bihar and Odisha. Transport of any part of banana (*Musa sp.*) except the fruit is not permitted form these states to other states in India.

Banana Mosaic is present in Maharashtra and Gujarat. Transport of any part of banana (*Musa sp.*) except the fruit is not permitted form these two states to other states in India.

Wart disease of potato is present in Darjeeling area of West Bengal therefore potato seed tubers are not transported from West Bengal to other states of India.

2. Foreign quarantine:

It includes rules and regulations which are framed to prohibit import of plants, plant materials, insects and fungi into India from foreign countries by air, sea or land. Rules for foreign quarantine are of two types- General and Specific. General rules help to prevent or avoid introduction of pests and diseases into a country where as specific rules aim at specific diseases and insects pests.

3. Total embargoes:

It means an official ban on trade or other commercial activity with a particular country i.e. total restriction on import and export of agricultural commodities.

Components of Plant Quarantine

Plant health or quarantine services in most countries usually have three components (Kahn, 1991) such as:

1. Exclusion of pathogens and pests of quarantine and economic significance that might inadvertently be moved along manmade pathways when articles are imported, or induction of the risk of introducing such hazardous organisms to as acceptable level.

2. Containment, suppression and eradication of exotic pathogens and peats recently introduced along natural and manmade pathways.
3. Assistance to exporters of plant products, such as fruits, vegetables, plants, cut flowers, commodities, etc. in meeting the quarantine or exclusion requirements of improving countries and therefore, based on plant health, biologically facilitating the acceptance of imports.

Organisms of quarantine significance:

Any pathogen or pest that a government considers to pose a threat to the agriculture and environment of that country or region is considered as organism of quarantine significance. Such organisms are exotic to that country or region. Some fungal genera and species and plant bacteria and viruses of quarantine significance have been published by Kahn, 1989.

Problems in plant quarantine:

Quarantine serves as a filter against the introduction of dangerous pathogens but pathogens are still introduced, the reasons behind this are-

1. Detection of all types of infectious pathogens by conventional methods is quite difficult
2. Methods may not be enough sensitive to detect traces of infection
3. Latent infections may pass undetected under post entry quarantine
4. Destruction of entire suspected material
5. Sensitive methods for testing fungicide treated seeds may be lacking (Waterworth, 1993).

Discussion:

Plant diseases have become a permanent threat since human societies started to relay agriculture as major food provider. Nearly 15-20 % reduction in crop yield worldwide is due to attack of various plant pathogens. It has been reported that a number of plant pathogens have been introduced in India by various countries through agricultural trade knowingly or unknowingly and caused heavy losses to farmers. Therefore efforts to reduce these losses through proper management of crops and through pest and disease management are essential. Methods like exclusion and eradication are employed to eliminate pathogen which is a fundamental concept of plant quarantine.

The main aim of Plant quarantine is to provide protection to the agriculture of a country or region from alien pests and pathogens. Adding of new species (pest and disease) to an environment can affect the well-being of people. The Plant Quarantine processes acts as an important device in keeping away pests from the crop. The importance of plant quarantine has

ISBN: 978-81-953600-3-1

increased because of the increase in exchange of seeds or grains for consumption along with better means of transportation. The international exchange of plants or their parts is practiced widely to improve crops of a country and their genetic base.

The success of plant quarantine measure depends on the proper composition of the state service for plant quarantine.Better quarantine programs need to be developed and coordinated and strengthen regional organizationsis of great importance.

References:

Agarwal V.K. and James Sinclair (2012).Principles of Seed Pathology (2nd Edition).Lewis Publishers, pp.-466-483.

Chock A.K. (1979). The international plant protection convection, in Plant Health, the Scientific Basis for Administrative Control of Plant Diseases and Pests, Ebbels D.L. and King J.E., Eds., Blackwell Scientific, Oxford, 1.

Erica M. Goss, Javier F. Tabima, David E. L. Cooke, Silvia Restrepo, William E. Fry, Gregory A. Forbes, Valerie J. Fieland, Martha Cardenas, and Niklaus J. Grünwald (2014). PNAS, Vol. 111, 24, 8791–8796.

Fathipour, Y. and Maleknia, B. (2016).Integrated Pest Management. In: Omkar (Ed.), Ecofriendly Pest Management for Food Security. Elsevier, San Diego, USA, pp. 651--684.

Kahn R.P. (1988). The importance of seed health in international seed exchange, in Rice Seed Health, Int. Rice Res. Inst., Manila, 7.

Kahn R.P. (1989). Plant protection & Quarantine, Vol. I, Biological concepts, CRC Press, Boca Raton, FL, 1989, 226.

Kahn R.P. (1991). Exclusion as a plant disease control strategy. Annu. Rev. Phytopathol.,29, 219.

Mathys G. and Baker E.A. (1980). An appraisal of the effectiveness of quarantines, Annu. Rev. Phytopathol.,18, 85.

Morrison L.G. (1977). Quarantine Principles and Policy, SPC Workshop and Training Course in Plant Quarantine, Suva, Fiji, SPC, Suva, 2.

Neergaard P. A. (1980). A review on quarantine for seed, in Golden Jubilee Commemoration Volume, National Academy of Sciences, New Delhi, India.

Neergaard P.A. (1984). Seed health in relation to the exchange of germplasm, International Board of Plant Genetic Research, 1.

Waterworth H. (1993). Processing foreign plant germplasm at the National Plant Germplasm Quarantine Center, Plant Dis., 77,854.

https://plantquarantineindia.nic.in/pqispub/docfiles/Schedule-I.html

TISSUE CULTURE TECHNIQUES OF MEDICINAL PLANT *PLUMBAGO ZEYLANICA*

Noopur Singh[1] and M. Praveen Kumar[2]

[1]Plant Biotechnology, Graphic Era deemed to be University,
Dehradun, Uttarakhand, India

[2]Agriculture Officer,
ACFS, Govt. of Kerala

*Corresponding authors E-mail: noopurs1090@gmail.com, pkumarapm@gmail.com

Abstract:

The present investigation was carried out on an herbaceous angiosperm namely *Plumbago zeylanica* belonging to family *Plumbaginaceae*. It is commonly known as ''chatrak'' and is grown as a medicinal plant. Different vegetative parts like root, stem, leaves, and shoot apices were excised from both the in vivo and in vitro raised plants and thereafter planted on Murashige and Skoog's medium supplemented with various growth adjutants for callus induction, organogenesis and multiple shoot formation. Multiple shoot proliferation was effected from shoot apices on MS medium supplemented with various growth adjutants in different combinations. A maximum number of shoots (8 +_ 1.3 per explants) was obtained from nodal explants when cultured on MS liquid medium supplemented with 1.0 mg/L BAP, 0.5 mg/L IBA and 2.0 mg/L adenine sulphate. The highest percentage of callus induction was obtained when stem explants cultured on MS medium supplemented with 2.0 mg/L BAP and 1.5 mg/L IAA. The greatest percentage of shoot induction (100%) with a mean of 34.2 shoots obtained from callus was cultured on MS medium supplemented with 0.75 mg/L BAP, 1.0mg/L IAA, NAA and adenine sulphate each. Regenerated shoots were rooted best on half-strength MS medium containing 0.5 mg/L NAA and 3% (w/v) sucrose. The regenerated plantlets were acclimatized in the culture room and successfully transferred in soil. After shoot and root development, attempts were made to establish regenerated plantlets into soil through a series of hardening stages.

Keywords: *Plumbago zeylanica*, Plant tissue culture

Introduction:

Human beings have been utilizing plants for basic preventive and curative health care since time immemorial. Recent estimates suggest that cover 9,000 plant species have known medicinal applications in various cultures and countries and this is without having conducted comprehensive research amongst several indigenous and other communities (Farnsworth and Soejarto 1991). The widespread use of herbal remedies and health care preparations, as described

ISBN: 978-81-953600-3-1

in ancient texts including the Vedas, holy Koran and the Bible are obtained from commonly used traditional herbs and medicinal plants. In India, approximately 1700 plant species are used in Ayurveda, 500 for Siddha, 400 for Unani, 300 for Amchi systems of medicine with substantial overlaps of common plants among these systems.

The trend of using natural products is increasing steadily. The use of traditional medicines and medicinal plants in most developing countries as a normative basis for maintenance of good health has been widely observed. Further an increasing reliance on the use of medicinal plants in the industrialized societies has been related to the development of several drugs and chemotherapeutics from plant species as well as from traditionally used rural herbal preparations. Herbal remedies have attained much more popularity in the treatment of minor ailments, due to increasing awareness of personal health maintenance through natural products. Indeed, the market and public demand has been so great that there is a great extinction risk to many medicinal plants and obviously the loss of genetic diversity.

In view of the growing world population, increasing anthropogenic activities rapidly eroding natural ecosystem etc natural habitat for a grate number of herbs and trees are dwindling. Many of them are facing extinction. To cope up with alarming situation the recent exciting developments in biotechnology have come as a boon. One of them is the use of plant tissue culture technique.

Plant tissue culture has emerged as potential tool and forms the back born of plant biotechnology**.** Tissue culture techniques are widely applied for the improvement of field crops, forests, horticulture and plantation crops for increased agricultural and forestry production . This technique has be commercialized globally and contributed significantly towards the enhanced production of high quality planting material. Clonal propagation of selected phenotypes is an essential step in most of the plant breeding programs. It is a faster method of asexual reproduction in comparison to propagation through seeds. Plants raised through seeds are highly heterozygous and one has to select plant from a wide population which has the best qualities. Owing to heterozygosity, the seed raised plants show high variation in growth, habit, and yield and they may have to be discarded because of the poor quality of their flowers and fruits for commercial release. Likewise majority of the plants propagated by vegetative means contain systemic bacteria, fungi and viruses which affected the yield, quality and appearance of selected plants. Moreover majority of plants are not amenable to vegetative propagation through cutting, budding and grafting, thus limiting multiplication of desired cultivars. In the recent years,tissue culture has emerged has a promising technique to obtain genetically pure elite populations under in vitro propagation also called micro propagation is infecting the miniature version of conventional propagation, which is carried out under aseptic conditions. The advent of in vitro

tissue culture technique has offered a new approach to the morphogenetic investigations. It allows a living system to be studied under controlled environmental condition.

Plants rose through Tissue culture technique:

- To obtaining plant of uniform in quality
- To obtaining disease free or pathogen free plants
- Can be produced much more rapidly as new cultivars could become commercially available with in 2 to 3 years from development rather than 5to 10 years needed using conventional propagation.
- Produce uniformly superior seeds.
- Show improved vigor and quality.
- Rapid tissue culture of plants which are difficult to culture.
- Somatic hybridization.
- Genetic improvement of commercial plants
- Obtaining haploid plants from pollen cultures or another culture for breeding programs

Most outstanding advantages offered by aseptic methods tissue culture over conventional methods are:-

1. In a relatively short time and space a large numbers of plantlets can be produced starting from the single explant.
2. Unlike conventional methods multiplication can be carried out throughout the year.
3. Tissue culture technique growing plants are usually free from bacterial and fungal diseases virus eradication maintenance of plantlets in virus free state can also be rapidly achieved in culture

Tissue culture technique:

Most of the plants rose through seeds show tremendous genetic variation and the grower have to select from wide population the plants having the best characteristics. However, it is a very cumbersome and time consuming activity. Likewise majority of the plants are not amenable to vegetative propagation by culturing or grafting. In vitro culture technique also called tissue culture technique has become an important tool to obtain genetically pure elites rather than having indifferent populations.

Advantages of tissue culture technique:

- Tissue culture technique can be use as an alternative to conventional methods of vegetative propagation with the objective of enhancing the rate of multiplication.

ISBN: 978-81-953600-3-1

- ❖ Through in withdraw clonal propagation, large number of plants can be raised from a small even microscopic piece of plant tissue with in a short span of time.
- ❖ Tissue culture technique provide reliable and economical methods of maintaining pathogen free plant in a state that can allow rapid multiplication and also allows international exchange of germplasm.
- ❖ Plant multiplication can continue throughout the year irrespective of season.
- ❖ Stocks of germplasm can be maintained to many years.

Techniques of tissue culture technique:

1. Directly from the explants like roots, stem,petiole, leaf lamina,flower parts etc or
2. Indirectly from callus cultures obtained from these explants. Plants obtained through calli may not be true elites because of high incidence of polyploidy and aneuploidy associated with callus cells & plants obtained from it.
3. Somatic or nonzygotic embryogenesis involves the formation of bipolar embryos which can develop into fully functional plants under appropriate conditions

Stages of tissue culture technique:

Tissue culture technique involves for definite stages. These are as follow:-

Stage I: Initiation and establishment of aseptic cultures

This involves explant isolation, surface sterilization & establishment on an appropriate culture medium.Cultures are initiated from explants of several organs but shoot apices and axillary buds are most often used for commercial tissue culture technique.

Stage II: Shoot multiplication using a defined culture medium

It can be achieved through any one of the following for method:

1. Multiplication through calli obtained from different organs and tissues and their subsequent sub culturing leading to organogenesis.
2. Multiplication through direct induction of shoots on the explants.
3. Multiplication through growth and proliferation of existing apical shoots & adventitious buds.
4. Somatic or non zygotic embryogenesis directly on the explants or in callus cultures.

Stage III: Rooting of regenerated shoots in vitro conditions

This stage is characterised by preparation of stage II shoots or shoot clusters for successful transfer to the soil. The process may involve:-

- ❖ Elongation of shoots prior to rooting.
- ❖ Root formation :-

Adventitious root formation involves 3 stages

- Induction of rooting – it is the first stage in the process of rooting.

In this process the fate of cell is changed to root formation and results in the formation of root primordium.

- Emergences of roots- root primordium grows and emerges out from the epidermis.
- Elongation of roots- further elongation of root primordium results in root formation.

Stage IV:

The climatic adaptation of a plant when moved to a new environment is known as acclimatization.

- Plants which are produced under cultural conditions (high humidity, low light, constant temperature) when transferred to field conditions are required to be acclimatized.
- The ultimate success of any tissue culture technique protocol depends upon the ability to transfer and re-establish plants from in vitro to green house conditions.

Tissue cultured plants are difficult to transplant for two primary reasons:-

1. A heterotrophic mode of nutrition.
2. Poor control of water loss.

To overcome these limitations, plantlets should be transplanted into a well drained, sterile growing medium and maintained initially at high relative humidity (90%) and reduced light at 20 to 27 oC. for the first 10-15 days by keeping them under mist or covering them with clear plastic bags. After spending few days under high humidity the plants should be moved to the green house bench. Transplants should them acclimatized by gradually lowering the relative humidity over 1 to 4 weeks period. Plants are gradually moved to higher light intensive to promote vigorous growth.

Objectives:

The present investigation was carried out on an important medicinal plant *Plumbago zeylanica.*

The main objectives of the present investigation were:

- ❖ To develop a reliable protocol for the rapid and mass scale propagation of plants in short duration of time and space.
- ❖ To obtain genetically pure elites rather than having indifferent populations under tissue culture conditions.
- ❖ To get disease-free plants this will lead to qualitative improvement of the crop.

Medicinal Plant status in India:

Species in Northern and Central India and 42 species in the high altitude of Himalayas are threatened in the wild (Chopra RN, Nayar SL, Chopra IC). The need of the hour, then, is to

ISBN: 978-81-953600-3-1

replant India's participation in the expanding global market, in light of the interest of all the stakeholders who are affected and who play a role in this sector.There is a need to collect all the information regarding medicinal plants development in the country in order to obtain a comprehensive overview which will provide the necessary insight for coordinated and effective action (Uma DP, Solomon FE, Sharda AC).

Such an overview could form the basis of a renewed development of India's medicinal plants sector, and a strategic exploitation of her comparative advantage in the global market on a sustainable and equitable basis. Important medicinal plants in India The Indian system of medicine particularly Ayurveda, Siddha, Unani, and Homeopathy largely use plant based materials, minerals, metals, marine and products of animal origin. The domestic market of Indian system of medicine and Homeopathy is of order of Rs 4000 crores which are expanding day by day. The Ayurveda drug market alone is of the order of the Rs 3500 crores, besides this there are demands from food supplements and cosmetics. However, the sector is not well organized and needs special attention. Hence, the National medicinal Plants Board was set up under the ministry of Health and Family welfare, Govt. of India during 2000 and the board has initially identified 31 species of importance. In a report to the scientific Advisory Committee to the cabinet (SAC-C), Govt.of India, Technology information, Forecasting and Assessment Council (TIFAC) has mentioned 45 medicinal plant species and specifically recommended 7 plants for immediate attention during 2001-2005.

***Plumbago zeylanica* a medicinal plant:**

Today, Ayurvedic, Hoemoeo and Unani physicians utilize numerous species of medicinal plants. (Narayana and Thamanna, 1987).Many compounds used in today's medicine have a complex structure and synthesizing these bioactive compounds chemically at a low price is not easy (Majumdar *et al.*, 2000; Madhava, 1998). The increasing awareness about the side effects of drugs had made the western pharmaceutical industries to turn towards the plant based Indian and Chinese medicine (Balandrin and Klocke, 1998*). Plumbago* popularly known as chittiramulam in Tamil and white leadwort in English. *Plumbaginaceae* is distributed as a weed throughout the tropical and subtropical countries of the world. The family *Plumbaginaceae* consists of 10 genera and 280 species. The genus *Plumbago* includes species, namely *Plumbago indica* L, *Plumbago rosea* L, *plumbago capensis*, and *Plumbago zeylanica* L, which are distributed in several parts of India. In *P. zeylanica* root and bark is an active part used as a traditional herbal medicine to treat several diseases. Compounds isolated from *p. zeylanica* L. are composed of naphthoquinone, such as plumbagin, 3-biplumbagin, 3-chloroplumbagin, chitranone, elliptinone, isoshinanolone and coumarins such as seselin, 5-methoxyseselin, suberosin, and xanthyletin.

Other compounds such as 2, 2-dimethyl-5-hydroxy-6- acetylchromene, plumbagin acid have also been isolated and identified (yuan-Chuen Wang 2005; Michael, 1956).

The whole plant and its roots have been used as a folk medicine in Taiwan for the treatment of rheumatic pain, menostasis, carbuncle and injury by bumping (Okoli and Akah, 2005). Roots and root barks of this plant are the most frequently used plant parts which have traditionally been used for the treatment of various ailments, such as dyspepsia, piles, diarrhea, skin diseases, leprosy and also reported to posses antibacterial, antifungal, (Uma *et al.,* 1999) and vesicant diuretic properties and further may be used as a substitute for cantharides (Nguyen *et al.,* 2004).

Antibacterial and antimycotic activity of *Plumbago zeylanica*:

Alcoholic crude extracts of *p. zeylanica* were investigated for their ability to inhibit the growth of multiresistant (16-23 β-lactam antibiotics) strains of *E. coli* and *Shigella*, compared with other plant extracts they showed high activity with MIC value of 0.64-10.24 mg/ml.

Infections with *Helicobacter pylori* were inhibited *in vitro* by ethyl acetate extracts of *p. zeylanica* with a minimum bactericidal concentration (5.12-20.48 mg/ml).

When tested against the resistant strain of *Mycobacterium tuberculosis* (H37RV) the inhibitory activity of Plumbagin was <12.5 µ/ml. The antimycotic activity of isonicotin acid hydrazide against. *Mycobacterium intracellurum, M. smegmatis, M. xenopei* and *M. chelonei* combined with Plumbagin was lowered from a MIC value 0f 1.25-2.5 to 0.15-0.3µg/ml.

Antiviral activity of *Plumbago zeylanica*

Between other Ethiopian medicinal plants *p. zeylanica* is used for skin disorders. The antiviral activity and the cytotoxicity were examined with 80% methanolic extract. The antiviral activity was tested with plaque reduction assay, the cytotoxicity with a crystal violet uptake assay. There was a weak anti-influenza A-activity and an inhibition against Coxsackie virus B3(CVB3).

Antiplasmodial activity of *Plumbago zeylanica*

80% ethanolic extracts from 47 Indian plant species were tested in vitro for antiplasmodial activity. 31 extracts were found to be active, five of them, together with *p. zeylanica*, are of special interest for further antimalarial studies.

Cytotoxicity by *Plumbago zeylanica*

Beta-sitosterol and plumbagin isolated by a bioassay guided fractionation of the dichloromethane extract from aerial parts of *p. zeylanica* were toxic against the cancer cell lines : β-sitosterol against MCF7 and Bowes cancer cell lines (IC50 113µM and 152µM) and inhibited Bowes cell growth with(IC50 36.µM).

ISBN: 978-81-953600-3-1

Biochemical effects of *P. zeylanica*

In rat liver mitochondria antioxidant effects of the aqueous and alcoholic root extracts were tested, corresponding to medicinal preparations. In the method using ferric reducing /antioxidant powder boiled ethanolic extracts were the most effective ones. In the method with 2, 2-azobis-3-ethylbenzthiazoline-6-sulfonic acid, boiled aqueous extracts were most efficient. In conclusion, the extracts and plumbagin have significant antioxidant abilities which may explain reported therapeutic effects.

Pharmacological effects of *Plumbago zeylanica*

Swiss Albino mice pre-treated with an alcoholic root extract of *P. zeylanica (*250 and 500 mg/kg body weight) showed protection against cyclophosphamide induced genotoxicity, reduced the frequency of micronucleated polychromatic erythrocytes, and increased the normochromatic erythrocyte ratio in the bone marrow.

Materials and Methods:

The laboratory set-up for tissue culture depends on the nature of the research undertaken and availability of funds. Regardless of the fact that the technique employed may be for simple plant propagation, synthesis of secondary metabolites or genetic manipulations, the basic facilities that should be available to an individual or group for tissue culture usually include.

1. Washing and storage facilities.
2. Media preparation, sterilisation storage room.
3. Transfer area for aseptic manipulation.
4. Culture room or incubators for maintenance of culture under controlled conditions of temperature, light and humidity.
5. Observation or data collection area.

Washing and Storage Facilities:

The main requirement for washing and storage in an area sufficient to accommodate large sinks (some lead- lined to resist acid or alkalis) with provision for running water, draining boards or racks and ready access to a de-ionised distilled and double-distilled water apparatus. Space should also be available to set-up drying ovens, washing machines, plastic or steel buckets for soaking labware, acid or detergent baths, pipette washers, dries and cleaning brushes. For storage of washed and dried labware, the washing area may be provided with dust proof cupboards or storage cabinets. A tissue culture facility requires good running cold and hot water as well as provision for waste water disposal.

Glassware:

The glassware used for culture work comprised of 6”•1” Riviera and Borosil test tubes, 100ml, 250ml, 500ml and 1000ml Corning and Borosil flasks, pipettes and measuring cylinder

(100ml, 500ml). Before use, glasses were thoroughly brushed with alkaline detergent teepol and then washed in running water these were then treated with hot chronic acid (mixture of K2Cr2O7 + H2SO4 + H2O) followed by thorough washing with tap water.

The glasses were than inverted in a clean tray and left to dry in the oven. Plugs for the tubes and flask were made out of absorbent surgical cotton wrapped in Muslin, 5-10ml water was then poured into every culture vessel which was tightly plugged. The glassware were than steam sterilised in an autoclave at a pressure of 15 lb/in2 at 121oC for 15-20 minutes.

Media Preparation Room:

The area earmarked for media preparation should have ample storage and bench space for chemical lab ware culture vessels, closure and miscellaneous equipment required for media preparation and dispensing. There should be provision for placing hot plates or stirrers, pH meters, balance, water baths Bunsen burners with a gas source. A microwaves oven, autoclave or domestic pressure cooker for sterilising media, culture vessels and instruments are appliances most needed for media preparation. Others requirements to be included in the media room are vacuum sources, refrigeration and freezer for safe storage of chemical and media stocks.

Transfer Area:

It is very essential that all precautions be taken to prevent the entry into the culture of a contaminant when its mouth is opened either for subculture or for planting fresh tissues. To achieve this all transfer operations are carried out under strictly aseptic conditions. Laminar airflow cabinets are used. This has a small motor to blow air which first passes through a coarse filter, where it loses large particles and subsequently through a fine high-efficiency particulate air (HEPA) filter, which ultraclean the air. The velocity of the air coming out of this filter is about 27+-3m per minute that is adequate for preventing the contamination of the working area. Presence of UV light also prevents contamination.

Culture Room:

Environmental factor greatly influence the process of growth and differentiations of tissues in cultures. All types of plant tissues are therefore incubated under conditions of well-controlled temperature, humidity, illumination and air circulation. A typical culture room should have both light and temperature programmable for a 24 hour period. Usually air conditioners and heaters are used to maintain the temperature around 25+-2oC. Lighting is adjusted in terms of quality and photoperiod duration by using automatic clocks.Specially designed shelves made of glass,rigid wire mesh, or wood are provided in the culture room for storing cultures. Each shelf is illuminated by a separate set of fluorescent tubes.Individual shelves may also be ventilated by fitting a small fan at one end of the shelf and blowing air through a plastic pipe running along the length of the shelf. Holes drilled along the sides of the pipe at appropriate distances will further allow an even airflow and thus prevent build –up of hot air in the shelves due to lamps. A

ISBN: 978-81-953600-3-1

generator should kept as a stand-by and emergency power points in the culture room, incubators, or growth chambers attached to it in order to maintain the necessary light and temperature conditions.

Data collection area and specialized facilities:

The growth and development of tissues cultured *in vitro*are generally monitored by observing culture at regular intervals in the culture room or incubators where they have been maintained under controlled environmental condition. Data based on the observation under aseptic condition may be controlled using a laminar airflow cabinet or Suitable transfer area designed for the purpose. Whereas for the microscopic examination of culture the usual laboratory Space is used.

Transplantation area:

Plants regenerated from *in vitro*tissues culture at transplanted to soil in pots. The potted plants are ultimately transferred to given houses or growth, cabinets and maintained for further observations under controlled conditions of light, temperature and humidity. Prior to transfer of potted plants to greenhouse the acclimatization of these plants may begin in growth chambers under mist benches, or in a standard greenhouse bay. Plugs for the tubes and flasks were made out of absorbent surgical cotton wrapped in muslin. 5-10ml water was then poured into every culture vessel which was tightly plugged. The glasswares were then steam sterilized in an autoclave at a pressure of 15lb/in2 at 121oC for 15 to 20 minutes.

Cleaning Glassware:

In tissue culture, glassware should be resistant to heat. Generally, a goodquality borosilicate glass is recommended as it also appears to be less prone to breakage and scratching. The conventional method of cleaning laboratoryglassware is to soak it in acid for sometime Then washing it with detergent.To dry the cleansed glassware, it is placed in an oven at high temperature.

Media preparation room:

The area earmarked for media preparation should have ample storage and bench Space for chemicals, labware, culture vessels, closures and miscellaneous equipment required for media preparation and dispensing. There should be provision for placing hot plates or stirrers, pH meters, balances, water baths and Bunsen burners with a gas source. A microwave oven, autoclave, or domestic pressure cooker for sterilizing media, culture vessels and instruments ate appliances most needed for media preparation.

Other requirements to be included in the media room arevacuum sources, refrigerator and freezer for safe storage of chemicals and media stocks.

Sterilization of instruments, plant materials and medium:

Glass culture vials are monthly sterilized together with the medium. For pre sterilized medium autoclaving or heating in an oven at 160-180^{o}C for 3 hours may sterilize the glassware. Disadvantages of dry heat sterilization are poor circulation and slow penetration. Therefore, proper loading of the oven is essential Glassware is allowed to cool before removal from the oven. Certain types of places lab ware can also be heat sterilized (Bhojwani S.S and Razdan M.K). The instruments used for aseptic manipulations. Such as forceps, scalpels, needles and spatula are normally sterilized by dipping in 95% ethanol followed by flaming and cooling. This is done at the start of transfer work and several times during the operation (text book of biotechnology 12th class).Surface of plant carries a wide range of microbial contaminants. To avoid this source of infection the tissue must be thoroughly surface sterilized before planting it on the nutrient medium. To disinfect plant tissues various sterilizing agents have been used. Hypochlorite and Mercuric chloride solutions have proved to be the most effective in most cases. Ethyl and isopropyl alcohol have been used to surface sterilize some plant tissues.The microbial contaminant is normally present in the medium from the start. To destroy them, the mouth of the culture vessels should be closedwith a suitable bacterial proof closure and the vessels ate autoclaved. Sterilization depends on the temperature and not directly on the pressure.Medium should after autoclaving, tubes placed in slanting stands to preparethe slants. These were then left to cool and solidify.

Tissue Culture Media:

The most important factor governing the growth and morphogenesis of planttissue culture is the composition of culture medium. Nutritional requirementsfor optional growth of a tissue *in vitro* may vary with the species. Even tissuesfrom different parts of a plant may have different requirements for satisfactorygrowth. As such no single medium can be suggested as being entirely satisfactoryfor all types of plant tissues and organs but generally all culture media are made up of Macronutrients, Micronutrients**,** Vitamins, Growth regulators**,** Carbohydrates (Sucrose) Formulation designed by Murashige & Skoog (1962). Revised by Linsma & Skoog (1965) can be regarded as standard.

Media constituents (Kumar. A and Kumar. V.A):

Inorganic Nutrients Mineral elements are very important in the life of a plant.

- Mg is a part of chlorophyll molecules.
- Ca is a component of cell wall.
- N is an essential part of amino acid, vitamins, proteins and nutlike acid.
- Fe, Zn, and Mo are part of certain enzymes.
- Besides, C, H, &O 12 elements are known to be essential for plat growth.
- N,P,S,K, Ca, Mg,Fe, Mn, Cu, Zn, B and Mo.

ISBN: 978-81-953600-3-1

First six elements are required in large quantities and termed as macro or majorelements. Six other elements required in small quantity and termed as microelement.

Media combination:

Table 1: **compositioon of murashige and skoog's medium** (Stock solution table)

Constituents	**Concentration N of stock in (mg/l)**	**Volume of stock per Litre of medium (ml)**	**Storage Temp of stock solution**
Stock soln. I Macrosalts		50ml	+4^{o}C
NH4NO3	33000		
KNO3	38000		
CaCl2.2H2O	8800		
MgSO4.7H2O	7400		
KH4PO4	3400		
Stock soln. II Microsalts		5ml	+4^{o}C
KI	166		
H3BO3	1240		
MnSO4.4H2O	4460		
ZnSO4.7H20	1720		
CuSO4.5H20	5		
CoCl2.6H20	5		
Na2MoO4.2H2O	50		
***Stock soln. III Iron sources**		5ml	+4^{o}C
Na2EDTA	7460		
FeSO4.7H2O	5560		
Stock soln. IV Vitamins		5ml	-20^{o}C
Myo-Inositol	20000		
Nicotinic Acid	100		
Pyridoxine HCl	100		
Thiamine HCl	100		
Glycine	400		

* Dissolve FeSO4 7H20 separately in 450ml of water by heating and constant stirring. Mix the two solutions and adjust pH to 5.5 and complete he volume to one litre.

Supplements/Additive	
Sucrose	30g/lit
Agar	7-8
Water (Double distilled)	1litre

Culture Medium:

The media formulation described as Murashige and Skoog (1962) referred as MS medium was selected as the optimal culture medium. Stock solutions of generally 4 times major elements. 1000 times minor elements, 100 times organic constituents were prepared. These stock solutions were stored in a freeze chest at 4oC and were stored for more than 15 days. The reagents used ere of Analytical Reagent Grade Each salt was dissolved separately one after one to avoid precipitation. Coconut milk (liquid endosperm)when used was extracted from young green coconuts and stored at 4o C (Murashige T.Skoog F). All the constituents except agar were mixed and then the pH of the solution was adjusted to 5.5-5.8 Later agar was added and the medium was heated to boil so as to homogenize agar. After the preparation of the medium, water was poured out of the autoclaved glassware. Definite aliquots of the medium were then added depending upon the capacity of the culture vessel. Generally, 25ml, 50ml, 100ml of the medium was distributed into the test tubes,100ml and 250ml flasks respectively. After plugging the glassware with cotton plugs, media ware steam sterilized at 15lb/in2 (121^0C) for 15-20 minutes.

Gelling Agents:

In static culture if liquid medium is used the tissue would get submerged and die due to lack of oxygen. A gelling agent is generally used to circumvent this problem. The most desirable property of a gelling agent is that it should withstand sterilization by autoclaving and the medium should be liquid when hot but form a semisolid gel when cool. Agar is commonly used gelling agent.

pH:

The pH of medium is adjusted to 5.8- 6.0 using 0.1N NaOH or 0.1N HCL.

Inoculation:

All the experimental manipulations were carried out under strictly aseptic conditions in laminar air flow bench fitted with a bactericidal U.V. tube (15 W. Peak emission 2637 Ao). The floor of the chamber was thoroughly scrubbed with cotton dipped in alcohol. The surface of all the vessels and other accessories such as instruments (spatula, forceps, scalpels, blade etc.).Gas burner, lighter, tube containing absolute alcohol etc were also cleaned with alcohol. The fresh material to be inoculated was kept in a petri dish covered with a piece of black paper in order to

ISBN: 978-81-953600-3-1

protect it from the harmful effects of U.V rays. Alcohol was then sterilized with U.V rays continuously on for one hour. The explants were then washed properly to remove the detergent. The explants were then treated with Bavistin (fungicide) for another 20-30 minutes to remove the fungus and then washed properly to remove the fungicide. Fresh seeds of Nasturtium were used to raise the seedlings under sterile conditions. Seeds with testa were presoaked overnight and the seeds which settled down were selected and taken to be viable. Like explants, seeds were washed under running tap water, then liquid detergents and finally under Bavistin to remove dust particles, microbes and fungi.Hands and arms which were to be used inside the inoculation chamber were scrubbed with alcohol before inoculation.The rims of the test tubes and the sides of the plugs were flame sterilized instruments (like forceps, scalpels, spatula etc.) were all sterilized by dipping in the alcohol and flaming a number of times. Care was taken to cool the instruments before putting into operation.The explants taken from field borne plants were treated with 0.01-0.1% mercuric Chloride solution for 5-10 minutes respectively depending upon the explants.Shoot apices of Nasturtium were treated with 0.1% mercuric chloride for 4-5 minutes. The explants like stems and leaves were treated with 0.1% Hgcl2 for 5-6 minutes.The explants were then thoroughly washed (4-5 washings) with sterilized distilled Water to remove the traces of Hgcl2.Fresh cuts were given to the stem explants after sterilization to remove undesirable or dead portions.The explants were then planted on variously augment MS medium. Seeds were surface sterilized with 0.1% Hgcl2 for 7-8 minutes.

Observation and Results:

The present studies pertained to the *in vitro* studies for micropropagation of important medicinal plant: ***Plumbago zeylanica*** *linn*. This aspect dealt with followingstudies for the micropropagation of the *Plumbago zeylanica linn.*

1. Selection of explants
2. Standardization of sterilization techniques.
3. Establishment of explant.
4. To get maximum rate of shoot proliferation.
5. To get maximum percentage of root induction.
6. *In Vitro* hardening.
7. Acclimatization of in-vitro growth plantlets.

In vitro propagation experiments were carried out by aseptically culturing the nodal explants on media MS supplemented with different plant growth hormones.

Selection of explants:

Micropropagation is preferred because of genetic stability. For this experiment nodal explants were collected from mature *Plumbago zeylanica* plant. Explants were collected from non-wood forest product division of herbal garden&Germplasm Conservatory F.R.I.

Sterilization of explants:

Different concentration of Hgcl2 (0.1-0.3%) were used for surface sterilization, 0.1% HgCl2 treatment gave fruitful results and at this concentration up to 70% nodal remained green and uncontaminated on the basis of these finding 0.1%HgCl2 for 2-3min was used for surface sterilization of nodal explants. Constant shaking was done during. This period to get through sterilization. Rinsing with sterile distilled water 4-5 times was necessary for the remove off sterilant from the seeds. These were than planted on Basal MS medium for germination various parts like root, stem, leaves and shoot apieces were excised from weeks old seedlings and transferred separately to different experimental media.

Cultural conditions:

All the cultures were maintained in an air-conditioned culture room at a temperature of 25+.4oC. The source of illumination consisted of 2.5 feet wide fluorescent tubes (40 watt) and incandescent bulb (25 watt). The intensity of illumination was 3500 lux at the level of cultures and a 12-hour light regime was followed by 12-hour darkness (s. Chand publication text book of biotechnology 12th

Table 2: Different percentages of sterilant and survival % of Explants

Sr. No.	Explant	sterilant	Conc.	Time duration	Survival %age	Condition of Explant
1	Node	$HgCl_2$	0.1 **%**	2-5 min	70-80%	Green and healthy

Initial establishment of explant (shoot induction /Bud break):

Bud break was achieved in 20 days in different media combinations.Maximum percentage of bud break was achieved MS medium supplemented withKn (.04 mg/l) and NAA (.02 mg/l). Multiple shoots were separated and regular sub culturing was done on pre-established cultures on MS medium supplemented with different concentrations of BAP.

Discussion:

The present project work was undertaken in order to learn the tissue culture technique for medicinally important medicinal plant *Plumbago zeylanica* which has several therapeutic uses. *Plumbago zeylanica* as explant material for vitro propagation. Nodal segments were inoculated in MS media with different hormonal concentration under aseptic conditions.The bud break was

ISBN: 978-81-953600-3-1

observed after two weeks &axillary bud break was found to be best on (1.0 mg/l) concentration of BAP. These axillary shoots were excised and cultivated in MS media supplemented with different hormonal concentration of BAP (0.1,0.5,0.1). A very high rate of multiplication occurs on 1mg/1 BAP concentration.The shoots were rooted in MS media supplemented with different concentration of BAP (0.5,1.0, 1.5). Best rooting occurs on 1.0mg/1IBA. Old culture on rooting medium developed healthy roots in mist chamber and there after transferred in shade house for acclimation. Field transfer of tissue culture raised plant was successfully carried out. So this technology is effective as it produces thousands of plants in a short span of time.

Table 3: effect of plant growth regulator (Kn) ON number of shoots buds per explants formed (after 3 weeks)

Sr. No.	Concentration Kn (mg/l)	No.of explants inoculated	No.of shoots induced	Response %
Control	0.0	1	1	20%
1	0.5	1	2	40%
2	0.1	1	4	20%
3	1.0	1	3	40%
4	**1.5**	**1**	**8**	**60%**
5	2.0	1	6	40%

Table 4: Effect of plant growth regulator (BAP) on number of shoots buds per explants formed (AFTER 3 WEEKS)

Sr. No.	Concentration BAP (Mg/l)	No.of explants inoculated	No.of shoots induced	Response %
Control	0.0	1	1	20%
1	0.1	1	2	40%
2	0.5	1	3	60%
3	**1.0**	**1**	**6**	**80%**
4	1.5	1	4	60%
5	2.0	1	5	60%

References:

Abdul KM, Rachender RP (1995) Modulatory effect of plumbagin of macrophage function in Balb/c mice. I. Potentiation of macrophage bactericidal activity*Immunpharmacol* 30, 3:231-6 Pub Med 8557 523

Ahmad I, Aquil F (2006) In vitro efficacy of bioactive extracts of 15 medicinal plants against Esbetal-producing multidrug resistant enteric bacteria Microbiol Res 2006.

Aitkin- Chirstic J(1991). Automation In: Debergh PC, Zimmerman RH(eds). Micropropagation, Dordrecht: Kluwer Academic Publishers. Pp 342 354

Alluri KV, Tayi RVN, Dodda S. Mulabagal V (2005). Assessment of bioactivity of Indian medicinal plants using brine shrimp (Artemia Salina) lethality assay,32: 125-134.

Anonymous, (1989) In : The wealth of India : A Dictionary of Indian Raw Materials and industrial products. CSIR New Delhi India, 2; 163-164.

Aquil F, Ahmad I, Owais M (2006) Evalution of anti-methicillin resistant *staphylococcusaureus* (MRSA) and synergy of some bioactive plant extracts Biotechnol J(10): 1093-1102.

Azad c, sushanta KC, Azadkhan AK (1982). Antifertility activity of *plumbago zeylancia* Linn Root, Indian J, Med.Res 76: 99-101.

Azad chowdhury AK, sushanta KC, Azadkhan AK (1982).Antifertility activity of *plumbago zeylancia* Linn Root, Indian J.Med. Res 76: 99-101.

Azad chowdhury AK, sushanta KC, Azadkhan AK (1982).Antifertility activity of *plumbago zeylancia* Linn. Root, Indian j.Med.Res 76; 99-101.

Aziz MH, Dreckschmidt NE, verma AK (2008). Plumbagin, a medical plant derived naphthoquinone, is a novel inhibitor of the growth and invasion of hormone refractory prostate cancer. Cancer Res. 68: 9024-9032.

Balasubramanun, D., Bryce, C.F.A., Dharmalingum, K and jayaram, K (1998) Concepts in biotechnology university press, Hyderbad.

Barna, K.S and whakhlu,A.K (1994) Whole plant regeration of Cicer arietium from Callus culture via organogesis, Plant cell Reports, 13:510-513.

Bhargava SK (1984). Effects of plumbagin on reproductive function of male dog. Indian J. Exp. Biol. 22: 153-156.

Bhargava SK (1984). Effects of plumbagin on reproductive function of male dog.Indian J. Exp. Biol. 22: 153-156.

Bhojwani S.S and Razdan, M.K (1992) plant tissue culture: Theory and practice, Elsevier, Amsterdam, London, New York, Tokyo.

Bhojwani S.S (1980) Micropropagation method for hybrid willow (salix matsudana X alba NZ-10002)N.Z.J. Bot., 18:209-21.

ISBN: 978-81-953600-3-1

ROLE OF LADYBIRD BEETLES (COCCINELLIDAE: COLEOPTERA) FOR THE MANAGEMENT OF SUCKING PESTS IN DIFFERENT ECOSYSTEMS

Kshitiz[1], Vinod Kumar Dubey[2]* and Radha Koranga[3]

[1]Department of Nematology, OUAT, Bhubaneswar, Odisha, India

[2]Department of Entomology, RPCAU, Pusa, Samastipur, Bihar, India

[3]Department of Entomology, GBPUAT, Pantnagar, Uttarakhand, India

*Corresponding authors E-mail: vinodkumardubey42@gmail.com

Abstract:

Coccinellid beetles are most ecological diversified, species richness group of agriculturally important and major natural enemies of sucking insect pests occurred in different of terrestrial ecosystems. Current appraisal was assessing to know the biological important of coccinellids beetles against sucking pests. However, 6000 species of ladybird beetles were reported from different parts of the worlds, out of 6000 species 90 per cent species are belonged to the beneficial category and they were divided into seven subfamilies *viz.*, Sticholotidinae, Chilocorinae, Scymninae, Coccidulinae, Coccinellinae, Epilachninae and Ortaliinae. Ladybird beetles are significant predators for sucking insect pests like aphids, scales, mealybugs, whiteflies, bugs, jassids, thrips, psyllids and mites. Now days, the ladybird beetles have been used in broad range in the field of biological control programmes in both forms such as classical as well as in augmentative biological control. They are having great importance in organic cropping and in integrated pest management systems.

Keywords: Coccinellids, Biological Control, Ecology, Biology, Insect pests, Natural enemies

Introduction:

Ladybird beetles belong from the family Coccinellidae. It is one of the most diversified predators in their habitat. They are prevalent in most of the terrestrial ecosystems. This is one of the significant groups of the natural enemies from agricultural and horticultural crop pests. Ladybird beetle belong to order Coleoptera, suborder Polyphaga, super-family Cucujoidea and family Coccinellidae. Coccinellidae family includes 6,000 reported species from worldwide and it is divided into six subfamilies i.e., Sticholotidinae, Chilocorinae, Scymninae, Coccidulinae, Coccinellinae and Epilachninae and in latest phylogeny suggested that a seventh subfamily, Ortaliinae. Out of these seven families, the subfamily (Epilachininae) is phytophagous and rest

of the families having predatory in nature on sucking insect pests. Most of the taxonomist divided the family into two major parts, based on the feeding potential i.e., phytophagous or predatory in nature ladybird beetles fulfill their diet with pollen and nectar and (Halyziini) group is mainly mycophagous) (Dyadechko, 1953).

Ladybird beetles are one of the most beneficial predatory insects in the worlds. Coccinellidae beetles are predatory in nature against most of the sucking insect pests, which were belong to different families such as Aphididae, Pseudococcidae, Aleyrodidae, Thripidae, Cicadellidae, and Psyllidae and suborder sternorrhyncha (Blackman, 1967). They are feeding on different stage of insect pests like insect eggs, small larvae and phytophagous mites; they are causing economic damage to agricultural crop and forest plantations in the India as well as in worlds. Coccinellids are mainly recorded as useful insects and one of the most important friend insects of the farmer. Ladybird are nature own insect pest management and more efficient than pesticdes chemicals (Matti, 1976). It was recorded approximately 5200 species of ladybird beetle from the different parts of the world. Vandenberg gave the list of 6000 species from 370 genera of ladybird beetle in worldwide, and then Ślipiński recorded 6000 species from 490 genera. Poorani recorded 400 species under 79 genera of ladybird beetles from different Indian subcontinent (Samadov, 1963).

Newly, Shah and Khan reported 17 species of predaceous coccinellids from different agro ecosystems of Kashmir; they were belonging to 15 genera and three subfamilies *viz.*, Coccinellinae, Chilocorinae and Platynaspinae. Preyed by the adults and larvae of the ladybird beetles have a major impact on populations of immature stages of these insect pests. Even though their prey of choice, most predatory Coccinellid include other non-prey items in their diet such as honeydew, pollen, sap, nectar and various fungi (Nasirova, 1978).

A brief description about the identification, biology and Ecology of Coccinellids beetle

Ladybirds beetle life stage varies from eggs to adult, always female is larger compared to male. Coccinellid beetle from eggs produce larva that undergo into four different instars before transforming into pupa and in adult stage. Larval stage colours are varied from species-to-species level. Newly hatched larva is grayish or black with yellow or orange color spots are present on the upper side of the body. The length of the fully grown-up larvae ranged from less than 1 mm. to about 1 cm in the length and the large larvae they may be able to travel up to 12 m in search of prey, the larvae stage completes in 2-3 weeks. The last instar of the larva goes for pupation when they are attaching to a leaf, stem or other surface and emerge into the other ladybird beetle.

ISBN: 978-81-953600-3-1

Cannibalism in the eggs, larva and pupa are common in nature and mainly whenever the scarcity of prey (Debaraj and Singh, 1990).

Most of the species of the ladybird beetle are completely white yellowish in nature after emergence. The body shape of adult varies from species to species like elongate, circular to oval. Head mainly buried under thoracic pronotum, mouth parts are found biting and chewing type and clavate type of antenna. Pubescence is absent or present over the body, eyes large, maxillary palpi are four-segmented, galea and lacinia are separated, mandible with reduced mola; five pairs of abdominal spiracles are present, front clypeal sutures are absent, tarsal formula 4-4-4 or 3-3-3, tarsal segment two usually strongly dilated below. On the head have three ocelli present on either side of the head. The legs are long and delicate in nature. The head has three ocelli on either side. The legs are long and slender. Pupation takes place on the same spot where larva is feeding. Ladybird beetles are multivoltine in nature and they go under hibernation stage in the winter. Adult life cycle is only few hours to over a year. The bright coloration of the body it's not making them attractive visitor in the different ecosystem it may also help to protect themselves by warning potential to predators of their tastelessness (Gilkeson and Kelin, 2001).

Ladybird beetle secrets yellow substances from legs. The adult and larval stage of ladybird beetles is feeding on aphids. One ladybird beetle's adult may can eat approximately 5000 aphids in complete life cycle. The ladybird beetles' prey on aphid is more active and the development rate is also faster than the ladybird beetles eating on scales and the scales consuming ladybird beetles the rate of development is slow (Muzammil *et al.*, 2008).

Coccinellid beetles hundreds of eggs on the aphid colonies and some other plant eating pests. After the emergence of larvae of ladybird beetle immediately they go for feeding. Coccinellid beetles are reddish orange in colors are eating on aphids and dark colour aphids are consuming on spider mites, whiteflies and scale insect pests. More efficient feeding time of Coccinellid in the different ecosystem is in afternoon and in evening condition they may push them to stay up to night and to get it suitable food and protection. Under the temperate condition the adult Coccinellid beetles going in the hibernation condition. The adult ladybird beetles also eat on pollen, nectar, water and honeydew which may constitute up to 50 per cent of the total food. Whenever the scarcity of the prey they may become the cannibalistic. The duration of the life cycle of ladybird beetles depends on various abiotic factors like temperature, rainfall, relative humidity and prey density. The larva is most active during the day time. They exhibit positively phototrophic toward the light and negatively geotaxis in nature toward the gravity (Saleem *et al*., 2014).

Dry condition and in summer season there were negative impact on the population dynamics of aphids and which in turn dramatically reduces the fertility of coccinellids beetles (Ahmadov and Hasanova, 2016**).**

Importance of Coccinellids beetle in biological control:

The term biological control was defined as the action of parasites, predators or pathogens in maintaining another organism's population density at a lower average than would occur in their absence. In the ecological sense, it refers to the 'up to down' action of predators, parasites or pathogens on organisms occupying a lower trophic level, action that maintains their populations at lower levels than would occur in the absence of these natural enemies, i.e., a natural process that does not depend on any human intervention. In the applied sense, it refers to the specific use or manipulation of population of natural enemies to reduce the pest population to tolerable level. Biological control is economically viable, minimum environmental impact and there is no environmental risk of contamination. There is no risk present for human and animal health. On the economic point of view, the effective natural enemies are able to control the insect pests population density and maintaining it below the economic damage threshold level in crops. The important characteristics of natural enemies are such as specificity towards the prey, maximum searching efficiency, short life cycle, do not become hyperparasite, efficient feeding potential, high rate of reproductive capability (Harde, 2000).

Now a days Ladybird beetles are important part of integrated pests management due to its high feeding activity and highest reproductive rate. In classical biological control programmes, the ladybird beetles are mass produced and release for the control of various insect pests (Notario, 1978). Approximately 90 per cent of coccinellids are beneficial predators. Predators are basically chewers or suckers, but there can be combinations of these habits. In general, predators can be considered generalists in relation to their prey. Three major categories of feeding habits generally are recognized for coccinellids predation (zoophagy), plant feeding (phytophagy), and fungus feeding (mycophagy). Biological control programmes involve three major techniques such as introduction, conservation and augmentation. Introduction refers to the intentional introduction of an exotic biological control agent for permanent establishment and long-term pest control Pushpendra (Sharma *et al.*, 2010).

Augmentation reveals to the mass production and periodic release of natural enemies of the pest, in order to increase their effectiveness of control, but without the goal of permanent establishment of the population. Augmentation classified into two major group i.e., Inundative and inoculative biological control. Inundative biological control means to the use of living organisms to control pests when control is achieved by exclusively by the organisms themselves that have been released. Therefore, inoculative biological control means refers to the intentional

ISBN: 978-81-953600-3-1

release of living organisms as a biological control agent with the expectation that it will multiply and control the pest for an extended period, but not that it will do so permanently (Zia, 2010). All the three major techniques are applicable in coccinellids. *Cryptolaemus montrouzieri* Mulsant is one of the most important biological control agents have been used. The *C. montrouzieri* have some important features like rapid development rate, high reproductive potential, good adaptation to a range of tropical and subtropical climates, high prey consumption rates by both adults and larvae and ease of rearing. The first introduction of this ladybird beetles for biological control dates back to 1891 when Albert Koebele brought it into California for control of *Planococcus citri* Risso. Recently, it was introduced in parts of the Caribbean and Central and South America for control of the hibiscus mealybug, *Maconellicoccus hirsutus* Green (Saharia, 1982).

Ladybird beetles is having polyphagous predatory in nature and they feed on minimum eight different type of hemipteran families. In some countries, it is also used extensively for augmentative releases, for instance in citrus orchards in the Mediterranean, the former USSR and USA. In India, it is used in coffee plantations, fruit orchards and vineyards. In South Africa, it is used against mealybugs in citrus. In India, maximum control of *M. hirsutus* on grape was attained 6–8 weeks after initial release of 1000–1500 (10 per vine) ladybirds per hectare. In the Black Sea area of the former USSR, 5000 ladybirds per hectare were used with good results in tea plantations to control *Chloropulvinaria floccifera* Westwood. *Chloropulvinaria aurantii* Cockerell was controlled in citrus plantations in Azerbaijan when 5000 *C. montrouzieri* were released in 3 ha. of orchards. Similar kind of results was obtained for *P. citri* on citrus in Italy (Saharia, 1982).

Repeated releases of the predator-controlled mealybug pests on ornamental plants in European glasshouses were recorded. In New Delhi, 2–3 larvae and adults of *C. montrouzieri* per tobacco plant controlled the mealybug, *Ferrisia virgata* (Cockerell) successfully within 1 month under glasshouse conditions. For most agricultural systems, conservation techniques for coccinellids are lacking, even though they are abundant in these habitats. The provision of overwintering habitats and refuges from pesticides in and near cropland are the core practices for the use of coccinellids in conservation biological control. In diversified ecosystems the diversity of natural enemies is also quite high. Factors that contribute to high level of natural enemies in these ecosystem are the availability of diverse microhabitats, alternative hosts and shelter all of which encourage colonization and population buildup of natural enemies besides, greater availability of food sources (prey, nectar, pollen); provision of refuge, the abundance of floral nectar and alternative prey (aphids), shelter, mating and oviposition sites harbored in the border crop compared with monoculture having lesser biodiversity (Muzammil *et al.*, 2008).

References:

Ahmadov B A, Hasanova S H. (2016). The role of coccinellid (Coccinellidae) in suppression of the number of *Aphis craccivora* Koch, 1854 and Aphis gossypii Glover,1877. Journal of Entomology and zoology studies 2016; 4(3): 234-237.

Blackman RL. (1967). The effects of different aphid foods on Adalia bipunctata L. and Coccinella 7-punctata L Ann. Appl Biol. 59(2):207-219.

Debaraj Y, Singh TK. (1990). Biology of an aphidophagous coccinella predator, Coccinella tranversalis. J Biol Cont 4: 93-95.

Dyadechko NP. (1953). Meaning of predators and parasites of the ermine moths and orchard ermine, depending on the chemical treatments in gardens. Pr Ukr MIIZP. 8:199-204.

Gilkeson L, Kelin M. (2001). Natural enemies of crop insect pests. Coop. Ext, Cornell Univ. Ithea, N.Y. 63 pp.

Harde KW. (2000). A field guide in colour to beetles. Silverdale Books, Leicester.

Matti M. (1976). Rearing the univoltime lady beetles Coccinella septempunctata and Adalia bipunctata (Col., Coleoptera) all year around in the laboratory. Ann agr fenn. 15(1):66-71.

Muzammil S, Hamad M, Nadeem S. (2008). Biology of *Cocinella septempunctata* Linn. (Coleoptera: Coccinellidae) and its predatory potential on cotton aphid, Aphis gossypii Glover (Hemiptera Aphididae). Pak J Zool 40(4): 239-242.

Nasirova NZ. (1978). Predatory beetles (Coleoptera, Carabidae) of the Absheron Peninsula: Avtoref cand diss Baku, 26.

Notario A. (1978). Contribucion al studio biologico de Coccinella septempunctata L. (Col., Coccinellidae) Ann. INIA ser Prot veg. 8:63-67.

Pushpendra Sharma K, Joshi PC. (2010). Biology of a predatory coccinellid Coccinella septumpunctata Linn. (Coleoptera: Coccinellidae). J. Env. & Biosci. 24 (2):235-238.

Saharia D. (1982). Field evaluation of granular systemic insecticides on Lipaphis erysium and its predator Coccinella repanda. J. Res. Asam Agric. Univ., 2: 181-185.

Saleem M, Hussain D, Anwar H, Saleem M, Ghouse G, Abbas M. (2014). Predation Efficacy of Menochilus sexmaculatus Fabricus (Coleoptera: Coccinellidae) against *Macrosiphum rosae* under laboratory conditions. JEZS 2014; 2 (3): 160-163.

Samadov NG**. (1963).** Fauna and biology of beetles injuring agricultural plants in Azerbaijan. Baku: Publishing house of the Academy of Sciences of Azerbaijan SSR, 352.

Zia A, Hassan SA, Shehzad A, Naz F. (2010). Diversity of Aphidoidea in Rawalpindi Division (Punjab)Pakistan, with a list of host plant studied. Halteres 1: 40-57.

ISBN: 978-81-953600-3-1

L- CARNITINE AS A MITOCHONDRIA BASED NUTRACEUTICAL AGAINST OXIDATIVE STRESS, MITOCHONDRIAL DYSFUNCTION IN NEURODEGENERATIVE DISEASES - A MINI REVIEW

Jyothsna Karanth[1]* and Jamuna M.[2]

[1] Department of Biochemistry,
Government College for Women (Autonomous), Mandya-571401, Karnataka

[2] Postgraduate Department of Chemistry,
Maharani's Science College for Women, Mysuru-570005 Karnataka

*Corresponding authors E-mail: jyothsnakarant@gmail.com

Abstract:

Aging and age –associated neurodegenerative diseases are the most important challenges to research. Neurodegenerative ailments are becoming increasingly widespread as the global population ages. Several lines of evidence imply that the oxidative stress induced mitochondrial dysfunction is one of the main drivers of these processes. Increasing reactive oxygen species (ROS) levels and products of the oxidative stress, which develop with age, were also detected in age-related neurological diseases. ROS may be generated by ineffective oxidative phosphorylation, which encompasses a variety of redox processes, resulting in mitochondrial dysfunction and tissue injury. The brain requires more energy than other organs in the body, and it consumes a large amount of oxygen resulting in excessive production of ROS.The mitochondrial dysfunction and oxidative stress can cause biochemical alteration in biomolecular components leading to decline in the neuron functions thereby its death attribute to various neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, Huntington's disease.A major cause of age-related dementia is Alzheimer's disease. Currently, there are no appropriate medications to reduce, stop, or reverse the progression of this disease. Many studies have demonstrated, however, that modifying lifestyle factors, such as nutrition, can help to delay or prevent the onset of this disease. Considering the ability of crossing the blood–brain barrier, L-carnitine supplementation may also be beneficial in preventing neurological damage derived from oxidative injury. This review focuses on the therapeutic potential of L-carnitine and its derivative, acetyl-L-carnitine against neurodegenerative diseases. However further studies are required to better explore this potential.

Keywords: Carnitine, neurodegenerative diseases, Mitochondrial dysfunction, Neutraceutical, Antioxidants.

Introduction:

Aging is the major risk factor for many diseases but little is known about the factors that cause age-related alterations and promote dysfunction. Slowing or even postponing these processes in humans could lead to prolonged periods of good health (1). A better understanding of ageing has enormous implications for the realm of medicine. It raises the prospect of a slower illness progression and a longer human lifespan. A decline in the efficiencies of various biochemical and physiological functions takes place in most of the organs during aging. The main impact of aging is on the cardiovascular and nervous systems. Degenerative disorders such as atherosclerosis and senility may result from age-related problems in these physiological systems. The ageing of the brain, which is part of the central nervous system, is manifested by grey and white matter atrophy due to neuronal loss, neuronal morphology changes, and dendritic and synaptic reductions resulting in neurodegenerative diseases like dementia, Parkinson's disease, Alzheimer's disease etc. (2-7). Alzheimer's disease is currently five times more common than Parkinson's disease, the second most common neurodegenerative disease, and this margin is predicted to widen further (1). Many studies are currently being conducted in attempt to better understand the fundamental mechanisms of ageing and age-related neurodegenerative diseases, as well as to find ways to combat this biological process (7). The pathophysiology of neurodegenerative diseases are far from being fully explained.

Oxidative stress (OS) has been proposed as one factor that plays a potential role in the pathogenesis of neurodegenerative disorders (8). The brain uses around 20% of energy in the whole adult human body and derives ATP, energy currency of the cell, through the mitochondrion (9). Mitochondria are actively involved in the intermediary metabolism, heat production, storage of calcium ion, regulation of the membrane potential and transports, and programmed cell death signaling i.e. apoptosis (10). Pathophysiological changes in mitochondria during ageing and many other metabolic disorders are now recognised as being linked to impaired mitochondrial functions such as decreased oxidative capacity and antioxidant defence due to increased production of reactive oxygen species (ROS), reduced OXPHOS, and decreased ATP production. The other factor is reduced mitochondrial biogenesis with age may be due to alterations in mitochondrial fission and fusion processes and the inhibition of mitophagy, a process which eliminates dysfunctional mitochondria (8, 11). Although the production of ROS within cells is an unavoidable process, cells have a number of defence mechanisms in place to combat it. The overproduction of ROS has been linked to oxidative damage to lipids, DNA, and proteins (12, 13). Previous research has shown that oxidative stress is linked to a variety of pathophysiological diseases (14).

ISBN: 978-81-953600-3-1

Nutrition is critical in delaying the ageing process, according to gerontologists. Attempts have been made to delay the onset of ageing by giving antioxidant supplements. L-carnitine (LC), a naturally occurring compound, facilitates β oxidation of fatty acids in mitochondria (15). Also it is reported that LC is a potent antioxidant and exerts cytoprotective effects. LC therapy is used to treat a variety of diseases, including diabetes mellitus, atherosclerosis and myocardial myopathy (16-18). According to research, a prolonged treatment with carnitine preserved pyramidal cells in the hippocampus (19). The neuroprotective role of L- carnitine is explored in this review, with the goal of presenting a new method for using this substance as a neutraceutical in the treatment of age-related neurodegenerative illnesses such as Alzheimer's disease.

Oxidative stress:

Increased production of ROS and reactive nitrogen species (RNS) is linked to oxidative stress, which is characterised as "an imbalance in pro-oxidants and antioxidants with accompanying disturbance of redox circuitry and macromolecular damage" (20). ROS and RNS include superoxide radical anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^{\cdot}$), nitric oxide (NO), and peroxynitrite ($ONOO^-$). ER, peroxisomes, a family of NADH oxidases, and other enzymes such as monoamine oxidases are all sources of ROS production in the cell, however mitochondria constitute the major contributor to ROS production (21- 25). In mitochondria during oxidative phosphorylation, complexes I and III produce $H_2O_2$and $O_2^{\cdot-}$ as byproducts (26). ROS/RNS perform crucial regulatory and mediator functions at physiological concentrations; however an uncontrolled increase in ROS/RNS concentrations triggers a sequence of free radical events, increasing the risk of damage to biological components in a living organism (8). Nitric oxide plays an important role in the brain and, when combined with ROS, such as superoxide, can generate RNS such as peroxynitrite that can nitrate tyrosine residues of proteins and alter their function (27). Because of its high consumption of oxygen and its high lipid content, the brain is particularly vulnerable to damage caused by ROS and RNS.

To limit the amount of ROS/RNS from endangering the integrity of biological systems, an antioxidant barrier system is maintained (Fig. 1). These include antioxidant enzymes which scavenge ROS and RNS, superoxide dismutases (SOD), glutathione peroxidase (GPX), glutathione reductase (GR), thioredoxins, and catalase and the nonenzymatic antioxidants include glutathione (GSH), thioredoxin (Trx), vitamins A, E, and C, flavonoids, trace elements, and proteins such as albumin, ceruloplasmin, and metallothionein (8).In OS, nuclear factor erythroid-2-related factor 2 (Nrf2) gets activated that further activates transcription of

cytoprotective genes via promoter sequences containing conserved antioxidant response elements (AREs) thereby increasing cellular level of antioxidant enzymes (26).

Excessive generation of ROS/RNS that surpasses the antioxidant barrier's maximal capacity causes a disruption in the pro-/antioxidant balance and, eventually, the development of OS. It may be triggered by either exogenous processes (e.g., xenobiotics, cold, viral and bacterial infections, ionizing radiation, ultrasound or photo-oxidation, poor diet, alcohol consumption, and smoking) or endogenous processes involving biochemical reactions in the body as mentioned earlier (28-30).

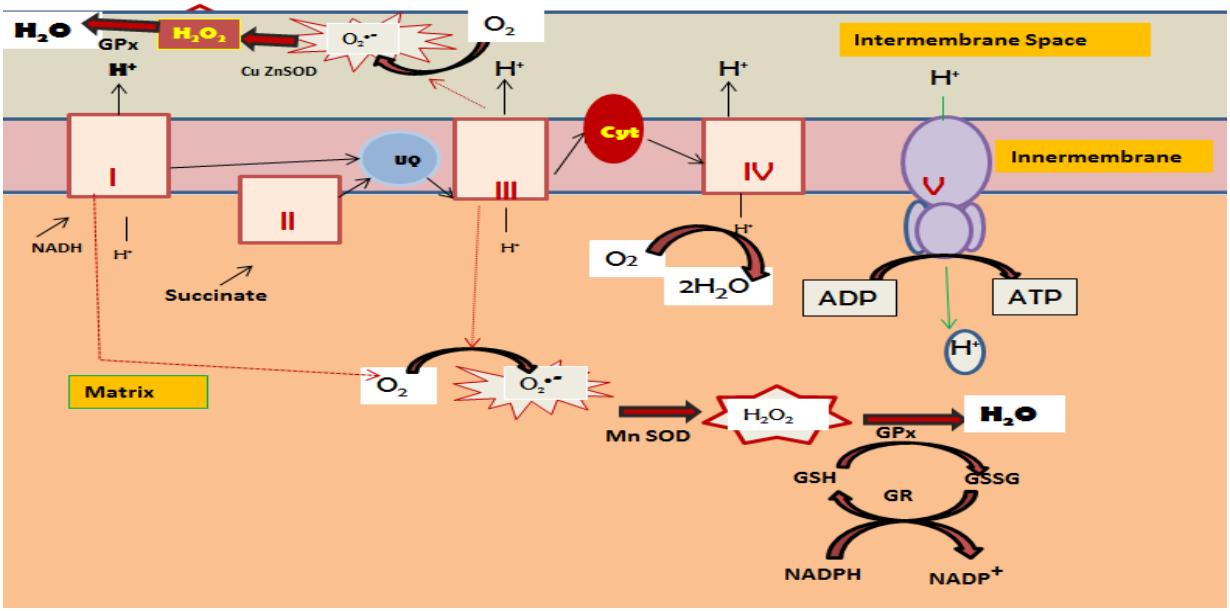


Figure 1: ROS production in mitochondria during oxidative phosphorylation and antioxidant mechanisms [Modified T¨onnies et al. (26)]

Oxidative stress in neurodegenerative diseases:

Due to the high rate of oxygen consumption, elevated levels of polyunsaturated fatty acids (which are easily targeted by free radicals), and relatively high levels of redox transition metal ions, the brain is especially vulnerable to oxidative damage; additionally, the brain has very low antioxidant levels and hence the hotspot of neurodegeneration (31). ROS modulate the functioning of cellular proteins, DNA, RNA and lipids by triggering their oxidation, which contributes to the development of neurodegeneration (27). According to multiple studies, the accumulation of various oxidative damage markers of proteins, lipids, and DNA causes brain ageing (32). A decline in DNA repair enzymes with ageing associated with oxidative damage-induced somatic mutations in mtDNA in the aged brain causes down regulation of protein

ISBN: 978-81-953600-3-1

expression involved in oxidative phosphorylation (33). Oxidative damage to mitochondrial proteins and lipids causes mitochondrial dysfunction, which obstructs bioenergetic activity. Overall, it results in reduced ATP production, calcium dysregulation, mitochondrial permeability transition pore opening, perturbation in mitochondrial dynamics, and deregulated mitochondrial clearance and ultimately hinders biological processes leading to various diseases (27).The involvement of mitochondria and mitochondrial dysfunction owing to ROS in various diseases is schematically represented in Fig.2. As age advances mitophagy declines resulting in accumulation of defective mitochondria, increased oxidative damage, eventually apoptosis. Neurodegenerative diseases are specifically characterized by apoptosis/necrosis and dysfunction of neuronal cells, leading to a malign effect on the neural system (32).

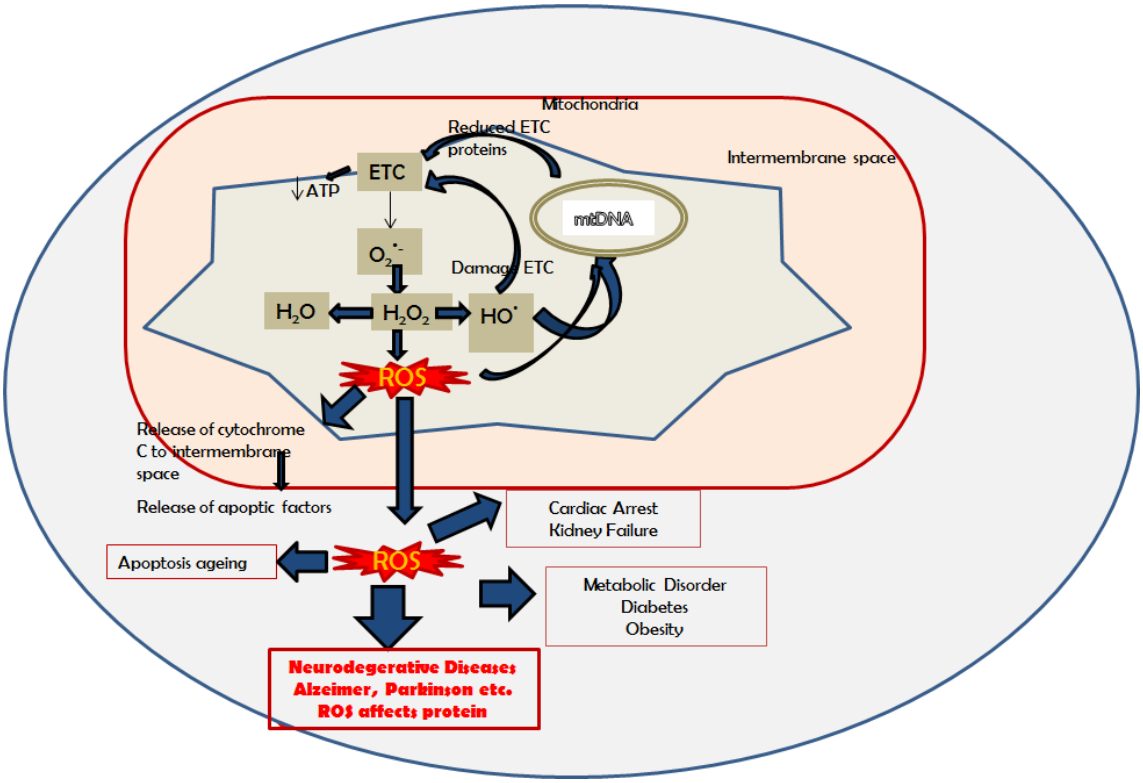


Figure 2: Involvement of mitochondria in oxidative stress and diseases [Modified Singh *et al.* (32)]

Oxidative stress in Alzheimer's disease (AD):

Alzheimer's disease, often known as dementia is defined by increasing cognitive and behavioural decline, which causes daily routine activities to be impaired. It's one of the most common neurodegenerative diseases, affecting around 45 million individuals around the world (32). Data from transgenic animal models of AD in which markers of protein and lipid peroxidation are elevated in the cortex and hippocampus before the formation of plaques or

tangle pathology (34). It is illustrated that Aβ protein acts as an antioxidant undergoes aggregation complexing with copper in its redox state. It becomes more toxic by forming fibrillary Aβ aggregates due to interaction with zinc. Accumulation of Aβ leads to oxidative stress, mitochondrial dysfunction, and energy failure prior to the development of plaque pathology (35). Aβ is able to induce opening of mitochondrial permeability transition pore (mPTP) in isolated mitochondria (36) and primary astrocytes (37). The activation of mPTP results in decreased $\Delta\psi$ also release of cytochrome c, thereby activating caspases that induce apoptosis of neuronal cells as well as mitophagy in the same cell (38). However, under pathogenic conditions, mitophagy or protective mechanisms are significantly compromised (39) which ultimately leads to cell death leading to neurodegeneration (36).

Use of Antioxidant Therapy in Neurodegenerative Disease:

As a result of modern societies' ongoing ageing process, the occurrence of neurodegenerative diseases is becoming a global public health concern (20). There have been attempts to delay the beginning of ageing and age-related disorders. Due to the ineffectiveness of Alzheimer's drugs, researchers are looking for alternative therapies (27). The argument for employing antioxidants as treatments is straight forward, as it is focused on the concept that oxidative stress causes neurodegenerative disease. Gerontologists are well aware of the importance of nutrition in delaying the ageing process (19).Indeed, first evidence of antioxidant effects in this disease in animal and cell models appeared promising. Vitamin E (a significant scavenger of lipid peroxidation in the brain), vitamin C (an intracellular reducing molecule), and coenzyme Q_{10} (transfers electrons from complexes I and II to complex III in respiratory chain) are among the most thoroughly investigated antioxidant therapies (27). However, the clinical use of classical antioxidants in these diseases has been poorly evaluated, with no evidence of benefit thus far.

Mitochondria-targeted antioxidants:

Several lines of evidence suggest that mitochondrial dysfunction is involved in the pathogenesis of metabolic disorders, ageing, and age-related neurodegenerative diseases. The commonly used antioxidants are vitamin E, vitamin C, coenzyme Q, α-lipoic acid, N-acetylcysteine (NAC) that have been shown to lower ROS production in a variety of metabolic conditions (40- 42). In the recent years, antioxidant compounds incorporating ubiquinone (MitoQ) or vitamin E (MitoVit E) specifically targeted to mitochondria have been successfully

ISBN: 978-81-953600-3-1

used against mitochondrial dysfunctions (43). MitoQ found to reduce ROS in the mitochondria and protect against age-related mitochondrial insult in brain tissue (14, 44,45).

L-Carnitine is an endogenous substance involved in the lipid metabolism in mitochondria, and also acts as a potent antioxidant (free radical scavenger) and thus may protect tissues from oxidative damage. Moreover, L- Carnitine has the ability to cross the blood brain barrier and its supplementation may help to prevent oxidative neuronal damage (20).

L- Carnitine:

L-Carnitine (LC, β-hydroxy-y-trimethylaminobutyric acid) is a water soluble molecule important in mammalian lipid metabolism. LC performs important cellular functions in the mitochondrial and peroxisomal metabolism. Carnitine is essential for the transport of long-chain fatty acids into mitochondria passing through the inner mitochondrial membrane in cells and thus facilitating β-oxidation and thereby ATP production. Besides, LC buffers level of CoA by shuttling the shortchain acyl groups from the inside of the mitochondria to the cytosol, thus balancing the levels of mitochondrial free CoA (46). l-carnitine as well as its derivatives i.e., propionyl-l-carnitine and acetyl-l-carnitine, are bioactive compounds (47).

Antioxidant properties of L-carnitine:

According to recent in vivo and in vitro studies LC has shown to effectively protect different cells against oxidative injury. Gülçin (2006) reported that LC is an efficient antioxidant agent in several in vitro experiments, and found to scavenge hydrogen peroxide and superoxide radicals, as well as chelate transition metal ions (48). In vitro experiments using SH-SY5Y neuroblastoma cells (49) and human hepatocyte HL7702 cell line (50) showed the protective effect of LC against H_2O_2-induced toxicity. LC found to protect the endogenous antioxidant defense system such as GPx, CAT and SOD activities (50-52) from peroxidative damage induced by aspartame in Wistar rats (53) and is very effective in normalizing age-associated alterations of oxidative status (54-56).After prolonged hypoperfusion, rats given LC had lower oxidative DNA damage and lipid peroxidation (57), thicker myelin sheaths, and higher expression of oligodendrocyte markers (58).

Neuroprotective role of L- Carnitine:

LC may have cytoprotective properties in addition to its involvement in energy metabolism. This chemical has been shown to have several neuroprotective, neuromodulatory, and neurotrophic characteristics in recent years. Despite the fact that the brain has a low degree of β -oxidation, blood levels of free acetyl-CoA and ketosis may become significant for brain

function in metabolically compromised conditions (59).In this instance, fatty acids are also needed for the integration of brain structural lipids. LC is transported across the blood–brain barrier along with the organic cation transporter OCTN2 and stored in neural cells, primarily as acetyl-L-carnitine (ALC)(60). Although the exact effects of LC in the brain are not clear, it is known that ALC can enhance cholinergic neurotransmission by mediating the transfer of acetyl groups for acetylcholine production in neurons (15, 61, 62) and also it influences signal transduction pathways and gene expression (63). In numerous in vitro and in vivo studies, ALC was found to be able to inhibit neuron degradation and expedite regeneration (64).It has been reported that ALC prevents the loss of muscarinic receptors as well as nerve growth factor (NGF) and directly or indirectly modulates N-methyl-D-aspartate receptor (NMDA), which maintains Ca^{2+} ion homeostasis in neurons, by stimulating the synthesis of nerve growth factor receptors in the hippocampus and basal forebrain (62).

Palmitoylcarnitine, other ester of carnitine, found to stimulate the expression of GAP-43 (named also B-50, neuromodulin, F1, pp45), a protein involved in neural development, neuroplasticity, and neurotransmission (65). LC treatment for neonates with inborn metabolic disorders has been shown to be effective (20, 66). ALC's neuroprotective efficacy in adults facing the conditions which may lead to central or peripheral nervous system injury has been proven in a number of clinical trials and case studies (66, 67).

Low concentrations of free carnitine, ALC and other acylcarnitines were found in plasma and tissues in many studies on AD (68). Subjective memory complaints (SMC), mild cognitive impairment (MCI), and AD were all correlated to a progressive decrease in ALC and other acyl-carnitine blood levels in healthy people (62). It is concluded that the decreased serum concentrations of ALC and hence its disturbed functions may predispose to AD and contribute to neurodegeneration. Clinical research in humans revealed that ALC had beneficial effects on brain function, cognition, and memory, leading to the hypothesis that ALC could reduce or correct moderate cognitive impairment and dementia progression in Alzheimer's disease (69). Dietary supplements applied for maintaining mitochondrial homeostasis include L-carnitine; coenzyme Q_{10}; mitoquinonemesylate; and other mitochondrion-targeted antioxidants such as N-acetylcysteine; vitamins C, E, K_1 and B; sodium pyruvate; and lipoic acid (14, 62).

Role of carnitine and its derivatives in AD:

Recent research suggests that LC may protect cells from oxidative damage in important neurodegenerative disorders like Parkinson's and Alzheimer's (70). In a study by Bavari *et al.* (71) the neuroprotective effect of LC (5 mM) inhibited caffeine cytotoxicity within 18 hours by regulating apoptosis-related caspase-3 activity, reducing DNA fragmentation, inhibiting reactive

ISBN: 978-81-953600-3-1

oxygen species (ROS) generation, elevating endogenous anti-oxidant defence systems, and preventing lipid oxidation (62).LC (50 mg/kg/day) therapy for 7 months in rats showed antioxidant effects via modulating Bax and Bcl-2, decreasing caspase-3 activity, increasing total antioxidant activity, and scavenging oxygen free radicals (71). In animal studies, it was discovered that ALC supplementation reduces homocysteine-induced protein hyperphosphorylation (a novel hallmark of AD) and inhibits the phosphorylation of β-amyloid (Aβ)(73). ALC and L-carnitine found to reduce apoptosis through the mitochondrial pathway (74).Suchy et al. reported that carnitine supplementation reduces free radicals induced murine brain damage and improves cognitive performance. ALC has been shown to improve cognition and behaviour in ageing and AD patients (64). ALC's probable methods of action in AD include enabling cell membrane repair, boosting synaptic function, increasing cholinergic activity, replenishing brain energy supply, guarding against toxins, and exerting neurotrophic effects via NGF stimulation and protein acetylation(72).In a number of tissues such as heart, skeletal muscle, and brain, co-supplementation of L-carnitine and -lipoic acid reduced age-related increases in reactive oxygen species (ROS), lipid peroxidation, protein carbonylation, and DNA strand breaks (73). The number of total and intact mitochondria, as well as the mitochondrial ultrastructure of neurons in the hippocampus, increased after three months of co-supplementation (62). ALC may reduce key pathogenic elements in AD, such as amyloid processing by up-regulating kinesin light chain 1 (KLC1) gene expression in the brain of rats chronically treated with ALC (61, 74). In an Alzheimer's disease mouse model, lowering KLC1 raises A*β*levels in the brain and speeds up and intensifies amyloid deposition (75). ALC was able to attenuate oxidative stress, ATP depletion, and cell death in cases of neurotoxicity caused by Aβ fragments (62, 76).

L-Carnitine and Acetyl L- Carnitneas nutraceuticals:

LC and ALC supplementation is advised for boosting brain and nervous system activity, memory, brain energy, and the impacts of brain neurodegenerative disorder therapies (20). Carnitine supplementation is critical in the prevention and treatment of Alzheimer's disease because it enriches intracellular and extracellular carnitine resources. Presently, there are no published recommended carnitine reference values. It is necessary to introduce carnitine-rich foods or carnitine dietary supplementation, based on rational dietary recommendations that support good health (47). The American Food and Drug Administration (FDA) approved LC in powder, fluid, pills, or capsules for the treatment of primary and secondary carnitine deficiency. Experimental data from in vitro and in vivo studies found no evidence of LC toxicity.After oral

administration of LC in humans, no adverse effects (including allergic reactions) were found. However, some persons who used LC had indications of gastrointestinal intolerance (62). However, in aging or its associated disease, ALC is better absorbed and more efficiently crosses the blood-brain barrier as compared to L-carnitine hence found to be more efficient diet supplement (74).

Conclusion:

The central role of mitochondrial OS in aging and age associated diseases has long been recognised. OS induced structural and functional changes in mitochondria are reported to involve in aging, cancer, metabolic syndromes and neurodegenerative diseases. Though the production of ROS is an unavoidable physiological event that occurs during mitochondrial catabolism, an imbalance between pro and antioxidants becomes a detrimental factor to the brain, ultimately aggravating neurodegeneration (8). Despite the disappointing outcomes of non-targeted antioxidants in clinical trials, there is growing evidence for the beneficial effects of mitochondrial-targeted antioxidants in aging and age-related diseases. LC and its derivatives are prospective mitochondrial-targeted nutraceuticals in reducing OS-induced neurological damage due to their capacity to cross the blood–brain barrier and their role in mitochondria. The therapeutic potential of LC and its acetylated derivative, ALC for neuroprotection in a variety of disorders, including hypoxia-ischemia, traumatic brain injury, Alzheimer's disease, and conditions leading to central or peripheral nervous system injury, has captivated interest in recent years (59). According to a recent meta-analysis of 21 double-blind clinical trials, LC is beneficial in the treatment of mild cognitive impairment and mild Alzheimer's disease (76). LC/its derivatives supplemented diets show a positive therapeutic effect on patients with neurodegenerative diseases especially in AD. Therefore, it is necessary to implement foodstuffs rich in LC and its derivatives for the prevention and/or alleviation of neurodegerative symptoms particularly dementia and other AD symptoms (47, 62). It should be emphasised that proper diet is a key aspect of a healthy lifestyle that may play a role in promoting healthy, gradual, and beneficial ageing as well as delaying the onset of neurodegenerative disorders such as dementia and Alzheimer's disease.

References:

1. ThelenM, Brown-Bor HM: Does Diet Have a Role in the Treatment of Alzheimer's Disease? Front Aging Neurosci2020, 12: 617071.

2. Terry RD, Katzman R: Life span and synapses: will there be a primary senile dementia? Neurobiol Aging 2001, 22; 347–348.
3. Dumitriu D, Hao J, Hara Y, Kaufmann J, Janssen WG, Lou W, Rapp PR, Morrison JH: Selective changes in thin spine density and morphology in monkey prefrontal cortex correlate with aging-related cognitive impairment. J Neurosci 2010, 30; 7507–7515.
4. Alexander GE, Ryan L, Bowers D, Foster TC, Bizon JL, Geldmacher DS, Glisky EL. Characterizing cognitive aging in humans with links to animal models. Front AgingNeurosci 2012, 4: 21.
5. Dykiert D, Der G, Starr JM, Deary IJ. Age differences in intra-individual variability in simple and choice reaction time: systematic review and meta-analysis. *PLoS ONE* 2012, 7:e45759.
6. Levin O, Fujiyama H, Boisgontier MP, Swinnen SP, Summers JJ. Aging and motor inhibition: a converging perspective provided by brain stimulation and imaging approaches. NeurosciBiobehav Rev 2014, 43, 100–117.
7. Evaluation of neuroprotective role of L-carnitine in aged rats. Juliet arockia rani P. http://hdl.handle.net/10603/195239.
8. Oxidative Stress in Neurodegenerative Diseases. Niedzielska1E, Smaga1 I, Gawlik1M, Moniczewski A, Stankowicz P, PeraJ, Filip M: MolNeurobiol 2016, 53; 4094–4125.
9. Power Failure of Mitochondria and Oxidative Stress in Neurodegeneration and Its Computational Models: Woo J, Cho H, Seol E, Kim S, Park C, Yousefian-Jazi A, Hyeon SJ, Lee J, Ryu H: Antioxidants 2021, 10; 229.
10. Mitochondrial Dysfunction and Cell Death in Neurodegenerative Diseases through Nitroxidative Stress. Akbara M, Essab MM, Daradkehb G, Abdelmegeeda MA, Choia Y, Mahmoodd L, Song B: Brain Res. 2016, 1637; 34–55.
11. Chistiakov DA, Sobenin IA, Revin VV, Orekhov AN, Bobryshev YV: Mitochondrial aging and age-related dysfunction of mitochondria. Biomed Res Intl 2014, 2014; 238463.
12. Halliwell B, Gutteridge JMC: Free Radicals in Bio Med 4th ed. Oxford University Press; Oxford: 2007.
13. Finkel T, Holbrook NJ: Oxidants, oxidative stress and the biology of ageing. Nature 2000, 408; 239–247.
14. Bhatti JS, Bhatti GCP, Reddy H: Mitochondrial dysfunction and oxidative stress in metabolic disorders — A step towards mitochondria based therapeutic strategies. BiochimicaetBiophysicaActa 1863, 2017; 1066–1077.

15. Nalecz KA, Nalecz MJ: Carnitine— A known compound a novel function in neural cells. Acta Neurobiol Exper 1996, 56; 597–609.
16. Paulson DJ, Schmidt MJ, Traxler JS, Ramacci MT, Shug AL: Improvement of myocardial function in diabetic rats after treatment with L-carnitine. Metabol, 1984, 33;358–363.
17. Dayanandan A, Kumar P, Kalaiselvi T, Panneerselvam C. Effect of L-carnitine on blood lipid composition in athrosclerotic rat. J ClinBiochemNutr 1994,17; 81–89.
18. Angelucci, Ramacci MT, Taglilatela G, Hulsebosch C, Morgan K, Werrbach-Perez, Perez-Polo R: Nerve growth factor binding in aged rat central nervous system: effect of acetyl-L-carnitine. J Neurosci Res 1988, 20; 491–496.
19. Packiasamy AR, Juliet, Balasubramaniam D, Balasubramaniam N, Panneerselvam C: Carnitine: A Neuromodulator in Aged Rats. J Geront: Biological Sciences 2003, 58A; 970–974.
20. Ribas GS, Vargas CR, Wajner M: L-carnitine supplementation as a potential antioxidant therapy for inherited neurometabolic disorders. Gene 2014, 533: 469–76.
21. Jones DP: Redefining oxidative stress. Antioxid Redox Signal 2006,8; 1865-1879.
22. Di Meo S, Reed TT, Venditti P, Victor VM: Role of ROS and RNS sources in physiological and pathological conditions. Oxid Med Cell Longev2016, Article ID 1245049.
23. Ray PD, Huang BW, Tsuji Y: Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. Cell Signal2012, 24, 981-990.
24. Holmstrom KM, Finkel T: Cellular mechanisms and physiological consequences of redox-dependent signalling. Natl Rev Mol Cell Biol2014, 15; 411-421.
25. Quinlan CL, Perevoshchikova, Hey-Mogensen M, Orr AL, Brand MD: Sites of reactive oxygen species generation by mitochondria oxidizing different substrates. Redox Biol 2013,23; 304-312.
26. T¨onniesa E, Trushina E: OxidativeStress, Synaptic Dysfunction, and Alzheimer's Disease. J Alz Dis 2017, 57; 1105–1121.
27. Gandhi S, Abramov AY: Mechanism of oxidative stress in neurodegeneration. Oxid Med Cell Longev 2012, Article ID 428010.
28. Butterfield DA, Drake J, Pocernich C, Castegna A. Evidence of oxidative damage in Alzheimer's disease brain: central role for amyloid beta–peptide. Trends Mol. Med. 2001, 7; 548–554.

29. Llanos-Gonzalez E, Henares-Chavarino AA, Pedrero-Prieto CM, Garcia- Carpintero S, Frontinan-Rubio J, Sancho-Bielsa FJ, et al. Interplay between mitochondrial oxidative disorders and proteostasis in Alzheimer's diseases. Front Neurosci, 2019.

https://doi.org/10.3389/fnins.2019.01444

30. Cassidy L, Fernandez F, Johnson JB, Naiker M, Owoola AG, Broszczak DA: Oxidative stress in Alzheimer's disease: A review on emergent natural polyphenolic therapeutics. ComplTher Med 2020, 49; 102294.
31. Halliwell B; Reactive oxygen species and the central nervous system. J Neurochem1992, 59; 1609–1623.
32. Singh A, Kukreti R, Saso L, Kukreti S. Oxidative Stress: A Key Modulator in Neurodegenerative Diseases. Molecules 2019, 24; 1583.
33. Tan FC, Hutchison ER. Eitan E. Mattson MP: Are there roles for brain cell senescence in aging and neurodegenerative disorders? Biogeront2014, 15; 643–660.
34. D. Pratic ` o: Evidence of oxidative stress in Alzheimer's disease brain and antioxidant therapy: lights and shadows. Annals of the NY AcadSci 2008, 1147; 70–78.
35. Caspersen C, Wang N, Yao J, et al., Mitochondrial Aβ: a potential focal point for neuronal metabolic dysfunction in Alzheimer's disease. The FASEB J 2005, 19; 2040–2041.
36. Shevtzova EF, Kireeva EG, Bachurin SO: Effect of β-amyloid peptide fragment 25-35 on nonselective permeability ofmitochondria. Bull Expt Bio Med. 2001, 132; 1173–1176.
37. Abramov AY, Canevari L, Duchen MR. Beta-amyloid peptides inducemitochondrial dysfunction and oxidative stress in astrocytes and death of neurons through activation of NADPH oxidase. JNeurosci 2004, 24; 565–575.
38. Hyung Kim S, Kim H: Inhibitory effect of Astaxanthin on oxidative stress-induced mitochondrial dysfunction-A mini-review Nutrients 2018, 10; 1137.
39. Geisler S, Holmstr¨om KM, Skujat D, et al., PINK1/Parkinmedziatedmitophagy is dependent on VDAC1 and p62/SQSTM1. Nature Cell Biology 2010, 12; 119–131.
40. Conte V, Uryu K, Fujimoto S, et al., Vitamin E reduces amyloidosis and improves cognitive function in Tg2576 mice following repetitive concussive brain injury. J Neurochem, 2004, 90; 758–764.
41. Yorek MA. The role of oxidative stress in diabetic vascular and neural disease. Free radical Res 2003, 37; 471–480.

42. Yi X, Maeda N: alpha-Lipoic acid prevents the increase in atherosclerosis induced by diabetes in apolipoprotein E-deficient mice fed high-fat/low-cholesterol diet. Diabetes. 2006, 55; 2238–2244.
43. Mehta K, Van Thiel DH, Shah N, Mobarhan S: Nonalcoholic fatty liver disease: pathogenesis and the role of antioxidants. Nutr Rev 2002, 60; 289–293.
44. Kelso GF, Porteous CM, Coulter CV, Hughes G, Porteous WK, Ledgerwood EC, Smith RA, Murphy MP: Selective targeting of a redox-active ubiquinone to mitochondria within cells: antioxidant and antiapoptotic properties. The J Bio Chem 2001, 276; 4588–4596.
45. Smith RA, Porteous CM, Coulter CV, Murphy MP. Selective targeting of an antioxidant to mitochondria. Eur J Biochem / FEBS 1999, 263; 709–716.
46. Evangeliou A, Vlassopoulos D: Carnitine metabolism and deficit — when supplementation is necessary? Curr Pharm Biotechnol 2003, 4; 211–219.
47. Durazzo A, Lucarini M, Nazhand A, Souto SB, Silva AM, Severino P, Souto EB, Santini A: The nutraceutical value of carnitine and its use in dietary supplements. Molecules 2020, 25; 2127.
48. Gülçin: Antioxidant and antiradical activities of L-carnitine. Life Sci 2006, 78 803–811.
49. Jing L, Zhou L, Li W, Zhang F, Yuan L, Li S, Song J, Sang Y: 2011. Carnitine regulates myocardial metabolism by Peroxisome Proliferator-Activated Receptor-alpha (PPARalpha) in alcoholic cardiomyopath. Med SciMonit 2011, 17; BR1–BR9.
50. Li JL, Wang QY, Luan HY, Kang ZC, Wang CB: Effects of L-carnitine against oxidative stress in human hepatocytes: involvement of peroxisome proliferatoractivated receptor alpha. J Biomed Sci 2012, 19, 32.
51. Augustyniak A, Skrzydlewska E: The influence of L-carnitine supplementation on the antioxidative abilities of serum and the central nervous system of ethanolinduced rats. Metab Brain Dis 2010, 25; 381–389.
52. Binienda ZK, Ali SF: Neuroprotective role of L-carnitine in the 3-nitropropionic acid induced neurotoxicity. ToxicolLett 2001, 125 (1–3), 67–73.
53. Reham Z, Hamza1, Rasha A, Al-Eisa, Nahla S, El-Shenawy: L-carnitine acts as a neuroprotecor against aspartame injury in Wistar albino rat. The J Basic Appl Zoo 2020, 81; 28
54. Rosca MG, Lemieux H, Hoppel CL: Mitochondria in the elderly: is acetylcarnitine a rejuvenator? Adv Drug Deliv Rev 2009, 61; 1332–1342.

55. Sethumadhavan S, Chinnakannu P: L-Carnitine and alpha-lipoic acid improve age-associated decline inmitochondrial respiratory chain activity of rat heart muscle. J Gerontol A BiolSci Med Sci 2006, 61; 650–659.
56. Thangasamy T, Jeyakumar P, Sittadjody S, Joyee AG, Chinnakannu P: L-Carnitine mediates protection against DNA damage in lymphocytes of aged rats. Biogerontology 2009, 10; 163–172.
57. Ueno Y, Koike M, Shimada Y, Shimura H, Hira K, Tanaka R, Uchiyama Y, Hattori N, Urabe T: L-carnitine enhances axonal plasticity and improves white-matter lesions after chronic hypoperfusion in rat brain. J Cereb Blood Flow Metab 2015,35; 382–91.
58. Ferreira GC, McKenna MC: L-Carnitine and acetyl-L-carnitine roles and neuroprotection in developing brain. Neurochem Res2017, 42; 1661–1675.
59. Virmani A, Binienda Z: Role of carnitine esters in brain neuropathology. Mol Aspects Med 2004; 25, 533–549.
60. Mroczkowska JE, Galla HJ, Nalecz MJ, Nalecz KA: Evidence for an asymmetrical uptake of L-carnitine in the blood–brain barrier in vitro. BiochemBiophys Res Commun 1997; 241; 127–131.
61. Traina G. The neurobiology of acetyl-L-carnitine. Front Biosci. (Landmark Ed) 2016, 21;1314–1329.
62. Kepka A, Ochocinska A, Borzym-Kluczyk M,Skorupa E, Stasiewicz-Jarocka B, Chojnowska S, Waszkiewicz N: Preventive role of L-Carnitine and balanced diet in Alzheimer's disease Nutrients 2020, 12; 1987.
63. Binienda ZK, Ali SF: Neuroprotective role of L-carnitine in the 3-nitropropionic acid induced neurotoxicity. ToxicolLett 2001, 125; 67–73.
64. Manfridi A, Forloni GL, Arrigoni-Martelli E, Mancia M: Culture of dorsal root ganglion neurons from aged rats: effects of acetyl-L-carnitine and NGF. Int J DevNeurosci 1992, 10; 321–329.
65. Nałecz KA, Miecz D, Berezowski V, Cecchelli R: Carnitine: transport and physiological functions in the brain. Mol Aspects Med 2004, 25; 551–567.
66. Jones LL, McDonald DA, Borum PR: Acylcarnitines: role in brain. Prog Lipid Res. 2010, 49; 61–75.
67. Zhang R, Zhang H, Zhang Z, Wang T, Niu J, Cui D, Xu S: Neuroprotective effects of pre-treatment with l-carnitine and acetyl-L-carnitine on ischemic injury in vivo and in vitro. Int J MolSci2012,13; 2078–2090.

68. Cristofano A, Sapere N, La Marca G, Angiolillo A, Vitale M, Corbi G, Scapagnini G, Intrieri M, Russo C, Corso G, et al. Serum levels of acyl-carnitines along the continuum from normal to Alzheimer's dementia. PLoS ONE 2016,11; e0155694.
69. Del Rio D, Zimetti F, Caffarra P, Tassotti M, Bernini F, Brighenti F, Zini A, Zanotti I: The gut microbial metabolite trimethylamine-N-oxide is present in human cerebrospinal fluid. Nutrients 2017, 9; 1053.
70. Abdul HM, Butterfield DA: Involvement of PI3K/PKG/ERK1/2 signaling pathways in cortical neurons to trigger protection by cotreatment of acetyl-L-carnitine and alpha-lipoic acid against HNE-mediated oxidative stress and neurotoxicity: implications for Alzheimer's disease. Free RadicBiol Med 2007, 42; 371–384.
71. Kelly GS: L-Carnitine: Therapeutic applications of a conditionally essential amino acid. AlternMed Rev 1998, 3; 345–360.
72. Zhou P, Chen Z, Zhao N, Liu D, Guo ZY, Tan L, Hu J, Wang Q, Wang JZ, Zhu LQ: Acetyl-L-carnitine attenuates homocysteine-induced Alzheimer-like histopathological and behavioral abnormalities. Rejuvenation Res 2011,14; 669–679.
73. Grodzicki W, Dziendzikowska K: The role of selected bioactive compounds in the prevention of Alzheimer's disease. Antioxidants 2020, 9; 229.
74. Onofrj M, Ciccocioppo F, Varanese S, di Muzio A, Calvani M, Chiechio S, Osio M, Thomas A: Acetyl-L-carnitine: From a biological curiosity to a drug for the peripheral nervous system and beyond. Expert Rev Neurother 2013, 13; 925–936.
75. Ames BN, Liu J:Delaying the mitochondrial decay of aging with acetylcarnitine. Ann NY AcadSci 2004, 1033: 108–116.
76. Ramli NZ, Yahaya MF, Tooyama I, Damanhuri HA: A mechanistic evaluation of antioxidant nutraceuticals on their potential against age-associated neurodegenerative Diseases. Antioxidants (Basel) 2020, 9; 1019.

ISBN: 978-81-953600-3-1

BIOREMEDIATION POTENTIAL OF NITROGEN FIXING BACTERIA

Hemlata Bhosale* and Sakshi Khode

School of Life Sciences,

Swami Ramanand Teerth Marathwada University,

Nanded, M.S., India

*Corresponding authors E-mail: bhoslehemlata@gmail.com

Abstract:

Bioremediation is one of the best remedial techniques in which living organism like plants and microbes are used to reduce, eliminate or detoxify contaminants from surrounding environment through all intensive action. The main principle of bioremediation is degradation, eradication, immobilization , detoxification or transformation of pollutants such as heavy metal, pesticide, hydrocarbon, oil, dye's that is carried out in enzymatic way through different metabolism. So it has greater contribution in solving many environmental problems. Nitrogen fixing organism play important role in bioremediation and enhance nutrient content in the soil due to having capacity of biological nitrogen fixation. Recently legume Rhizobium symbiosis play important role in heavy metal contamination. In soil legumes accumulate heavy metal mainly in roots and show low level of translocation to the shoot (Phytostabilization). Various Rhizobium species show resistance to the heavy metals and converts toxic form of metal into non toxic form. Free living Azotobacter species possess different enzymes and functional genes for degradation of pesticide. These bacteria also promote the plant growth by producing growth hormones. Cyanobacteria are able to survive in extreme environment because of unique adaptation such as the capacity to fix Nitrogen and resistant to desiccation and also play important role in removing xenobiotic compounds from environment that's why it is also used in waste water treatment. In this review the role of different nitrogen fixing organism in bioremediation and their ability to remove contaminants are discussed.

Keywords: Bioremediation, nitrogen fixing bacteria, Rhizobium, Azotobacter, cyanobacteria (BGA).

Introduction:

Worldwide pollution is increasing due to various anthropogenic activity of human leading to cause imbalance in nature. Global industrialization, excess use of fertilizers, pesticide, bio solids (sewage sludge) and waste water irrigation are the main sources of releasing toxic compounds such as heavy metals (Cr, Pb, Cd, As, Zn, Cu, Hg, Ni), organic pollutants,

hydrocarbon into the biosphere which possess greater risk to the human health, wildlife and environment. So, bioremediation is the process where hazardous waste biologically degraded under control condition. This technique uses living organism specially plant and microbes to reduce, eliminate, transform and detoxify unwanted products present in soil, water and air.

The most effective and complete degradation of the pollutants in the environment is carried out under aerobic condition. Main enzymatic reactions of aerobic biodegradation - oxidation catalyse by oxygenase and peroxidase. Under strictly anaerobic conditions, soluble carbon compounds are degraded stepwise to methane, carbon dioxide, ammonia and hydrogen sulphide [1].

There are two types of bioremediation. I) Ex situ – this technique involve excavating pollutant from polluted site and subsequently transforming them to another site for treatment. This is costly, time consuming process and depends on depth of pollution, types of pollutant and Geographical location. II) In situ- this technique involves treating polluted substances at the site of pollution. Therefore it is accompanied by little or no disturbance to the soil structure. Ideally this technique is less expensive than ex situ. Due to no extra cost required for excavation process [2].

Many microorganisms play important role in bioremediation such as species of *pseudomonas, bacillus, rhizobium, klebsiella, Azotobacter, azospirillium, clostridium, Streptomyces, enterobacter, cyanobacteria* (BGA). The first patent for a biological remediation agent was registered in 1974 that was *Peudomonas putida.* Pseudomonas strains isolated from tea rhizosphere also have the ability to biodegrade triazole propiconazole in vitro [3].

Nitrogen fixing bacteria play important role in bioremediation. These organism carried out nitrogen fixation in which atmospheric nitrogen is converted into organic compound (ammonia) and enhance the nutrient in soil which is utilize by plant for their growth. Nitrogen is essential for biosynthesis of amino acid, protein and nucleic acid. The soil rich in nitrogen has a good metabolic activity and good microbial biomass. In addition microbes able to metabolites hydrocarbons will quickly immobilize the mineral nitrogen that is available, leaving unfavourable condition to other plants. The absence of sufficient nitrogen in the soil will in turn slow the degradation process resulting from microbial metabolism therefore adding nutrient in the form of organic or inorganic fertilizers can stimulate contaminant degradation [4]. In different types of environmental conditions, types and pattern of vegetation have been shown to increase microbial degradation rate of organic residues in soil [5].

Among different bioremediation techniques, rhizoremediation is the combination of Phytoremediation and bio augmentation [6]. Phytoremediation includes three strategies: Phytoextraction (uptake and accumulation of metals from soil into the plant harvestable part),

ISBN: 978-81-953600-3-1

Phytostabilization (complexation of metals in rhizosediments decreasing it's solubility or bioavailability) and Rhizofiltration (absorption of metals by roots) [7].

So, rhizoremediation refers to the combine use of plant and rhizospheric microorganisms in order to improve the bioremediation capacity. The term rhizosphere was first introduced by Hiltner in 1904 and refers that it is interaction between plant root, soil and community of rhizospheric microorganisms associated with plant roots. The rhizosphere show highest microbial density 10^2 to 10^4fold. Plant releases variety of organic compound in the rhizosphere that serve as carbon source for many organism. The organic compound increases the metabolic activities of microbes and their population. The colonization of plant root allow organism to move deeper into the soil layer and there after increases contact of detoxifying microorganism and soil contaminant [8].

High concentrations of pesticides alter the soil ecosystem, soil-microbe interaction, plant growth and biogeochemical cycling of elements. So many species of Azotobacter play important role in degradation of pesticides and the cyanobacterial species also used for waste water treatment. Bioremediation is considered as natural, efficient, inexpensive and environmentally safe technology. it is used in the enhancement of food, seed, fertilizer production and pollution control.

Use of microorganisms in bioremediation:

Different microorganisms play important role in bioremediation and help to remove contaminants from soil by different mechanism. Those are given in following table.

Benefits of PGPR:

Plant growth promoting rhizobacteria are produce different types of secondary metabolites. Such as vitamins, amino acids, plant growth hormones, anti fungal metabolites, hydrogen cyanide and siderophores. They help to increase nutrient in soil, stimulate the plant growth, control or inhibit the activity of plant pathogens, improve the soil structure and help in solving many environmental problems.

Vitamins: *Azotobacter vinelandii*(ATCC 12837) and *Azotobacter chroococcum* (CECT 4435) strains produced B-group vitamins which include niacin (Vit B3), pantothenic acid, riboflavin, and biotin. Vitamins are essential compounds for the physiological functions of the living beings. Riboflavin Vitamin B2 required for a wide variety of cellular processes.

Amino acids: Species of *A. vinelandii* and *A. chroococcum* are known to produce aspartic acid, serine, glutamic acid, glycine, histidine, threonine, arginine, alanine, proline, cysteine, tyrosine, valine, methionine, lysine, iso-leucine, leucine, and phenylalanine.

Natural biocontroling agent: Azotobacter produces antifungal antibiotic compounds (2, 3dihydroxybenzoic acid, aminochelin, azotochelin, protochelin, and azotobactin) which inhibit the growth of plant pathogenic fungi.

Table 1: Plant growth promoting bacteria applied in bioremediation strategies [4]

Characteristics of PGPB	Microorganism	Plants associated
Nitrogen fixation		
Freely associated bacteria	*Azotobacter chroococcum*	*Brassica juncea*
Symbiotic bacteria	*Sinorhizobium meliloti*	*Medicago truncatula*
Enhanced the nodule numbers, seed yield, grain protein, root and shoot	*Bradyrhizobium sp. (vigna)*	*Greengram (Vigna radiate)*
Phosphate mobilization		
Inorganic P source	*Pseudomonas aeruginosa*	*Vigna mungo*
Organic P source	*Bacillus amyloliquefaciens*	*Zea mays*
Phosphate solublizing bacteria	*Pseudomonas putida, Azospirilium, Azotobacter*	*Artichoke (Cynara scolymus)*
Siderophore release		
Hydroxamates	*Streptomyces acidiscabies*	*Cicer arietinum*
Phenol catecholates	*Rhizobium sp.*	*Sesbania procumbens*
Carboxylates	*Pseudomonas fluorescens*	*Arachis hypogaea*
Salicylic acid	*Arthrobacter oxidans*	*Pinus sp.*
Stimulated plant growth, reduced Cd uptake	*Pseudomonas aeruginosa*	*Indian mustard and pumpkin*
Auxin production		
Indole acetic acid	*Enterobacter chloacae*	*Oryza sativa*
Gibberellin	*Bacillus pumilus*	*Alnus glutinosa*
Influence on metal toxicity		
Increased Ni accumulation	*Bacillus subtilis*	*Brassica juncea*
Increased Cd accumulation	*Xanthomonas sp.*	*Brassica napus*
Reduction of Cr(VI) to Cr(III)	*Ochrobactrum intermedium*	*Helianthus annuus*
Stimulated plant growth, facilitated soil Pb mobilization, enhanced Pb accumulation	*Bacillus edaphicus*	*Indian mustard (Brassica juncea)*

ISBN: 978-81-953600-3-1

Hydrogen Cyanide: Species of *Azotobacter*, *Alcaligenes*, *Aeromonas*, *Bacillus*, *Pseudomonas*, and *Rhizobium* capable of producing HCN a volatile, secondary metabolite that suppresses the growth of microorganisms and also influences the growth and development of plants.

Indole acetic acid production: Many symbiotic and free living bacterial species have ability to produce indole acetic acid (IAA) on the addition of tryptophan in the medium [9]. It is responsible for division, expansion and differentiation of plant cell, tissue and stimulate root elongation [7].

Gibberellic acid: Another important auxin, that is, gibberellins produced by *A. chroococcum* bacteriainclude a wide range of chemicals that are produced naturally within plant rhizosphere [10].

Siderophores: Many PGPR able to produce siderophores in iron deficient condition. It acts as iron chelating agent, drug delivery agent, anti microbial agent and use for soil remediation. Fe siderophore complex may not be available for other competing organism that's why they shows anti pathogenic activities and protect the plant form pathogenic attack [11]. A. vinelandii also have ability to bind metals other than Fe and allow the uptake of additional metals like molybdenum or vanadium that are needed in Nitrogenase [12].

Role of legume rhizobium in heavy metal bioremediation:

Impact of heavy metals:

a) Impact on soil: heavy metal exhibit toxic effect towards soil biota by affecting key microbial processes and decrease the number and activity of soil microorganisms. Ex. Cr (VI) is strong oxidising agent and highly toxic.

b) Impact on plant: some heavy metals like Fe, Mn, Cu, Zn are normally required for metabolism and growth of plant, but these elements can easily lead to poisoning when their concentration is greater than optimum value. These heavy metals inhibit some vital process of plants such as photosynthesis and mitosis, and also responsible for causing wilting, stunted growth and low yield. Uptake of heavy metal by plant and subsequent accumulation along food chain is a potential thread to animal and human health.

c) Impact on aquatic life: In aquatic system heavy metal stimulate the production of reactive oxygen species (ROS) that can damage fishes and others aquatic organisms. Methyl mercury formed in aquatic sediment through the bacterial methylation of organic mercury. It is toxic compound. Transport of metal in fish occurs through the blood where the ions are usually bound to a protein. Once a heavy metal is accumulated by aquatic organisms they can be transported to the upper class of the food chain including human.

d) Impact on human: Utilization of food crop contaminated with heavy metal is a major food chain route for human exposure. Heavy metal becomes toxic when they are not metabolising by body and accumulate in the soft tissues. Severe exposure to cadmium may cause

pulmonary edema, renal effect, osteoporosis, cancer. Lead poisoning may result in dysfunction in kidney, reproduction system; liver and brain damage also cause inhibition of haemoglobin synthesis. Mercury causes spontaneous abortion, congenital malformation, gastro intestinal disorder and neurological disorder. Arsenic coagulate protein, form complex with coenzyme and inhibit the production of ATP during respiration [13].

The legume rhizobium symbiotic interaction:

Legumes (Fabiaceae or Leguminosae) Is the third largest angiosperm family with more than 700 genera and 18000 species with wide range of habitat. Most legume have the ability to establish a symbiotic relationship with the soil nitrogen fixing rhizobacteria collectively known as rhizobia. Rhizobia currently include 13 genera with 76 species of alpha and beta proteobacteria. Ex. *Rhizobium*, *mesorhizobium*, *bradirhizobium* and *sinorhizobium*. These bacteria are collectively known as PGPR [14, 7 and 8].

Legumes excrete secondary metabolites mainly (iso) flavonoids that induce the expression of bacterial nodulation. (nod) gene involve within the production of lipochitooligosaccharide Nod factor. The Nod factor provokes several plant responses. like root hair curling and induction of cortical cell division resulting in nodule formation [8].

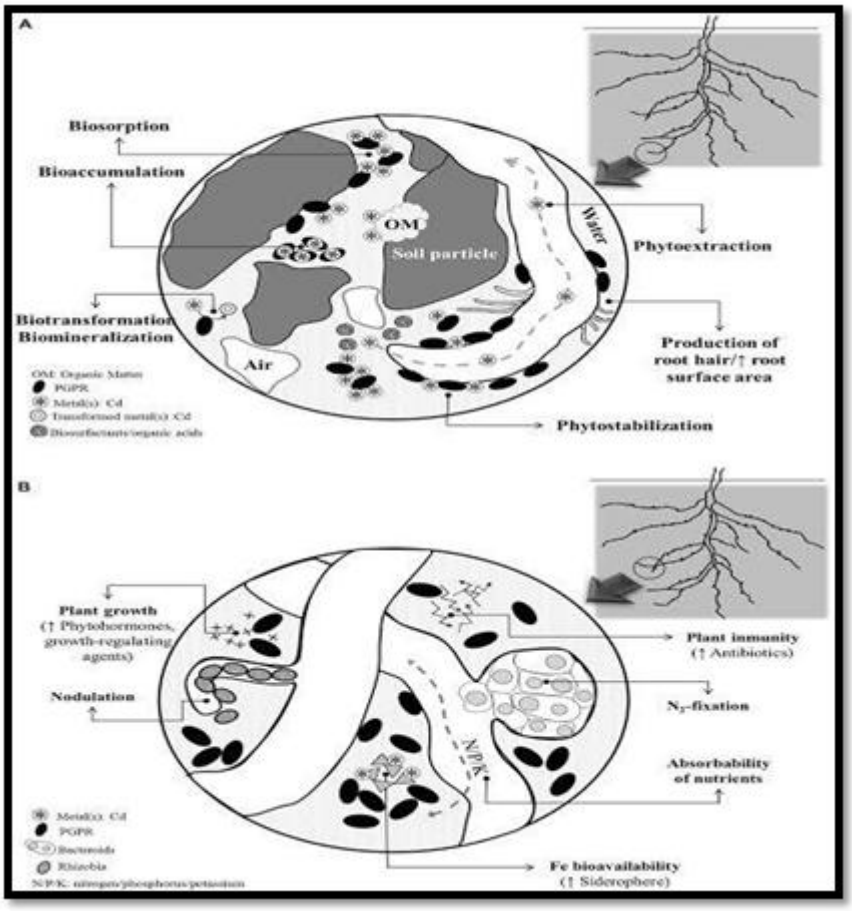


Figure 1: A) Influence of metal on bio / phyto remediation efficiency during PGPRS plant interaction (B) Benefitted from the interaction between PGPRS and nitrogen fixing legume [7].

ISBN: 978-81-953600-3-1

They are also essential for the expression of nodulin which is nodule specific plant gene. Nodules are form inside the roots of plants. Inside these nodules the symbiotic nitrogen fixation process takes place. Plant provides carbon source to the bacteria to fulfill the energy demand of the symbiotic nitrogen fixing bacteria and also micro aerophilic environment inside the nodule, which compatible with Nitrogenase complax is functioning. The enzyme nitrogenase reduce atmospheric dinitrogen to ammonia after that ammonia will be utilize by plant for their growth [15].

The microsymbiont and heavy metal uptake system:

Light metals such as Ca, Na, K, Mg, and other heavy metals like Co, Cr, Fe, Mn, Zn play important role in metabolism of microorganisms, some of the micronutrients are acting as cofactor for many enzymes and they participate in the osmoregulation process. Some of the heavy metals like Cd, Hg, Pb have no known biological function and adversely affect microbial cell through process like oxidative stress, they bind to the enzymes and other proteins and cause damage to the membrane and DNA.

Table 2: Rhizobial strains resistant to heavy metals and metalloids isolated from contaminated soils [8]

Rhizobium species	Metal (load) resistance
Azorhizobiumcaulinodans	4–5 mM Cd
Bradyrhizobium sp. RM8	5.1 mM Ni 21.4 mM Zn
Bradyrhizobium sp. STM2464	15 mM Ni
Mesorhizobium metallidurans	16–32 mM Zn 0.3–0.5 mM Cd
Mesorhizobium sp. RC1 and RC4	7.7 mM Cr
Rhizobium leguminosarumbv. viciae E20-8	2 mM Cd
Rhizobium sp. RP5	6 mM Ni 28.8 mM Zn
Rhizobium sp. VMA301	2.8 mM AsO_4^{3-}
Sinorhizobium medicae MA11	10 mM AsO2-

The microorganisms have different transport system for uptake of metal such as primary and secondary transport system. Primary transport system is inducible highly substrate specific and in which single molecule can be transported such as ABC-type or P-type ATPases. Secondary transport system which supplies the basic need for the range of metal ions and it is operating at high metal concentration. It is being constitutively express with lower specificity and it is based on chemoosmotic gradient across the plasma membrane.

Table 3: Genes for heavy-metal (and metalloid) tolerance in symbiotic rhizobia. A summary of the main genes whose function in tolerance was confirmed experimentally is reported [16]

Strain	*Host Plant*	Isolation Site	Method of Identification	Gene(s)	Metal(s) Tolera-nce
Bradhyrhizobium spp.	*Serianthes calycina*	Serpentine (New Caledonia)	PCRamplification, site-directed mutagenesis	cnr/nre systems	Co, Ni
Mesorhizobium Spp.	*Acmispon wrangelianus*	Serpentine (California)	Association mapping	Various	Ni
Mesorhizobium metalliduran	*Antyllis vulneraria*	Zinc mine (France)	Cosmid library	cadA (PIB-2-type ATPase)	Zn, Cd
Sinorhizobium meliloti 1021	*Medicago sativa*	Laboratory strain	Site-directed gene deletion	nreB (SMa1641)	Ni
Sinorhizobium meliloti 1021	*Medicago sativa*	Laboratory strain	Tn5 insertion, biochemical characterization	SMa1163 (P1B-5-ATPase)	Ni, Fe
Sinorhizobium meliloti CCNWSX0020	*Medicago lupulina*	Mine tailings (China)	Site-directed gene deletion and transcriptomics	P1B-type ATPases and others	Cu, Zn
Rhizobium leguminosarum bv. viciae UPM1137	*Pisum sativum*	Serpentine (Italy)	Transposon mutagenesis	14 loci (gene annotation corresponds to Rlv 3841 genome):	Ni, Co.

Microorganisms have evolved resistant mechanism in order to cop up with the high concentration of heavy metals and oxianions. The most common mechanism of heavy metals resistant is the extrusion of heavy metals and oxianions from bacterial cell avoiding accumulation to high level that possibly inhibit growth or cause cell death. Some of the efflux resistant systems are ATPases and chemoosmotic ion exchanger. In addition, accumulation and complexation of metal ion inside the cell, reduction of toxic metal to less toxic form, methylation, precipitation and chelation with S rich ligands like metallotioneins, glutathione etc. are other metal detoxification mechanisms adapted by microbes [8].

ISBN: 978-81-953600-3-1

Role of Azotobacter in pesticides bioremediation:

Usually Different types of pesticides are used worldwide to control the spread of plant disease and minimised the economic losses. The most common organic pesticides whichare used in agriculture are Atrazine and organophosphate. Atrazine is most widely used as herbicide in agricultural production of crops such as Maize, Sorghum and Sugar cane [1]. Near about 43.55% pesticides are used to protect cotton and 38.6% for protection of rice crop. These pesticides give better yield and reduce labour expenses by eradicating plant pathogen that would be harmful to the crop. Herbicide kills unwanted weeds, insecticide kill insect that feeds on crop, fungicide prevent numerous diseases caused by fungal pathogens[17].

Effect of pesticides:

Excess use of pesticide cause changes in soil microflora and decrease the number ofbeneficial microorganisms. It also effect on soil fertility and crop productivity also it concentration alter organic matter decomposition, soil structure, biogeochemical cycle of element. Due to its excessive use and distribution to the environment it pollute soil, water body and enter the food chain causing neurological disorder in animals, birds and human beings[18].
Different pesticides like 3,5,6 trichloro-2-pyridinol (TCP) inhibit the nitrogen mineralisation and arginine synthesis of rhizobacteria some pyrithroid group of pesticides cause reduction in phosphate solubilizing activity of phosphate solubilizing microorganisms. Due to toxic effect of herbicide fomesafen changes in phospholipid fatty acid level, growth and metabolic activities like TCA and rate of respiration in microorganism has been reported. Recently in a study conducted by Walvekar *et al.* Treatment of chlorpyriphos and cypermethrin pesticide at high dosage cause severe reduction in number of nitrogen fixers and phosphate solubilizers[19].

Some pesticides have been found to alter the plant machinery by interrupting the electron transport system of chloroplast, ATP level, effective quantum system, biomass production and reduction in antioxidant depends system through reactive oxygen species of enzymes [20 and19]. Entry of pesticides into the water body through percolation of soil, water runs off or by soil erosion all these factors affect suffocation of aquatic biota and zooplankton becauseof their toxicity. Low humidity, high temperature cause evaporation of pesticides in high amount some pesticides are applied for fumigation of soil can synthesize volatile organic compound which form a pollutant matter called tropospheric ozone by reacting with otherchemicals. Droplets of liquid pesticides spray to the field may adhere to the dust and transmitted as a dust particle. These pesticides are enter in our body through inhalation of aerosols or by consuming pesticides contaminated food and water. The severity of pesticides on human depends upon its toxicity, chemical nature and long term exposure to the pesticides. Acute effects cause respiratory tract infection and skin and eye problem, abdominal pain, vomiting and long term exposure cause neurological disorder, loss of fertility, cancer or hormone imbalance in the body. Due to this

several toxic and carcinogenic pesticides have been banned in many other countries including India [20].

Table 4: Degradation of Pesticides by Different Bacterial Species [20].

Sr. No.	*Bacteria*	Pesticides
1.	*Micrococcus*	Chloropyrifos
2.	*Flavobacterium sp.*	Glyphosate
3.	*Enterobacter strain B-14*	Chloropyrifos
4.	*Alcaligenesfaecalis*	Glyphosate
5.	*Rhodococcuserythropolis sp.*	Carbendazim
6.	*Klebsiella sp.*	Chloropyrifos
7.	*Pseudomonas sp.*	Monocrotophos
8.	*Bacillus pumillus strain C2A1*	Chloropyrifos
9.	*Lactobacillus bulgaris*	Chloropyrifos
10.	*Serratia sp.*	Chloropyrifos
11.	*Agrobacterium sp.*	Chloropyrifos
12.	*Azotobacter chroococcum*	Endosulfan
13.	*Bacillus circulans*	Pendimethalin
14.	*A. chroococcum*	Phorate

Biodegradation of pesticide:

Many microorganisms play important role in degradation of pesticides such as *Arthrobacter* spp., *Burkholderia* spp., *Bacillus* spp., *Azotobacter* spp., *Flavobacteria* spp., *Pseudomonas* spp., and *Rhodococcus* spp. are widely used in the degradation of pesticide. These bacterial genera possess enzymes and functional genes which are responsible for the degradation of such toxic pesticides.

Biodegradation of insecticide by *Azotobacter* species:

Azotobacter vinelandii is a free-living nitrogen fixing bacteria as compared to other nitrogen fixing bacteria, it's a novel feature that it possesses three separate nitrogen fixing enzymes called nitrogenase: Mo nitrogenase, V nitrogenase and Fe nitrogenase. The *Azotobacter armeniacus, Azotobacter tropicalis, Azotobacter chroococcum, Azotobacter vinelandii,* and *A. salinestris* are used for biodegradation of chlorpyrifos (1% concentration). It shows that every *Azotobacter* strains degrade echlorpyrifos compounds effectively. Among five isolates, A. tropicalis and A. salinestris showed highest biodegradation (95.2%–97.6%) of chlorpyrifos. *A. armeniacus* shows low percentage of degradation (85.1%) of chlorpyrifos. None of the isolates shows 100% degradation of chlorpyrifos. However, *A. armeniacus* and *A. salinestris* shows 95%–97% biodegradation. Over all, all the isolates

ISBN: 978-81-953600-3-1

degraded chlorpyrifos in an exceedingly range of 85%–97.6% and really minimum quantity of chlorpyrifos residues are recovered from the treatment samples[20 and 19].

Table 5: List of Bacterial Genera Encoded With Pesticide Degrading Enzyme[20].

Genes	Bacterial genera	Encoded enzymes
Opd	*P. diminuta* *Flavobacterium sp.* *F. blaustinum* *Pseudomonas sp.*	OPH OPH OPH OPH
opaA	*Alteromonas sp.* *A. haloplnaktis* *A. undina*	OPAA OPAA OPAA
hocA	*P. monteilli*	ND
Mpd	*Plesiomonas sp.*	ND
adpB	*Nocardia sp. B-1*	ADase
PdeA	*Delftia acidovorans*	Phosphor diesterase
PepA	*Escherichia coli*	AMPP
Phn	*Escherichia coli*	Phosphonatase
Glp A	*P. pseudomallei C-P*	lyase
pehA	*Burkholderia caryophilli*	PEH

***Azotobacter* species in oil bioremediation:**

Genus of *Azotobacter* use a broad range of substrate like Mannitol, various organic acid, benzoic acid, phenolic compounds of soil etc. as a source of carbon and energy and form several biological active compounds that stimulates the proliferation of rhizospheric microorganisms. *Azotobacter* species are also used in oil bioremediation. They have capacity to assimilate oil hydrocarbon both in presence of fixed nitrogen as well as during nitrogen fixation. *Azotobacter chroococcum* is found to activate proliferation of hydrocarbon oxidising bacteria existing in the microbial preparation of devoroil [21 and9].

An environment friendly bioremediation system of olive oil mill wastewater (OMWW) is studied with respect to its physicochemical characteristics and degradation efficiency. The method exploits the biochemical versatility of the dinitrogen fixing bacterium *Azotobacter vinelandii* (strain A) to grow in OMWW at the expense of its constituents and to transform it into an organic liquid fertilizer. The system eliminates the phytotoxic compounds from OMWW and enriches it with an agriculturally beneficial microbial load along with useful metabolites. The end product, branded “biofertilizer”, is used as soil conditioner and liquid organic fertilizer. Growth of A. vinelandii in OMWW results in the decline of content of most of the compounds associated with phytotoxicity, and this is confirmed by the assessment of degradation yields. In

parallel, during the process several other compounds noncommittally undergo degradation and biotransformation. More specifically, the biofertilization system is capable of achieving removal yields as high as 90 and 96% after 3 and 7 days respectively [4].

Role of cyanobacteria in waste water bioremediation:

Waste water is a global source of pollution, domestic waste water, agricultural, industrial effluent are directly discarded into open water system (river). Domestic waste water contain paper, urine, faces and synthetic detergents. Industrial effluent contains both organic and inorganic pollutants. Agriculture influent has animal waste, fertilizers and pesticides.

Subtended organic particles inhibit light penetration deep into the water and inhibit photosynthesis as well. They form bottom sediment which inhibits microbions and they create favourable environment for pathogenic organisms. Fat, oils, lubricants found spill on water surface which inhibit the gas exchange between water and atmosphere and lower oxygen content in water. Many of the organic substances like aliphatic carbohydrates and especially polycyclic aromatic hydrates possess transforming ability and able to cause cancer malformation or mutation. They can inhibit the growth, accelerated aging, disturb oncogenesis and change genome of organisms.

Cyanobacteria is one of the oldest photosynthetic prokaryotes on the Earth [23]. They are also known as blue green algae (BGA) and are considered as primary photosynthetic organisms found in fresh water, oceans, soil and bare rock. They are major nitrogen fixer in fresh water and marine sediments [22 and 24]. Cyanobacteria are microscopic in nature. They can be visible only when they are exists in the form of blooms [25]. Cyanobacterial mats are used as biofertilizer in modern agriculture. They can grow at faster rate and also have higher yield potential per acre relative to traditional food crops. Cyanobacteria produce variety of bioactive compounds like toxins also known as cyanotoxins. These cyanotoxins are used in drug development for certain cancer therapies [26]. Some cyanobacteria are used for biofuel production. Ex. *Synechococcus elongatus* [27] Cyanobacteria are considered as food and nutritional supplements which contains complex sugars, amino acids, proteins, vitamins and carbohydrates, minerals, fats, active enzymes Ex.Spirulina [25].

Recently, there has been increasing awareness about using cyanobacteria as bioremediation and pollution control agent. Cyanobacteria have great potential to take up external nutrients such as ammonium, nitrate, orthophosphate and heavy metals that's why it could be good candidate for treatment of urban, agricultural, industrial effluent and also helps in solving eutrophication and metal toxicity problem in aquatic ecosystem [28].

Cyanobacteria play an important role by supplying molecular oxygen to heterotrophic partner and thus support the initial step of degradation. Cyanobacteria able to remove xenobiotic from environments by sorption, transformation and degradation (4). Cyanobacteria have big

ISBN: 978-81-953600-3-1

advantages over other life forms, which allowed them to survive in different conditions. Their biochemical processes and specific adaptations of some representatives such as the desiccation resistance and the ability to fix nitrogen make them very flexible. At the same time cyanobacteria have the ability to switch easily to mixotrophic nutrition depending on the composition of the available nutrient medium. Cyanobacteria are able to metabolite various organic substrate or organic pollutants which they use as nutrients and breaking them down as non toxic or less toxic substances [29].

Table 6: Cyanobacterial species that can have the potential for bioremediation of numerous contaminants [25]

Potential Bio-Remediators	Contaminants
Cyanobacteria	Oxidize oil constituents and complexed organic compounds, i.e., herbicides and surfactants
Anabaena sp., Nostoc sp., Lyngya sp., Spirulina sp. and Microcystis sp.	Helpful in removing the contamination of glyphosate herbicide from agricultural sites
Cyanobacteria and chemotrophic Bacteria consortium	Wastewaters and oil-contaminated sites
Synechococcus elongatus, Anacystics nidulans, and Microcystis aeruginosa	Removal of insecticides contaminants, i.e., organo-chlorine and organo-phosphorus
Oscillatoria-Gammaproteo bacteria consortium	Phenanthrene, dibenzothiophene, and n-octadecane
Microcoleus chthonoplastes and organotrophic bacteria consortium	Nitrogen fixation, and degradation of aliphatic heterocyclic organo-sulfur compounds and hydrocarbons, i.e., alkylated monocyclic and polycyclic aromatic compounds
Consortium of Anabaena oryzae and Chlorella kessleri	Biodegrade crude oil under mixotrophic condition
Consortia of cyanobacteria and bacteria (Aphanocapsa sp. BDU 16, Oscillatoria sp. BDU 30501 and Halobacterium US 101)	Wastewater treatment and help to reduce calcium and chloride content in water bodies for fish survival
Oscillatoriaboryana BDU 92181	Removal of melanoidin from distillery effluents
Arthrospira genomes (Arthrospira maxima CS-328, Arthrospira sp. PCC8005, A. platensis NIES-39, A. platensis Paraca P0)	Tolerance to various environmental conditions such as high temperature, alkalinity, and salinization

Cyanobacteria having ability to adsorb and transport the microelements by the activation of membrane transporter system involving metal permease, metal oxidase and metal reductase is

essential in this case. These evolutionary emerging mechanisms for vital needs of cyanobacteria ensure in fact an active bioaccumulation and removing of metals from polluted environments. Heavy metal ions, especially those with multiple valences, are highly toxic to living cells including cyanobacteria, but could be converted into less toxic forms by biosorption and bioaccumulation. One of the mechanisms used by Cyanobacteria for this purpose is the formation of nanoparticles through reduction of metal ions. Thus, the accumulation of heavy metals by cyanobacteria from the polluted water, in addition to the positive environmental impact, may provide an opportunity to obtain metal nanoparticles both on cell surface and inside the cells. Another mechanism used in cyanobacteria protection strategies for reducing the toxicity of metal ions is their chelation and inclusion in the composition of certain organic structures, such as amino acids, oligopeptides, proteins, oligo- and polysaccharides.

Cyanobacteria can degrade different types of environmental pollutants including pesticides, crude oils, napthalene, phenphthalene, catachol, phenol and xenobiotics [30]. Wastewater contains high concentration of biogenic element which enhances the productivity of cyanobacteria. These compounds are utilised by cyanobacteria for their cultivation [31]. Cyanobacteria cultivation on media with the addition of wastewater carried out two important processes that are low cost accumulation of the biomass in large quantities and their purification. With the increasing amount of cyanobacterial biomass the metal concentration, the amount of ammonium ion, mineral phosphorous in water decreases [32]. The effectiveness of purification by using cyanobacteria can reach 90-97% [33].

Municipal as well as livestock wastewater efficient treatment can be done by using the species of the genera *Spirulina, Phormidium, Anabaena, Oscillatoria*. For example, the cyanobacteria *Phormidium bohneri, Phormidium tenue, Spirulina platensis, Oscillatoria brevis, Anabaena variabilis*etc can be used in purification of wastewater of different origin. Spirulina biomass can completely remove nitrogen from wastewaters; *Phormidium tenue* contributes water color reduction up to 60–90 %. In some cases, it has been foundthat high efficiency of waste water treatment is carried out by species of cyanophyta compared to the inoculation of Chlorophyta species. Also, it has been shown that the cyanophytes play a dominant role in the decontamination of oil substances from waters and contribute to the degradation of hydrocarbons.

Locally available eubacterium like *Oscillatoria limosa* and *Nostoc commune* have ability to remove variety of nutrientsfrom wastewater in order to enhance water quality. The phycoremediation experimentswere conducted at Department of Environmental Sciences, University of Pune, India using randomized complete block design with three replications of every treatment. The results of present investigation clearly indicated that the algal species, viz., *Oscillatoria limosa* and *Nostoc commune* are highly efficient for removal of NO_3^{-2}, PO_4^{-2},

ISBN: 978-81-953600-3-1

SO_4^{-2}, Cl^- and for reducing EC values. The common reduction was between 84 to 98%. The pollutant removal efficiency was increased with decreasing concentration of wastewater. Amongst the chosen algae *Oscillatoria limosa* was the simplest candidate as compare to *Nostoc commune*. It absolutely was concluded that the cyanophycea members would be the most effective options for phycoremediation [34].

Genetically engineered microorganisms in bioremediation:

Genetic material of organism is altered by using genetic engineering, also called as recombinant DNA technology.Such microorganisms are known as genetically modified organism (GMO) or genetically engineered microorganisms (GEM's). In this technique the desired gene from one organism introduced into the genome of another organism in order to enhance their potential i.e. insert the appropriate gene for production of particular enzyme which can degrade various pollutant. Recombinant living organisms able to obtain by recombinant DNA technology or by naturally exchanging genetic materials between two organisms. Genetically engineered microorganisms are effective in soil, activated sludge and ground water bioremediation. There are four principles are being constructed in development of GEM for bioremediation.

1) Modification of enzyme specificity and affinity.
2) Pathway construction and regulation.
3) Bioprocess development, monitoring and controls.
4) Bioaffinity, bioreporter sensor applications for chemical sensing, toxicity reduction and end point analysis [35].

A multi plasmid containing pseudomonas strains are successfully produced by using genetic engineering that can oxidize polyaromatic hydrocarbon, terpenic aromatic and alliphatic hydrocarbon.Ex. Pseudomonas aeruginosa (NRRLB-5472) and Pseudomonas putida (NRRLB-5473) were first patented by USA as genetically modified microorganisms [36].

Genetically engineered microorganisms are also play important role in the degradation of heavy metals. For example, chromium degradation from industrial wastewater was carried out by *Alcaligenes eutrophus* AE104 (pEBZ141) and *Rhodopseudomonas palustris* is used for expression of the Hg transport system and metallothionein for Hg2+ degradation from heavy metal and wastewater[37].

Genetically modified strain of *Mesorhizobium huakuii subsp. rengei* B3 by the expression of a gene which encode metal binding protein, synthetic tetrameric metallothionein (MTL4) on the surface under control of bacterial specific promotor (pnifH and pnolB). Inoculation of *Astragalus sinicus* plant with this strain results in increase Cd accumulation inside the nodule by (1.7 fold). The accumulation of Cd increases not only in nodules but also in roots *of A. Sinicus* by (3 fold) after infected with recombinant rhizobia [38].

For heavy metal *Arabidopsis thaliana* gene was introduced into *M. huakuii subsp. Rengai* strain B3 for phytochelatin synthase (pcs. pcsAt) increase Cd accumulation inside the nodule up to 1.5 fold as compared to non - modified rhizobial partner[39].

Conclusion:

Bioremediation is very fruitful and attractive technique for solving many environmental pollution problems through different microbial activities. In situ bioremediation is a simple, cost effective, less labour intensive and eco-friendly process. It is a natural and take a little time with minimum health hazards as compare to other physiochemical dependent strategies which are less eco-friendly and dangerous to life. But on the other hand this bioremediation is limited to those compounds which are Biodegradable. It is very highly specific because it requires suitable environmental conditions, appropriate level of nutrients and contaminants for microbial growth.

Many novel species of microbes have ability to degrade contaminants from environment. Especially heavy metal resistant rhizobia can increase legume heavy metal tolerance and promote legume growth in metal rich soil which results in great removal of heavy metal contaminants from soil. Different *Azotobacter* species having specific genes for production of enzymes are involved in degradation of contaminants. Along with bioremediation *Azotobacter* sp. also show ability to produce variety of vitamins like riboflavin and biotin, plant growth promoting hormones like gibberellin, indole acetic acid (IAA), Iron chelating agents like siderophores and improve soil fertility by adding nutrients to the soil through nitrogen fixation and phosphate solubilisation. Because of these abilities of *Azotobacter*, they are also used as good biofertilizer.

Cyanobacteria have ability to degrade different xenobiotic compounds from soil and water. Cyanobacteria can improve the water quality and remove excess contaminants effectively at minimum cost. Along with bioremediation, cyanobacteria are also used for biofuel production and as a food supplement. Nowadays, by using genetic engineering techniques, the potential of different microorganisms to tolerate contaminants are increased. The education, awareness and biotechnological research in this area may help to increase crop production and decrease environmental contaminants which give healthier environment.

References:

1. Das M., Adholeya A. (2012) Role of Microorganisms in Remediation of Contaminated Soil. In: Satyanarayana T., Johri B., Anil Prakash (eds) Microorganisms in Environmental Management. Springer, Dordrecht. https://doi.org/10.1007/978-94-007-2229-3_4

2. Azubuike CC, Chikere CB, Okpokwasili GC. Bioremediation techniques-classification based on site of application: principles, advantages, limitations and prospects. World J MicrobiolBiotechnol. 2016 Nov;32(11):180. doi: 10.1007/s11274-016-2137-x. Epub 2016 Sep 16. PMID: 27638318; PMCID: PMC5026719.
3. Sarkar, S.,SubbiahSeenivasan,PremkumarRobert. (2018). Biodegradation of propiconazole by Pseudomonas putida isolated from tea rhizosphere. Plant, Soil and Environment. 55. 196-201. 10.17221/2184-PSE.
4. Santra Subhas, Mallick A., Samal Alok. (2015). Biofertilizer for Bioremediation.
5. Anderson, J.M., Ingram, John. (1989). Tropical Soil Biology and Fertility: A Hand Book of Methods. XF2006286592.
6. Ali Hazrat, KhanEzzat, Anwar Sajad Muhammad. (2013). Phytoremediation of heavy metals—Concepts and applications. Chemosphere. 91. 10.1016/j.chemosphere.2013.01.075.
7. Gómez-Sagasti MT, Marino D. PGPRs and nitrogen-fixing legumes: a perfect team for efficient Cd phytoremediation? Front Plant Sci. 2015 Feb 25;6:81. doi: 10.3389/fpls.2015.00081. PMID: 25763004; PMCID: PMC4340175.
8. Pajuelo, E. et al. 2011. Legume–Rhizobium Symbioses as a Tool for Bioremediation of Heavy Metal Polluted Soils. Biomanagement of metal-contaminated soils. Environmental Pollution 20: 95-123.
9. Sumbul A, Ansari RA, Rizvi R, Mahmood I. *Azotobacter*: A potential bio-fertilizer for soil and plant health management. Saudi J Biol Sci. 2020 Dec;27(12):3634-3640. doi: 10.1016/j.sjbs.2020.08.004. Epub 2020 Aug 8. PMID: 33304174; PMCID: PMC7714982.
10. Brown M.E., Jackson R.M., Burlingham S.K. Growth and effects of bacteria introduced into soil. Ecol. Soil Bact**.** 1968:531–551.
11. Hayat Rifat, Ali Safdar, Amara Ummay, Khalid Rabia, Ahmed Iftikhar. (2010). Soil beneficial bacteria and their role in plant growth promotion: A Review. Annals of Microbiology. 60. 579-598. 10.1007/s13213-010-0117-1.
12. Eady RR. The vanadium-containing nitrogenase of *Azotobacter*. Biofactors. 1988 Jul;1(2):111-6. PMID: 3076437.
13. Dr. Jiwan Singh, Kalamdhad Ajay. (2011). Effects of Heavy Metals on Soil, Plants, Human Health and Aquatic Life. International Journal of Research in Chemistry and Environment. 1. 15-21.
14. Schmid Rudolf, Lewis Gwilym, Schrire Brian, MackinderBarbara, Lock Mike. (2006). Legumes of the World. Taxon. 55. 251. 10.2307/25065563.
15. Oldroyd Giles. (2013). Speak, friend, and enter: Signaling systems that promote beneficial symbiotic associations in plants. Nature reviews. Microbiology. 11. 252-263. 10.1038/nrmicro2990.

16. Fagorzi C, Checcucci A, diCenzo GC, Debiec-Andrzejewska K, Dziewit L, Pini F, Mengoni A. Harnessing Rhizobia to Improve Heavy-Metal Phytoremediation by Legumes. Genes (Basel). 2018 Nov 8;9(11):542. doi: 10.3390/genes9110542. PMID: 30413093; PMCID: PMC6266702.
17. Dr. Chennappa Gurikar,, Nidoni Udaykumar, Vidya M., Nagaraja H., Amaresh Y.S., Sreenivasa M.. (2019). *Azotobacter*—A Natural Resource for Bioremediation of Toxic Pesticides in Soil Ecosystems. 10.1016/B978-0-444-64191-5.00019-5.
18. Dallimer Martin, Irvine Katherine, Skinner Andrew, Davies Zoe, Rouquette, James, Maltby, Lorraine, Warren, Philip, Armsworth, Paul, Gaston, Kevin. (2012). Biodiversity and the Feel-Good Factor: Understanding Associations between Self-Reported Human Well-Being and Species Richness. BioScience. 62. 47-55. 10.1525/bio.2012.62.1.9.
19. Mohammad Shahida, Almas Zaidia, Aquib Ehtramb, Mohammad Saghir Khana (2019). In vitro investigation to explore the toxicity of different groups of pesticides for an agronomically important rhizosphere isolate *Azotobacter* vinelandii.
20. Fang, Y., Lu, H., Chen, S., Zhu, K., Song, H., Qian, H., 2015. Leaf proteome analysis provides insights into the molecular mech anisms of bentazon detoxification in rice.Pest Biochem. Physiol. 125, 45–52.Gasser, V., Baco, E., Cunrath, O., August, P.S., Perraud, Q., Zill, N., Schleberger, C., 2016.
21. Gradova NB, Gornova IB, Eddaudi R, Salina RN. Ispol'zovaniebakteriĭroda *Azotobacter* pribioremediatsiineftezagriaznennykhpochv [Use of bacteria of the genus *Azotobacter* for biodegradation of oil-contaminated soils]. PriklBiokhimMikrobiol. 2003 May-Jun;39(3):318-21. Russian. PMID: 12754830.
22. BagaliShridhar. (2012). Review: nitrogen fixing microorganisms. Int. J. Microbiol. Res.. 3. 46-52. 10.5829/idosi.ijmr.2012.3.1.61103.
23. Rasmussen, B.; Fletcher, I.R.; Brocks, J.J.; Kilburn, M.R. Reassessing the first appearance of eukaryotes andcyanobacteria. Nature 2008, 455, 1101–1104.
24. Whitton, B.A.; Potts, M. Introduction to the cyanobacteria. In Ecology of Cyanobacteria II; Springer: Dordrecht, The Netherlands, 2012; pp. 1–13.
25. Zahra,, Choo,, Lee, Kunwoo,Parveen,. (2020). Cyanobacteria: Review of Current Potentials and Applications. Environments. 7. 13. 10.3390/environments7020013.
26. Zanchett, G.; Oliveira-Filho, E.C. Cyanobacteria and cyanotoxins: From impacts on aquatic ecosystems and human health to anticarcinogenic effects. Toxins 2013, 5, 1896–1917.
27. Farrokh, P.; Sheikhpour, M.; Kasaeian, A.; Asadi, H.; Bavandi, R. Cyanobacteria as an eco-friendly resourcefor biofuel production: A critical review. Biotechnol. Prog. 2019, 35, e2835.

28. SubramaniyanVijayakumar, (2012). Potential Applications of Cyanobacteria in Industrial Effluents-A Review. Journal of Bioremediation and Biodegradation. 03. 10.4172/2155-6199.1000154.
29. Zinicovscaia**,** Inga**,** Cepoi, Liliana (Eds.)2016. Cyanobacteria for Bioremediation of Wastewaters.
30. Ebtesam A. El-Bestawy, Ali Z. Abd El-Salam, Abd El-Rahman H. Mansy,Potential use of environmental cyanobacterial species in bioremediation of lindane-contaminated effluents,InternationalBiodeterioration,Biodegradation,Volume 59, Issue 3,2007,Pages 180-192,ISSN 0964-8305, https://doi.org/10.1016/j.ibiod.2006.12.005.
31. Larsdotter K (2006) Wastewater treatment with microalgae – a literature review. Vattan 62:31–38.
32. Basso M, Cukierman A (2008) Biosorption performance of red and green marine macroalgae for removal of trace cadmium and nickel from wastewater. Int J Environ Pollut 34:340–352
33. Gonzalez C, Marciniak J, Villaverde S, Garcia–Encina P, Muñoz R (2008) Microalgae – based processes for the biodegradation of pretreated piggery wastewaters. ApplMicrobiolBiotechnol 80:891–898
34. Azarpira, Hossein. (2019). Potential use of cyanobacteria species in phycoremediation of municipal wastewater.
35. Endeshaw Abatenh, Birhanu Gizaw, Zerihun Tsegaye, Misganaw Wassie (2017). Application of microorganisms in bioremediation-review. Journal of Environmental Microbiology Vol.1 No.1 December 2017.
36. Chakrabarty, A.M., 1986. Genetic engineering and problems of environmental pollution. BioTechnology 8, 515–530.
37. Verma Samakshi, Kuila Arindam. (2019). Bioremediation of heavy metals by microbial process. Environmental Technology, Innovation. 14. 10.1016/j.eti.2019.100369.
38. Sriprang R, Hayashi M, Yamashita M, Ono H, Saeki K, Murooka Y (2002) A novel bioremediationsystem for heavy metals using the symbiosis between leguminous plant and genetically engineered rhizobia. J Biotechnol 99:279–293
39. Sriprang R, Hayashi M, Ono H, Takagi M, Hirata K, Murooka Y (2003) Enhanced accumulation of Cd2+ by Mesorhizobium transformed with a gene for phytochelatin synthase from Arabidopsis. Appl Environ Microbiol 69:1791–1796.

ACID TOLERANCE MARKER OF THE PROBIOTIC BACTERIA AT MOLECULAR LEVEL – A REVIEW

Deeksha Sharma*, Suman Kapila and Rajeev Kapila

ICAR- National Dairy Research Institute,

Karnal-132001 (Haryana) India

*Corresponding authors E-mail: amaraiberis@gmail.com

Abstract:

The gastrointestinal microbiota is a multifaceted ecosystem that harbors trillions of microbes. The interaction between the gut microbiota and the host promotes the cooperation and functional stability of the gut ecosystem. Moreover, an adequate addition of the beneficial microorganisms called probiotics can help in the maintenance of a healthy gut microbiota milieu. Among these microorganisms, *Lactobacillus* and *Bifidobacterium* species are commonly recognized as standard probiotics. In general, lactobacilli are found in an extensive range of ecological niches including the human gastrointestinal (GI) tract. Recently, the important criteria for the microbial culture to be used as a probiotic have been established which includes resisting the hostile acidic conditions of stomach and bile toxicity of the duodenum to express their health-promoting functions in the gut. Besides, the probiotic should also compete with other intestinal microorganisms for adhesion to mucus and epithelial cells which they do by secreting some specific adhesion proteins like mucus binding protein and mucus adhesion-promoting protein. In general, these Probiotics attributes have been shown to evaluate at the phenotypic level by checking, acid and bile tolerance and adhesion ability of the probiotic bacteria. However, very little is known about the molecular marker responsible for probiotic characteristics and their role in the survival & colonization of the gut. In the present review paper, we are specifically discussing the molecular marker responsible for the acid tolerance of probiotic bacteria.

Keywords: Probiotics, Lactobacilli, Probiotic attributes, acid tolerance, AtpD,

Introduction:

The mutual co-evolution among mammals and their gut microbes has lasted for hundreds of millions of years (Ley *et al.,* 2008). Scientific labors for approving the interaction between humans and human-associated microbial communities have made a great evolution in the

ISBN: 978-81-953600-3-1

achievements of the Human Microbiome Project (Human Microbiome Project Consortium, 2012) and Meta Hit Project (Qin *et al.,* 2010). We now have an improved understanding of an intricate ecosystem residing within our intestinal tract (Qin *et al.,* 2010). The human gut microbiota which is unruffled of approximately 10 to 100 trillion microorganisms is expected to outnumber human body cells by a factor of ten (Bäckhed *et al.,* 2005). These microbes play an imperative role in the physiology of their host, including the digestion and assimilation of nutrients, protection against pathogen colonization, modulation of immune responses, regulation of fat storage, and stimulation of intestinal angiogenesis (Gill *et al.,* 2006; Qin *et al.,* 2010; Yatsunenko *et al.,* 2012; Cabreiro *et al.,* 2013). A probiotic bacterium, i.e., "a live microorganism that, when administered in adequate amounts, confers a health benefit on the host". Probiotic bacteria must show some recommended properties such as specific health benefits, survival, and persistence in the host, proven safety, and stability. After ingestion probiotic bacteria start their journey in the gastrointestinal tract and come across various harsh environmental conditions of the host's gut such as exposure to the acidic pH of the stomach and gastric juice of the intestine, (Guchte *et al.,* 2002). Probiotic bacteria must endure this exposure and reach the lower intestine for colonization. Various strains of Lactobacilli from time to time have proven their ability to survive variable pH and bile conditions present in the gut and their ability to colonize the intestinal tract of humans and other mammals. These probiotic attributes can be checked phenotypically by the plating method. However, recent advances in genome sequencing projects, molecular tools, and genomic-based strategies have made it accessible for the identification of probiotic-associated factors at the gene expression level. Through genetic analysis of various well-known strains of probiotic lactobacilli, some molecular markers/genes responsible for the probiotic attributes have been discovered. Various studies showed the differential expression of various probiotic factors such as AtpD (F0-F1ATPase) responsible for acid tolerance, Bsh (bile salt hydrolase) involve in the bile tolerance mechanism, and cell surface proteins of bacteria involve in the adhesion of bacteria encoded by Mub and MapA genes. In the present review, we are specifically discussing the molecular markers/factors of probiotics responsible for acid tolerance of probiotics at the molecular level.

Probiotics their mechanism of action and health benefits:

Historically, lactic acid bacteria were used to improve the storage quality, flavor of consumable foods, and nutritive value, without knowing the existence of microorganisms. Noble Laureate Elie Metchnikoff (Metchnikoff, 1908), first recognized the beneficial effect of lactic

acid bacteria of yogurt and hypothesized that these bacteria played a crucial role in prolonging human life and later he wrote the book, the prolongation of life (Morelli, 2000). The word 'probiotics' was coined in the 1960s on the basis of substances produced by microorganisms, which promoted the growth of useful/helpful microorganisms (Lilley and Stillwell, 1965). The mode of action by which a probiotic demolishes a pathogen can be summarized as competitive exclusion, enhancement of epithelial barrier function by blocking adhesion site of pathogens, immunomodulation, and direct antagonism action by the production of antimicrobial components and forming biofilms, and interference with quorum sensing signaling (Schiffrin, 2002; Yan and Polk, 2006; Anderson, 2010; Vuotto *et al.,* 2014; Woo and Ahn, 2013).

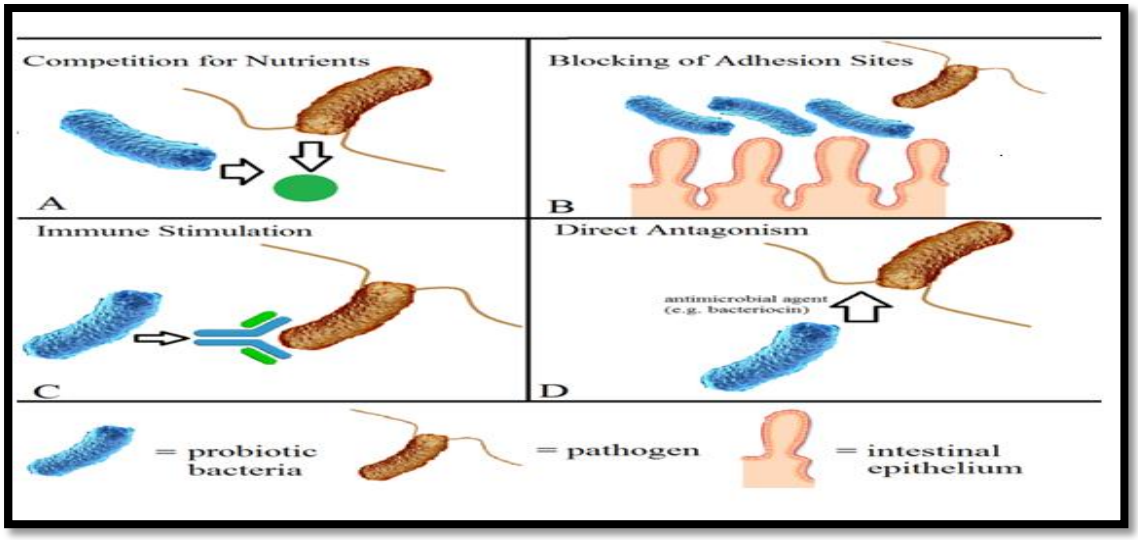


Figure 1: Mechanism of action of Probiotics (Brito *et al.*, 2012)

Several health benefits are put forth by probiotic products including antimicrobial activity and treatment of gastrointestinal infections, acute diarrhea, antibiotic-associated diarrhea, rotaviral diarrhea, improvement in inflammatory bowel diseases, anti-Helicobacter pylori activity, lactose intolerance, Improvement of tight junction, immune system modulation anti-mutagenic properties anti-carcinogenic properties, reduction in serum cholesterol, improve lipid profile, and also improved the sperm quality (Yan & Polk, 2006; Wenus, 2008; Baffoni *et al.*, 2012; Al- Muzafar & Amin, 2016; Valcarce *et al.*,2017).

Selection criteria of probiotic bacteria:

The selection of suitable probiotic candidates is the principal basis for improving the bio-therapeutic action and functional properties of probiotic foods and pharmaceutical products. For the selection of probiotic bacterial strain several attributes are already given (Begley, 2005 and

ISBN: 978-81-953600-3-1

Lin, 2006). The essential characteristics for Lactobacillus to be used as probiotics include the following-

1. Recognition as safe (GRAS; generally recognized as safe)
2. Viability during processing and storage
3. Antagonistic effect against pathogens
4. Tolerance to acid and bile challenge
5. Adherence to the intestinal epithelium of the host.

In the present work, we are discussing the important characteristic of probiotic bacteria that is acid and bile tolerance ability. In general, microorganisms are taken along the feed and their journey in the host begins with the mouth to the lower intestine. During this journey they exposed to the various harsh conditions of the gut and only a few microorganisms are survived. However, all probiotic bacteria must survive through this journey to impart their beneficial effect to the host.

Journey of the microorganism through the human gut:

Microorganisms taken with food commence their passage from mouth to the lower intestinal tract and during this passage, they are exposed to harsh conditions such as acidity in the stomach, bile in the small intestine that influence their survival (Simon and Gorbach, 1987; Marteau, 1993). Therefore, a probiotic strain should have the ability to resist these stressful conditions of the stomach and intestine and consequently colonize the gastrointestinal tract. So it can exert its beneficial effect. The time reported for ingested food from the entrance to release from the stomach is about 90 minutes but further digestive processes have longer residence times. During the microorganism journey in the gastrointestinal tract first cellular stress begins in the stomach, which has a pH as low as 1.5. Lankaputhra and Shah's (1995) experiments showed that lactobacilli growth was significantly decreased below pH 4.5, so acidity is the most critical factor affecting their growth and viability. Other than stomach acidity, probiotic bacteria must show bile tolerance ability in the small intestine and finally adhere to the small intestine (Gilliland 1987 and 1984). Therefore, the first step in the selection of a strain of probiotic is that the ability to survive in the gut and reached alive at its site of action. This is well supported by a previous study which showed that some lactic acid bacteria can exert beneficial effects, such as immune stimulation within the intestinal tract with better survivability in the acidic conditions and temporary colonization on increasing the residence time in the human gastrointestinal tract (Johansson, 1998).

Acid tolerance ability of probiotic bacteria:

After the complete genome sequencing of various recognized probiotic lactobacilli and bifidobacterial strains and their accessibility in the public domain, the location of various genes particularly those involved in probiotic attributes of acid tolerance have been identified on their genomes. The ability of microorganisms to grow at acidic pH is also linked to opposing harsh conditions in the GI tract as the gastric pH is less than 2.0 in healthy humans. Chou and Weimer (1999) isolated L. acidophilus strains based on acid and bile tolerance which were grown in MRS broth at pH 3.5 containing 0.2% mixed bile salts. In another study, L. acidophilus was reported to survive even after 5 hours of exposure to pH 3.0 (Azcarate-peril, 2004). The precedent study discovered the enzyme implicated in acid stress in lactobacilli such as ATPase i.e., F1F0 ATPase (Kullen and Klaenhammer, 1999).

F1F0 ATPase in the acid tolerance of probiotic bacteria:

The F1F0-ATPase is ubiquitous among bacteria and its molecular architecture and operation have been unraveled (Trejo, 2008: Cao, 2000; Sievers *et al.*, 2003; Corcoran *et al.*, 2008) For several microorganisms that inhabit the gastrointestinal tract, the F1F0-ATPase is an important element in response and tolerance to low pH. This enzyme plays an important role in those microorganisms which have a respiratory chain for the synthesis of ATP by using a proton gradient. In all these bacteria, the activity of the F1F0-ATPase increases as the pH of the growth media decreases. In LAB, the proton expulsion activity is enhanced at low pH and is crucial to maintain the intracellular pH (Nannen *et al.*, 1991). Sun (2012) also observed the role of F1F0-ATPase in the survival of E.coli under acidic conditions. Bacterial ATP synthases have two domains, the F1 and F0 domains, as indicated in Fig.2

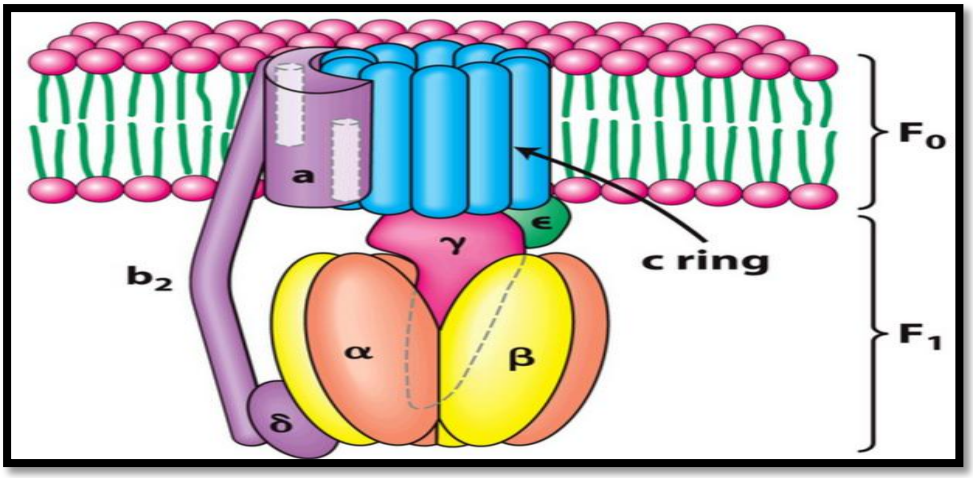


Figure 2: Structure of ATP synthases (Joshua, 2016)

ISBN: 978-81-953600-3-1

The cytoplasmically located, soluble F1 domain contains three catalytic α and β subunit pairs and single γ, δ, and ε subunits (Capaldi and Aggeler, 2002; Nakamoto *et al.,* 2008). The membrane-embedded F0 domain is composed of a single a-subunit, two b-subunits, and multiple c-subunits. The b and δ subunits comprise the stator for the rotary machine. The two b-subunits and the δ-subunit form a peripheral stalk. This stalk connects the two domains of the complex, spanning the ion-translocating a- and c-subunits of the F0 and the catalytic domain of the F1 that contains the three α- and β-subunit pairs (Rizzo *et al.,* 2002 and Clagget *et al.,* 2009). The rotor element found in the F0 is a homo-oligomer of hairpin-like c-subunits in most ATP synthases. The cytoplasmic loops that are exposed on the N-side of the membrane (the electrically negative side) interact with the γε sub-complex which forms the central stalk, with the γ-subunit extending asymmetrically into the F1 catalytic domain.

Bacterial ATP operon and molecular marker AtpD gene in the acid tolerance of probiotic bacteria:

F1F0- ATPase enzyme involved in the acid tolerance of bacteria has been regulated by ATP operon at the transcriptional level (Smith *et al.,* 1996; Kullen *et al.,* 1999; Wang *et al.,* 2018) Several studies showed the number of other ATP sequences as a result of LAB genome sequence projects (Koebmann *et al.,*2000). Previously, Das and Ljungdahl (1997) sequenced the ATP operon encoding F1F0 ATP synthase in the fermentative obligate anaerobic bacterium Clostridium pasteurianum consisted of nine genes that were found identical to other bacteria. Bacterial ATP synthases are most often encoded in a single operon namely ATP that have the eight structural genes in the order B, E, F, H, A, G, D, and C, respectively encoding the a, c, b, δ, α, γ, β and ε subunits. However, in the case of Streptococcus mutants and Streptococcus sanguis bacterial ATP operons, genes are ordered in a different manner e.g. ATP EBFHAGDC (Kuhnert and Quivey, 2003). The gene encoding the β subunit of the ATP operon (‘AtpD’) is considered to be a suitable molecular marker for bacterial phylogenetic investigations especially due to its ubiquitous distribution (Ventura, 2004). The role of the AtpD gene in the acid tolerance of probiotic bacteria is well supported by previous studies. Like in a previous study, it was observed that AtpD gene expression of L. Plantarum 91 induced significantly up to 4.7 fold at pH 2.5 after 90 min under in vitro conditions (Duary *et al.,* 2012). In one of the similar study, AtpD gene expression of *Lactobacillus Plantarum 91* was monitored under both in vitro and in vivo acidic environment conditions by real-time quantitative PCR and found significantly upregulated by 4.1 folds (Chandran *et al.,* 2014). The involvement of ATPase activity in the acid tolerance of

Lactobacillus rhamnosus strain GG was also found by Bang *et al.,* (2014) during long-term storage of different lactobacilli under low pH. Likewise, in another study AtpD gene of indigenous probiotic strain *Lactobacillus fermentum* and *Lactobacillus rhamnosus* were significantly increased under the simulated acidic conditions. In addition, these results were also confirmed in the *in vivo* conditions (Deeksha thesis, 2017). Overall these results established the role of the AtpD gene in the acid tolerance of probiotic bacteria. However, in the future, more *in-silico* studies are needed to evaluate the effect of acidity on the probiotic bacterial marker gene without the need for bacteria and simulated growth media.

Conclusion:

Overall, the present review showed the importance of the acid tolerance ability of the probiotic bacteria. This review shed the light on the molecular mechanism of acid tolerance ability of probiotics and established the relevance of the AtpD gene of bacteria.

References:

Amin, K.A., Al-Muzafar, H.M. and Elsttar, A.H.A., 2016. Effect of sweetener and flavoring agent on oxidative indices, liver and kidney function levels in rats. Indian Journal of Experimental Biology, 54, pp.56-63.

Anderson, R.C., Cookson, A.L., McNabb, W.C., Park, Z., McCann, M.J., Kelly, W.J. and Roy, N.C., 2010. Lactobacillus plantarum MB452 enhances the function of the intestinal barrier by increasing the expression levels of genes involved in tight junction formation. BMC microbiology, 10(1), pp.316.

Azcarate-Peril, M. A., Altermann, E., Hoover-Fitzula, R. L., Cano, R. J., & Klaenhammer, T. R. (2004). Identification and inactivation of genetic loci involved with Lactobacillus acidophilus acid tolerance. *Applied and environmental microbiology*, *70*(9), 5315-5322.

Bäckhed F, Ley RE, Sonnenburg JL, Peterson DA. Host-bacterial mutualism in the human intestine. | Science. 2005; 25;307(5717):1915-20.

Baffoni, L., Gaggìa, F., Di Gioia, D. and Biavati, B., 2012. Role of intestinal microbiota in colon cancer prevention. Annals of microbiology, 62(1), pp.15-30.

Bang, M., Oh, S., Lim, K.S., Kim, Y. and Oh, S., 2014. The involvement of ATPase activity in the acid tolerance of Lactobacillus rhamnosus strain GG. International Journal of Dairy Technology, 67(2), pp.229-236.

Begley, M., Sleator, R.D., Gahan, C.G. and Hill, C., 2005. Contribution of three bile-associated loci, bsh, pva, and btlB, to gastrointestinal persistence and bile tolerance of Listeria monocytogenes. Infection and immunity, 73(2), pp.894-904.

Cabreiro, F., Au, C., Leung, K.Y., Vergara-Irigaray, N., Cochemé, H.M., Noori, T., Weinkove, D., Schuster, E., Greene, N.D. and Gems, D., 2013. Metformin retards aging in C. elegans by altering microbial folate and methionine metabolism. Cell, 153(1), pp.228-239.

Cao, N. J. (2000). Characterization of reconstituted wildtype and mutant F (0) of the F (1) F (0) ATPase from Escherichia coli. Wayne State University.

Capaldi, R.A. and Aggeler, R., 2002. Mechanism of the F 1 F 0-type ATP synthase, a biological rotary motor. Trends in biochemical sciences, 27(3), pp.154-160.

Chandran, A., Duary, R.K., Grover, S. and Batish, V.K., 2013. Relative expression of bacterial and host specific genes associated with Bibliography iii probiotic survival and viability in the mice gut fed with lactobacillus plantarum Lp91. Microbiological research, 168(9), pp.555-562.

Chandran, A., Grover, S. and Batish, V.K., 2014. atp D expression of probiotic Lactobacillus plantarum 91 under acidic environment. Journal of Tropical Agriculture, 52(1), pp.21-30.

Chou, L. S., & Weimer, B. (1999). Isolation and characterization of acid-and bile-tolerant isolates from strains of Lactobacillus acidophilus. *Journal of Dairy Science*, *82*(1), 23-31.

Claggett, S.B., Plancher, M.O.N., Dunn, S.D. and Cain, B.D., 2009. The b subunits in the peripheral stalk of F1F0 ATP synthase preferentially adopt an offset relationship. Journal of Biological Chemistry, 284(24), pp.16531-16540.

Corcoran, B. M., Stanton, C., Fitzgerald, G., & Ross, R. P. (2008). Life under stress: the probiotic stress response and how it may be manipulated. *Current pharmaceutical design*, *14*(14), 1382-1399.

Das, A., Ivey, D.M. and Ljungdahl, L.G., 1997. Purification and reconstitution into proteoliposomes of the F1F0 ATP synthase from the obligately anaerobic gram-positive bacterium Clostridium thermoautotrophicum. Journal of bacteriology, 179(5), pp.1714-1720.

Del Rizzo, P.A., Bi, Y., Dunn, S.D. and Shilton, B.H., 2002. The "Second Stalk" of Escherichia coli ATP Synthase: Structure of the Isolated Dimerization Domain†. Biochemistry, 41(21), pp.6875-6884

Duary, R.K., Batish, V.K. and Grover, S., 2012. Relative gene expression of bile salt hydrolase and surface proteins in two putative indigenous Lactobacillus plantarum strains under in vitro gut conditions. Molecular biology reports, 39(3), pp.2541-2552.

García-Trejo, J. J., & Morales-Ríos, E. (2008). Regulation of the F 1 F 0-ATP synthase rotary nanomotor in its monomeric-bacterial and dimeric-mitochondrial forms. *Journal of biological physics*, *34*(1-2), 197-212.

Gill, S.R., Pop, M., DeBoy, R.T., Eckburg, P.B., Turnbaugh, P.J., Samuel, B.S., Gordon, J.I., Relman, D.A., Fraser-Liggett, C.M. and Nelson, K.E., 2006. Metagenomic analysis of the human distal gut microbiome. science, 312(5778), pp.1355-1359.

Gilliland, S.E., 1987. Importance of bile tolerance in lactobacilli used as dietary adjunct, Lyons, T.P., Ed. Biotechnology in the Feed Industry. Alltech Feed Co., Lexington, KY.pp.149–155

Gilliland, S.E., Stanley, T.E., and Bush, L.J., 1984. Importance of bile tolerance of Lactobacillus acidophilus used as a dietary adjunct. Journal of Dairy Science. 67, pp. 3045–3051

Johansson, M.L., Molin, G., Jeppsson, B., Nobaek, S., Ahrne, S. and Bengmark, S., 1993. Administration of different Lactobacillus strains in fermented oatmeal soup: in vivo colonization of human intestinal mucosa and effect on the indigenous flora. Applied and Environmental Microbiology, 59(1), pp.15-20.

Koebmann, B. J., Nilsson, D., Kuipers, O. P., & Jensen, P. R. (2000). The membrane-bound H+-ATPase complex is essential for growth of Lactococcus lactis. *Journal of bacteriology*, *182*(17), 4738-4743.

Koebmann, B.J., Nilsson, D., Kuipers, O.P. and Jensen, P.R., 2000. The membrane-bound H+-ATPase complex is essential for growth of Lactococcus lactis. Journal of bacteriology, 182(17), pp.4738-4743.

Kuhnert, W. L. (1999). *The F-ATPase operon from the oral streptococci S. mutans and S. sanguis: how structure relates to function*. University of Rochester.

Kuhnert, W.L. and Quivey Jr, R.G., 2003. Genetic and biochemical characterization of the F-ATPase operon from Streptococcus sanguis 10904. Journal of bacteriology, 185(5), pp.1525-1533.

Kullen, M. J., & Klaenhammer, T. R. (1999). Identification of the pH-inducible, proton-translocating F1F0-ATPase (atpBEFHAGDC) operon of Lactobacillus acidophilus by differential display: gene structure, cloning and characterization. *Molecular microbiology*, *33*(6), 1152-1161.

Kullen, M. J., & Klaenhammer, T. R. (1999). Identification of the pH-inducible, proton-translocating F1F0-ATPase (atpBEFHAGDC) operon of Lactobacillus acidophilus by differential display: gene structure, cloning and characterization. *Molecular microbiology*, *33*(6), 1152-1161.

Kullen, M. J., & Klaenhammer, T. R. (1999). Identification of the pH-inducible, proton-translocating F1F0-ATPase (atpBEFHAGDC) operon of Lactobacillus acidophilus by differential display: gene structure, cloning and characterization. *Molecular microbiology*, *33*(6), 1152-1161.

Ley, R.E., Lozupone, C.A., Hamady, M., Knight, R. and Gordon, J.I., 2008. Worlds within worlds: evolution of the vertebrate gut microbiota. Nature Reviews Microbiology, 6(10), pp.776-788.

Lilly, D. M., & Stillwell, R. H. (1965). Probiotics: growth-promoting factors produced by microorganisms. *Science*, *147*(3659), 747-748.

Lin, M., Gahan, C.G.M., and C. Hill., 2005. The interaction between bacteria and bile. FEMS Microbiol. Rev. 29:625–651

Marteau, P., Minekus, M., Havenaar, R. and Huis, J.H.J., 1997. Survival of lactic acid bacteria in a dynamic model of the stomach and small intestine: validation and the effects of bile. Journal of dairy science, 80(6), pp.1031-1037.

Marteau, P., Minekus, M., Havenaar, R. and Huis, J.H.J., 1997. Survival of lactic acid bacteria in a dynamic model of the stomach and small intestine: validation and the effects of bile. Journal of dairy science, 80(6), pp.1031-1037.

Morelli, L. (2000). In vitro selection of probiotic lactobacilli: a critical appraisal. *Current Issues in Intestinal Microbiology*, *1*(2), 59-67.

Morelli, L., 2000. In vitro selection of probiotic lactobacilli: a critical appraisal. Current Issues in Intestinal Microbiology, 1(2), pp.59-67.

Nakamoto, R.K., Scanlon, J.A.B. and Al-Shawi, M.K., 2008. The rotary mechanism of the ATP synthase. Archives of biochemistry and biophysics, 476(1), pp.43-50.

Nannen, N.L. and Hutkins, R.W., 1991. Intracellular pH Effects in Lactic Acid Bacteria1. Journal of Dairy Science, 74(3), pp.741-746

Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K.S., Manichanh, C., Nielsen, T., Pons, N., Levenez, F., Yamada, T. and Mende, D.R., 2010. A human gut microbial gene catalogue established by metagenomic sequencing. nature, 464(7285), pp.59-65

Schiffrin, E.J., Brassart, D., Servin, A.L., Rochat, F. and Donnet-Hughes, A., 1997. Immune modulation of blood leukocytes in humans by lactic acid bacteria: criteria for strain selection. The American journal of clinical nutrition, 66(2), pp.515S-520S

Sievers, M., Uermösi, C., Fehlmann, M., & Krieger, S. (2003). Cloning, sequence analysis and expression of the F1F0-ATPase β-subunit from wine lactic acid bacteria. *Systematic and applied microbiology*, *26*(3), 350-356.

Simon, G.L. and Gorbach, S.L., 1987. Intestinal flora and gastrointestinal function. Physiology of the gastrointestinal tract, 2, p.1729.

Smith, A. J., Quivey Jr, R. G., & Faustoferri, R. C. (1996). Cloning and nucleotide sequence analysis of the Streptococcus mutans membrane-bound, proton-translocating ATPase operon. *Gene*, *183*(1-2), 87-96.

Sun, Y., 2016. F1F0-ATPase Functions Under Markedly Acidic Conditions in Bacteria. In Regulation of Ca2+-ATPases, V-ATPases and FATPases (pp. 459-468). Springer International Publishing

Valcarce, D.G., Genovés, S., Riesco, M.F., Martorell, P., Herráez, M.P., Ramón, D. and Robles, V., 2017. Probiotic administration improves sperm quality in asthenozoospermic human donors. Beneficial Microbes, 8(2), pp.193-206.

van de Guchte, M., Serror, P., Chervaux, C., Smokvina, T., Ehrlich, S.D. and Maguin, E., 2002. Stress responses in lactic acid bacteria. Antonie Van Leeuwenhoek, 82(1-4), pp.187-216.

Ventura, M., Canchaya, C., van Sinderen, D., Fitzgerald, G. F., & Zink, R. (2004). Bifidobacterium lactis DSM 10140: identification of the atp (atpBEFHAGDC) operon and analysis of its genetic structure, characteristics, and phylogeny. *Applied and Environmental Microbiology*, *70*(5), 3110-3121.

Vuotto, C., Longo, F. and Donelli, G., 2014. Probiotics to counteract biofilmassociated infections: promising and conflicting data. International journal of oral science, 6(4), pp.189-194

Wang, C., Cui, Y., & Qu, X. (2018). Mechanisms and improvement of acid resistance in lactic acid bacteria. *Archives of microbiology*, *200*(2), 195-201.

Wenus, C., Goll, R., Loken, E.B., Biong, A.S., Halvorsen, D.S. and Florholmen, J., 2008. Prevention of antibiotic-associated diarrhoea by a fermented probiotic milk drink. European journal of clinical nutrition, 62(2), pp.299- 301.

ISBN: 978-81-953600-3-1

ENTOMOPATHOGENIC MICROORGANISMS AS BIOPESTICIDES: A REVIEW

Anupama Prabhakarrao Pathak[1]*, Mukundraj Govindrao Rathod[2], Ashwini Mahaling Devarshe[1], Mahesh Rajkumar Hundekar[1], Shivani Anil Tengse[1] and Gautam Tanaji Kamble[1]

[1]School of Life Sciences (DST-FIST phase-I & UGC-SAP DRS-II sponsored school), Swami Ramanand Teerth Marathwada University, Nanded 431606.

[2]Department of Biotechnology and Bioinformatics (U.G. & P.G.), Yeshwant College of Information Technology (Affiliated to S.R.T.M. University, Nanded), Parbhani 431401, Maharashtra

*Corresponding authors E-mail: anupama.micro@rediffmail.com

Abstract:

Biopesticides are able to protect crops from plant harming and damaging pests by the eco-friendly mode of action. Hence biopesticides are gaining attention of farmers in integrated pest management system. Some entomopathogens have been employed in modern agriculture to control pests and for improvement in productivity and now used immensely from last decade. Protection of crops from pests is an ultimate target of any biopesticide which is a bio-intensive tool of integrated pest management. The advantages of biopesticides include single target specific, multi target specific, effective in very small quantities, decompose quickly etc. The need and demand for biopesticides are rising gradually in agricultural sector. Biopesticides are used in Integrated Pest Management systems to protect the crops like fruits, vegetables, nuts and flowers from the attack of pest. This chapter has reviewed role and types of entomopathogenic microorganisms and their potential application as biopesticides are discussed.

Keywords: biopesticides, baculovirus, protozoa, nematode, entomopathogenic

Introduction:

In agriculture, control of pests with synthetic chemicals results in several problems as they show toxic effects on plants, birds, honeybees, wild animals, aquatic fauna etc. Synthetic chemical based pesticides can also be harmful to humans and domestic animals (Burges, 2012). Moreover they can contaminate ground water. Therefore, as an alternative, biological control agents have been introduced and screened for their efficacy and environmental safety (Dara,

2017). In this regard, some entomopathogens have been employed in modern agriculture to control pests and for improvement in productivity and now used immensely from last decade. In 2001, approximately 195 biopesticide active ingredients and 780 products were introduced. In general, biopesticides are derived from natural sources such as animals, plants, bacteria, and certain minerals. Simply, they are living organisms (natural enemies) or their products such as phytochemicals and microbial products or byproducts (semiochemicals) that can be used for the management of pests; however, some natural materials like canola oil and baking soda are reported to have pesticidal action and hence considered as biopesticides (Usta, 2013; Dara, 2017). Protection of crops from pests is an ultimate target of any biopesticide which is a bio-intensive tool of integrated pest management. The advantages of biopesticides include single target specific, multi target specific, effective in very small quantities, decompose quickly etc. Thus they are eco-friendly (Litwin *et al.,* 2020).

Microbial pesticides:

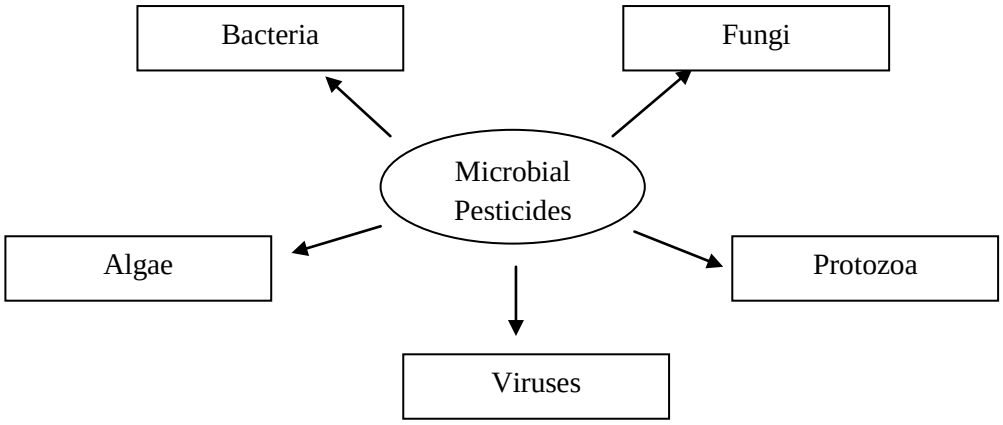


Figure 1: Naturally occurring or genetically engineered bacteria, fungi, algae, viruses and protozoa as sources of microbial pesticides

Sources of microbial pesticides are naturally occurring or genetically modified bacteria, fungi, algae, viruses and protozoa (Figure 1).

Entomopathogenic bacteria as biopesticides:

Many species of genus *Bacillus* have been isolated and identified from dead bodies of insects (Vos *et al.,* 2011; Pathak *et al.,* 2012; Cherekar and Pathak, 2015). Such bacterial entomopathogens invade through the integument or gut of the insect, multiply in host and produce insecticidal toxins. This results in the death of the host (insect). The toxins produced by

ISBN: 978-81-953600-3-1

microbial entomopathogens are peptides, but they vary greatly in terms of structure, toxicity and specificity. Bacterial pesticides are the most common and cheaper form of microbial pesticides (Usta, 2013). *Bacillus sphaericus* 2362(*Bs*) was found to be effective against mosquito and other dipteran larvae. *B. thuringiensis* ssp. *kurstaki* and *aizawai* exhibited the highest insecticidal activity against lepidopteran larval species. *Bacillus thuringiensis* produces crystalline proteins and kills a group of target insect pest species like lepidopteran species (Senthil-Nathan, 2015).

Entomopathogenic fungi as biopesticides:

Entomopathogenic fungi are the potential mycoinsecticidal agents that are effective against diverse insect pests in agriculture. These fungi infect their hosts by penetrating through the cuticle, gaining access to the hemolymph, producing toxins, and grow by utilizing nutrients present in the haemocoel and thus escape from insect's immune responses. Entomopathogenic fungi are applied either in the form of conidia or mycelium which sporulates after application. The combined application of insecticide with fungal entomopathogens could be very useful for insecticide resistant management (Usta, 2013). *Metarhizium anisopliae* is soil inhabiting and well studied entomopathogenic fungus. Spores of this fungus infect to soil-dwelling insects and produce insecticidal effects (Senthil-Nathan, 2015; Azizoglu *et al.*, 2020). Mode of action of entomopathogenic fungi against lepidopteran insects has been summarized in a following chart (Figure 2).

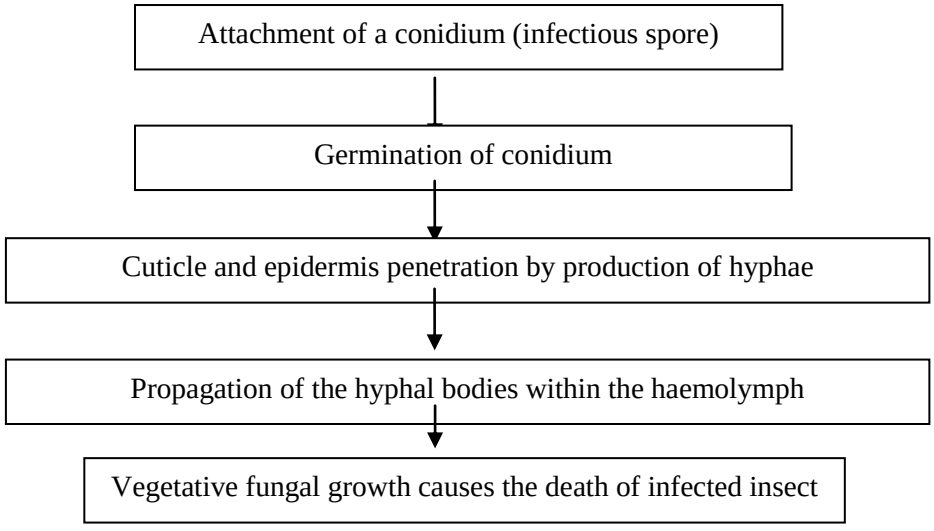


Figure 2: Insecticidal effect of entomopathogenic fungi

Entomopathogenic viruses as biopesticides:

About 1600 different viruses are known to infect 1100 species of insects and mites. Of these, a group of baculovirus infects to approximately 100 species of insect. This group of baculovirus accounts for more than 10% of all entomopathogenic viruses. Baculoviruses are rod-shaped particles which contain DNA as their genetic material. They are found in arthropods. Most viruses are enclosed in a protein coat to make up a virus inclusion body. Alkaline condition of insect's midgut helps to denature the protein coat. Then, viral particles are released from the inclusion body. These particles fuse with the midgut epithelial cells of insect, multiply rapidly and eventually kill the host. Many baculoviruses are host-specific. Therefore they cannot be used to control several different pests at a time. The action of baculoviruses on insect larvae is very slow to satisfy farmers and insecticides based on baculovirus are expensive. Moreover, such insecticides are not stable under the ultraviolet rays of the sun. Hence, to increase the efficacy, baculoviruses are encapsulated by UV protectants (Usta, 2013; Kachhawa, 2017). At present, many baculovirus based pesticides are available for the control of caterpillar pests (Senthil-Nathan, 2015). Mode of action of baculovirus against lepidopteran insects has been summarized in a following chart (Figure 3).

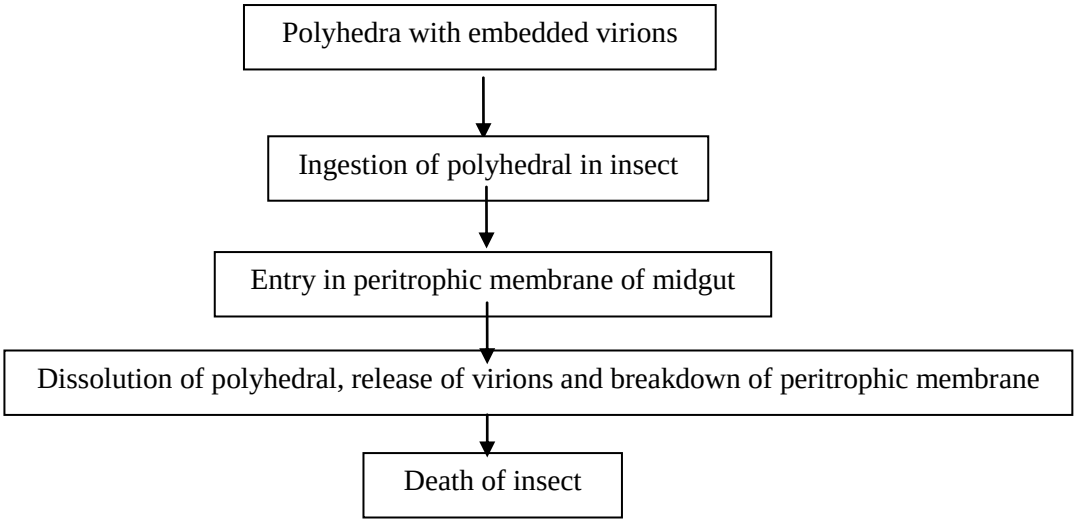


Figure 3: Flowchart for mode of action of baculoviruses against lepidopteran insects

Entomopathogenic protozoa nematodes as biopesticides:

A wide range of insect hosts are infected by protozoa. Microsporidia are popularly known for their insecticidal action. Some protozoa require many days or weeks to make harm their host. A protozoan species *Nosema locustae* is toxic for grasshoppers. Spores of protozoa induce massive infection and demolish organs and tissues of invertibrates (Senthil-Nathan, 2015). In 1990, entomopathogenic nematodes from two genera, namely, *Steinernema* and *Heterorhabditis*

ISBN: 978-81-953600-3-1

were found to be effective biocontrol agents against insects. Nonfeeding juvenile nematodes infest suitable insect and enter through the insect's natural body openings like the anus, mouth, and spiracles. Nematodes localize in the hemocoel and then release their symbiotic bacteria into the intestine. After that, the bacteria cause septicemia and kill the host within 24 to 48 h (Senthil-Nathan, 2015).

Formulation criteria:

The organisms selected in microbial insecticides should be non-toxic and non-pathogenic to wildlife and humans. The toxic action of microbial insecticides should be specific only to a single group or species of insects. The selected microbial insecticide should not affect beneficial insects (including predators or parasites of pests) in treated areas. The developed formulations of biopesticides should be protected from Heat and desiccation (drying out). Effectiveness of several types of microbial insecticides reduces after exposure to ultraviolet radiation; hence proper timing and application procedures are especially important (Usta 2013; Ruiu 2018).

Conclusion and future prospective:

The need and demand for biopesticides are rising gradually in agricultural sector. Biopesticides are used in Integrated Pest Management systems to protect the crops like fruits, vegetables, nuts and flowers from the attack of pest. A lot of hard work in the extreme extent is still to be carried out as numerous potential entomopathogenic microorganisms, have been identified by researchers and they have not scaled up as biopesticides. Research on biopesticides should be carried out to make available these products to economically weaken farmers at an affordable price.

Acknowledgement:

Authors are thankful to Hon. Dr. Udhav V. Bhosle, Vice-Chancellor of Swami Ramanand Teerth Marathwada University, Nanded for providing necessary facilities.

References:

Azizoglu, U., Jouzani, G. S., Yilmaz, N., Baz, E., and Ozkok, D. (2020). Genetically modified entomopathogenic bacteria, recent developments, benefits and impacts: A review. Science of The Total Environment, 139169.

Burges, H. D. (2012). Formulation of microbial biopesticides: beneficial microorganisms, nematodes and seed treatments. Springer Science and Business Media.

Cherekar, M. N., and Pathak, A. P. (2015). Studies on haloalkaliphilic gammaproteobacteria from hypersaline Sambhar Lake, Rajasthan, India.

Dara, S. K. (2017). Entomopathogenic microorganisms: modes of action and role in IPM. Agriculture and Natural Blogs, University of California, 7p.

Kachhawa, D. (2017). Microorganisms as a biopesticides. Journal of Entomology and Zoology Studies, 5(3), 468-473.

Litwin, A., Nowak, M., and Różalska, S. (2020). Entomopathogenic fungi: unconventional applications. Reviews in Environmental Science and Bio/Technology, 19(1), 23-42.

Pathak, A., Sardar, A., Kadam, V., Rekadwad, B., and Karuppayil, S. M. (2012). Efficacy of some medicinal plants against human dental pathogens.

Ruiu, L. (2018). Microbial biopesticides in agroecosystems. Agronomy, 8(11), 235.

Senthil-Nathan, S. (2015). A review of biopesticides and their mode of action against insect pests. Environmental sustainability, 49-63.

Usta, C. (2013). Microorganisms in biological pest control—a review (bacterial toxin application and effect of environmental factors). In Current progress in biological research (pp. 287-317). Rijeka: InTech.

Vos, P., Garrity, G., Jones, D., Krieg, N. R., Ludwig, W., Rainey, F. A., and Whitman, W. B. (Eds.). (2011). Bergey's manual of systematic bacteriology: Volume 3: The Firmicutes (Vol. 3). Springer Science and Business Media.

ISBN: 978-81-953600-3-1

EPIDEMIOLOGY, DIAGNOSIS AND TREATMENT OF MUCORMYCOSIS: A REVIEW

Sanyukta Kardekar and Narayan D. Totewad

Department of Microbiology,

B. K. Birla College of Arts, Science and Commerce (Autonomous),

Kalyan-421304, Thane, Maharashtra, India

*Corresponding authors E-mail: ndtmicro2013@gmail.com

Abstract:

Mucormycosis is an emerging fungal infection caused by group of filamentous molds belonging to the order Mucorales which usually begins in the nose and paranasal sinuses following the inhalation of spores. It usually occurs in the immunologically suppressed hosts. Risk factors include those with diabetes mellitus, neutropenia, malnutrition, antibiotic users, iron chelation therapy, etc. Infection may occur through inhalation, ingestion of contaminated food or direct contact through disrupted skin. Rate of progression is rapid. Mortality rate associated with invasion is high as compared to that of cutaneous mucormycosis. The disease is more recognized in recently developed countries such as India. Mucormycosis can be classified into one of the five forms: 1) Rhinocerebral mucormycosis, 2) Pulmonary mucormycosis, 3) Cutaneous mucormycosis, 4) Gastrointestinal mucormycosis and 5) Disseminated mucormycosis. Early diagnosis and adequate treatment are must for the recovery. This review describes the epidemiology, clinical forms, diagnosis and treatment of Mucormycosis.

Keywords: Mucormycosis, Inhalation, Spores, *Rhizopus Oryzae*, Steroides, Antibiotics

Mucormycosis:

'Fungus' the word is often heard in our day-to-day lives but only a few knows their real importance, existence apart from being found on the old breads. The only thing most people know is that fungus is found on decayed bakery products but fungi are way more than that. The formal study of fungi is termed as 'mycology', and the scientist who study some aspect of biology and ecology of these organisms are mycologists (Stephenson, 2010).The fungi originated as a distinctive group of unicellular eukaryotes in the Precambrian. Recent estimates of the origin of the fungal kingdom based on the analysis of molecular clocks range from 760 million years ago to 1.06 billion years ago (Watkinson *et al.,* 2016). Kingdom fungi comprises of diverse group of unicellular or multicellular, eukaryotic, heterotrophic microorganisms such as those of

molds, yeasts, etc important in terms of ecological and economical role. Fungi are ubiquitous in nature being found in air, soil, water, on plants, as lichens on rocks (Reiss *et al.,* 2012).

Characteristics of Fungi:

Fungi are eukaryotic, multinucleate or uninucleate organisms having heterotrophic mode of nutrition. Life cycle is both simple and complex (usually). Reproduction mode comprises of both sexual (nuclear fusion and meiosis) as well as asexual mode (pure mitotic division). They are ubiquitous in terrestrial and freshwater environments. In vegetative state, they typically exist as a non-motile mycelium of hyphae on or in the substratum. Ecological roles include saprotrophs, mutualistic symbionts, parasites and hyperparasites (Webster and Weber, 2012). Three peculiar characteristics unite the fungi: they are eukaryotes, which feeds by absorption and reproduce by forming spores (Money, 2016).

Fungi is beneficial in terms of economic importance such as metabolites of fungi has commercial importance, fungi help in the yield of antibiotics like penicillin, streptomycin, etc, fungi are useful in humus formation, acts as pesticides, bring about fermentation and has many more roles. Apart from the advantages, fungi have also been found associated with human infections. They are responsible for causingdiseases in host which are immunologically suppressed. Mostly human infections are caused by inhalation, ingestion or implantation of fungi that grow as saprophytes. Some yeasts are human commensals and cause endogenous infections (Richardson and Warnock, 2012). Opportunistic fungal pathogens are also present which may include common molds and yeasts. When fungus is capable of growing at 37˚C they may become opportunistic pathogens.

Different fungi belonging to different phyla are responsible for causing different fungal infections. Examples of different fungal infections include aspergillosis, blastomycosis, candidiasis, coccidioidomycosis, histoplasmosis, mucormycosis, mycetoma, ringworm, sporotrichosis and other fungal diseases. Currently mucormycosis which is one of them has shown a rise in number. Mucormycosis is seen to occur worldwide but it has geographical variations thus leading to the need of studying its epidemiology. Mucormycosis is rising but the rise is comparatively higher in India and China. A recent study of 851 cases between the period of 2000 to 2017 suggested that Europe was higher in terms of disease than Asia.According to them, 34% of the black fungus cases were reported from Europe, 31% were from Asia besides 28% from North and South America and 3% from Africa, Australia and New Zealand 3% (Prakash and Chakrabarti, 2019). India has been mostly affected by mucormycosis in many different states amongst which Gujarat is at the top with 2281 cases followed by Maharashtra (2000 cases). Other states' case numbers are Andhra Pradesh

ISBN: 978-81-953600-3-1

(910), Madhya Pradesh (720), Rajasthan (700), Karnataka (500), also have a high number of cases. States like, Haryana, Delhi, Chattishgarh, Bihar, Tamil Nadu, Kerala, Jharkhand, Odisha, Goa and Chandigarh have comparatively a smaller number of cases ranging upto 250 cases (Ians, 2021).

Mucormycosis! What is Mucormycosis? It is a rare but very severe rapidly progressing fungal infection which is commonly referred to as 'blackfungus.' Black fungus is not exactly black in colour as the name suggests but since it causes discoloration of skin by decreasing the blood supply, the skin appears to be gotten black in colour. The causative agents are a group of hyaline molds called as mucormycetes. Mucormycosis is generally seen in immunodeficient individuals however it can also occur in immunocompetent individuals. It ranks second among the most often occurring fungal infection in immunologically challenged individuals. Platauf is credited for the first description of zygomycosis in humans in his paper 'Mycosis Mucorina'. It is detailed enough to suggest that this first case of disseminated disease in a cancer patient was caused by *Absidia corymbifera* (Ribes *et al.*, 2000). Initially, the disease caused by these molds was known as 'Zygomycosis' and belonged to the phylum *Zygomycota* and order *Entomophthorales*. With the revised classification of fungi, phylum *Zygomycota* taxonomically does not exist anymore. In present, these molds or mucormycetes belong to Kingdom Fungi; phylum *Mucoromycota*; subphylum *Mucormycotina*; order *Mucorales*and family *Mucoraceae*. Most of the fungal pathogens involved in mucormycosis are placed under the order Mucorales and thus the disease was renamed as Mucormycosis instead of Zygomycosis.Majorly genera *Rhizopus*, *Mucor*, *Absidia* and rarely *Saksenaea* and *Cunninghamella* of the order *Mucorales* are responsible for the disease Mucormycosis.

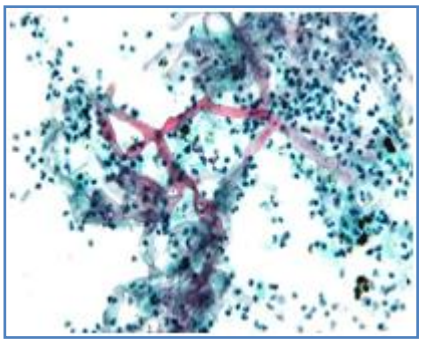


Figure 1: Mucormycosis

The diagnosis of Mucormycosis is quite strenuous thus leading to inadequate treatment and high mortality rate. However, early diagnostic measures, debridement (removal of infected tissue to help heal a wound), systemic antifungal therapy can help in the management of disease

(Gomes *et al.,* 2011). Patients contracting this infection uniformly suffer from predisposing conditions: having high levels of diabetes (particularly having ketoacidosis), organ/stem transplant, leukaemia, lymphoma, AIDS, renal failure, skin burns/cuts, people lacking neutrophils counts which helps in fighting foreign particles (Neutropenia), people undergoing chemotherapy for cancer, use of long-term corticosteroids, injecting drugs, malnutrition,liver problems, and dialysis patients on deferoxamine therapyand several other factors which lowers the immune response (Branscomb, 2002).Treatmentincludes a combination of antifungal agents and surgical intervention. The only new agent with activity against mucorales is isavuconazole, but it does not have advantages over historical first line therapy of amphotericin B-based drugs or Posaconazole (Skiada *et. al,* 2018).

Causative agents of Mucormycosis:

Several different organisms have been seen involved in the occurrence of Mucormycosis but the general cause of human infection is *Rhizopus oryzae (R. arrhizus)* and *Rhizopus microsporus.* Other causative agents include *Rhizomucor pusillus, Cunninghamella bertholletiae, Apophysomyces elegans, Actinomucorelegans, Cokeromyces recurvatus, Lichtheimiacorymbifera, Saksenaea vasiformis* and *Syncephalastrum racemosum.* The organisms belonging to order Mucorales are ubiquitary in environment (soil, food decaying vegetation, decomposing organic matter, air). *Rhizopus oryzae* is the most common organism isolated from patients detected with mucormycosis and is responsible for ~70% of all cases of mucormycosis (Ibrahim *et al.,* 2012).

Invasion of the Mucorales inside the host?

Pathophysiology of Mucormycosis involves inhalation, ingestion or direct inoculation of fungal spores through skin laceration. In case of inhalation and ingestion, the fungal spores germinate in the nasal sinuses and grow rapidly as aseptate, thus invading blood vessels and may further spread to paranasal sinuses and apparently to the orbit of the eye, meninges and brain by direct extension. Through skin, the mucorales experience a direct interaction with the epithelial cells and basement membranes (Afroze *et al.,* 2017).Another risk-factor for mucormycosis is the presence of high concentrations of iron in serum. Patients treated with deferoxamine have a high incidence of mucormycosis, probably because Mucorales use this chelant as a siderophore to obtain more iron (Bouza, 2006)

Clinical manifestations:

The causative organisms of clinical forms of mucormycosis have predilection for vascular invasion: thrombosis (coagulation of blood), infarction (death of tissue due to improper blood supply due to blockage by something. In this case by thrombus), tissue necrosis (death of

ISBN: 978-81-953600-3-1

tissue cells due to lack of oxygen supply or blockage of blood). Peculiarity of mucormycosis is the rapid beginning of necrosis and fever.

Different clinical forms of mucormycosis:

- Rhinocerebral Mucormycosis (Major Clinical form)
- Pulmonary mucormycosis
- Cutaneous mucormycosis
- Gastrointestinal mucormycosis
- Disseminated mucormycosis

Rhinocerebral Mucormycosis (major clinical form):

It is the most frequently occurring clinical form which is found in patients with poorly controlled diabetes with ketoacidosis. It is also seen in cancer patients and organ transplant patients. It is also termed as rhino-orbito-cerebral mucormycosis. It is initiated in the paranasal sinuses and then spread in the orbit, face, palate and brain. If untreated, the disease proves to be fatal. Initial symptoms include fever, unilateral headache, nasal/sinus congestion, unilateral facial swelling, and serosanguinous nasal discharge. Further spread into the orbit causes periorbital or perinasal swelling, hardening (induration) and discoloration, ptosis, proptosis, loss of vision, drainage of pus and later angio-invasive spread to brain is common. Black necrotic lesions and palatal perforation are the diagnostic symptoms. In case of diabetic patients, a red patch on the cheek is a signal of Rhinocerebral Mucormycosis (Reiss *et al.*, 2012).The extent of bone and soft tissue destruction are needed to be examined with the help of imaging studies. CT and MRI can be used in it. Computed tomographic (CT) scans are useful in detection of involvement of sinuses. Magnetic resonance imaging (MRI) is preferred over CT scan for determining the extension of infection to the delicate tissue of orbit and brain.

Pulmonary Mucormycosis:

Pulmonary mucormycosis is uncommon but a life threatening opportunistic fungal infection with a high mortality rate. The patients at high risk are those with neutropenia, such as those having leukemia and lymphoma, that receiving lung transplant. Transmission occurs via aspiration or inhalation of infectious fungal spores; from haematogenous spread during dissemination. If not treated, it turns fatal within 2-3 weeks (Richardson and Warnock, 2012). As such there are no specific symptoms but some may include fever, cough, dyspnea and pain in chest. Hemoptysis occurs due to the invasion of angio-invasive hyphae across the tissue. Compared to Rhinocerebral Mucormycosis, Pulmonary Mucormycosis is difficult to diagnose.

Also, it is difficult to distinguish it from other fungal infection such as aspergillus. Radiographic or imaging features in pulmonary mucormycosis are not specific but there are some radiological signs that can help predicting the disease. They include infiltrates, nodules, cavities and lobar consolidation (Reiss *et al.,* 2012). The presence of reverse halo sign is recognized as radiological sign of mucormycosis as that of aspergillosis. Pleural effusion is uncommon but suggests pulmonary mucormycosis (Richardson and Warnock, 2012).

Cutaneous mucormycosis:

Cutaneous mucormycosis usually occurs through direct traumatic inoculation of spores. When normal anatomic barrier of skin is disturbed, it provides an entry to organisms. This form of disease is seen in patients with wounds, insect bites, skin cuts, burns, non-sterile dressings and other injuries. Diabetic and neutropenic cancer patients are also at risk since infection may occur at the site of insulin injection and catheter insertion (Reiss *et al.,* 2012). Cutaneousmucormycosis can be divided as primary and secondary. In primary, the mode of infection is direct inoculation and the most common areas of infection are skin of arms and legs; the other areas include scalp, face, thorax, back, abdomen, neck and breast. Signs include purpuric lesions, swollen plaque, ulcers, cellulitis and necrosis. Secondary disease occurs by dissemination from other locations, mostly from a rhinocerebral infection. Signs include sinusitis, necrotic eschar, fever, periorbital cellulitis, periorbital edema, necrotic black or white ulcers and loss of vision. Diagnosis includes necrosis and infarction in burns (Castrejón-Pérez *et al.,* 2017).

Gastrointestinal mucormycosis:

Gastrointestinal disease occurs through ingestion of contaminated food though the use of contaminated herbal medicines has been linked to gastrointestinal disease development (Hernandez, 2020).The rare infection usually occurs in premature neonates and malnourished infants and children. The sites of infection include stomach, colon and ileum. Non-specific abdominal pain with nausea and vomiting are common symptoms along with other symptoms including fever, distention and haematemsesis. Gastric or intestinal perforation, perirenal abscesses and renal infarction may create complications. The disease is acute and progresses rapidly. Disease is seldom diagnosed and diagnosis is post-mortem. The intestinal mucormycosis disease is also fatal with death occurring within a several weeks due to bowel infarction, sepsis or haemorrhagic shock.

Disseminated mucormycosis:

Disseminated mucormycosis may occur from any four forms of mucormycosis mentioned above but is commonly found to occur in neutropenic patients with pulmonary form to the brain, heart, pancreas, bones, spleen, kidney and bladder. It occurs when the infection spreads through

ISBN: 978-81-953600-3-1

blood thus affecting other parts of the body. Brain is the main site of infection but necrotic lesions have also found in heart, spleen, other organs.It is seldom diagnosed and hence has a high mortality rate of approximately 100 %. Some patients also develop cutaneous lesions which help in the early diagnosis. Due to the high mortality rate, rapid diagnosis is very important for the successful treatment. Surgical debridement of necrotic tissue and antifungal therapy could help in the treatment (Richardson and Johnson, 2006).

Managementand Treatment:

For an effective management system, early diagnosis and treatment are important factors.Even after early diagnosis, management sometimes become quite difficult due to the rapid progression of fungal infection. Once diagnosis has been established, correction of hypoxia, acidosis, Hyper-glycemia, and electrolytic imbalance needs to be undertaken. After diagnosis use of steroids, antibiotics and immunosuppressive medicines should be stopped if possible (Branscomb, 2002). The management involves removal of infected necrotic tissue and administration of antifungal medicines. At an initial phase mucormycosis can be treated by prescribed antifungal medicines but at present those are quite limited in case of mucormycosis, since mucormycetes are less susceptible to common antifungal agents. Before treatment, identification of different species needs to be done because mucormycetes have different responses to antifungal medicines. Surgical debridement with amphotericin B therapy is also performed. For mucormycosis amphotericin B is the most widely used drug at a dosage of 5 mg/kg/day. Liposomal amphotericin B at the dosage of as high as 10 mg/kg/day is also used and is more effective and less toxic. In patients with Sino-orbital infection and cutaneous mucormycosis, surgical debridement of necrotic lesions and infected tissue area is important in treatment. Further there may be a need of repeated debridement's. In case of cutaneous mucormycosis, surgical debridement should be associated with amphotericin B therapy. The treatment should be administered according to the individual patient's clinical response and the intensity of improvement of infection (Richardson and Warnock, 2012)

Prevention:

Reduction to environmental exposures to pathogens though not easy but is an important measure for prevention of mucormycosis. Controlled diabetes and use of iron chelators would help in prevention. General environmental exposures to pathogen can be decreased by prohibiting the allowance of construction activity near the hospitals including immunosuppressed patients as the construction site may be a source of dust particles, polluted water, decaying organic matter (Gomes *et al.,* 2011). Also avoiding consumption of food which may have high

chances of being a source of fungal spores such as old breads; avoiding use of non-sterile surgical dressings. The main source of infection is inhalation of fungal spores through air, so the risk of nosocomial infection can be lessened by using High-efficiency particulate absorbing (HEPA) filters (Richardson and Warnock, 2012). Common prevention measures that should be taken at individual level includes- wearing a mask while stepping out; wearing clothes that least expose the skin; diabetic patients should regularly check sugar levels and blood glucose levels; people who are prescribed steroids should reduce their dosage in consultation with doctor if possible.

Covid-19 influence on Mucormycosis:

During the Covid-19 pandemic, mucormycosis is seen to be on rise. The number of cases of mucormycosis before Covid-19 was comparatively less than present. Not all the patients who have contracted with coronavirus are at risk of getting fungal infection but many of them have been observed to acquire mucormycosis. People who have been tested positive for coronavirus are on medication and hence their ability to fight with other environmental pathogens get reduced. This triggers the case of mucormycosis since mucormycetes majorly target those with the low immunity levels. Apart from having low immunity due to coronavirus,health experts point the sudden increase in mucormycosis to the extensive use of steroids, antibiotics and zinc tablets. Zinc and iron in the body provides a suitable environment for thegrowth of black fungus. According to many recent studies, zinc could be one of the main factors leading to mucormycosis.

References:

Stephenson, S.L. (2010). *The Kingdom Fungi: The Biology of Mushrooms, Molds and Lichens,*Timber Press, 1-272.

Watkinson S.C., Boddy L., and Money N.P. (2015). The fungi, 3rd edition. Academic Press: 1-466.

Reiss E., Shadomy H. J., and Lyon G. M. (2012). Fundamental Medical Mycology, Wiley-Blackwell, Hoboken, NJ, USA, 2011: 1-656.

WebsterJ.and Weber R. (2007). Introduction to Fungi, 3rd edition, Cambridge University Press: 1-817.

Money N. P. (2016). Fungi: A very short introduction. Oxford: Oxford University Press: 1-144.

Richardson M. D., and Warnock D. W. (2012). *Fungal infection: Diagnosis and management*4th Edition, Oxford: Wiley-Blackwell Publication, 1-476.

Prakash H., and Chakrabarti A. (2019). Global Epidemiology of Mucormycosis, Journal of Fungi, 5 (26): 1-19. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6462913/

Ians (2021). India reports 8,848 mucormycosis cases, more Amphotericin vials allocated. The tribune. https://www.business-standard.com/article/current-affairs/india-reports-8-848-mucormycosis-cases-more-amphotericin-vials-allocated 121052200583_1.html.

Ribes, J.A., Vanover-Sams, C.L, and Baker, D.J. (2000). Zygomycetes in human diseases, Clinical Microbiol Rev, Vol. 13: 236-301.

Marisa Z. R. Gomes, Russell E. Lewis, and Dimitrios P. Kontoyiannis (2011). Mucormycosis Caused by Unusual Mucormycetes, Non-Rhizopus, -Mucor, and -Lichtheimia Species, Clinical Microbiology Reviews, 24 (2): 411-445.

Branscomb R. (2002). An Overview of Mucormycosis. Laboratory Medicine,33(6): 453-455. doi:10.1309/ml2q-9jwn-krr7-366t.

Skiada A., Lass-Floerl C., Klimko N., Ibrahim A., Roilides E., and Petrikkos G. (2018). Challenges in the diagnosis and treatment of mucormycosis. Medical Mycology, 56: S93-S101. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6251532/

Ibrahim, A.S., Spellberg, B., Walsh, T.J., Kontoyiannis, D.P. Pathogenesis of Mucormycosis, *Clinical Infectious Diseases*, Volume 54, Issue suppl_1, F, 2012, Pg (16–22).

Afroze S. N., Korlepara R., Rao G. V., and Madala J. (2017). Mucormycosis in a Diabetic Patient: A Case Report with an Insight into Its Pathophysiology. Contemporary clinical dentistry, 8 (4): 862-866. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5754995/

Bouza, E. (2006). Mucormycosis: An emerging disease? [Review]. Clinical Microbiology and Infection, 12: 7-23.

Castrejón-Pérez A. D., Welsh E. C., Miranda I., Ocampo-Candiani J., and Welsh O. (2017).Cutaneous mucormycosis.An Bras Dermatol, 92 (3):304-11.https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5514567/

Richardson M., and Johnson E. (2008). Pocket Guide to Fungal Infection, 2nd Edition. Wiley-Blackwell publishing, 1-192.

Hernández, J. L. (2020). Mucormycosis: 1-13. https://www.ncbi.nlm.nih.gov/books/NBK544364/

https://www.cdc.gov/fungal/diseases/mucormycosis/index.html

https://images.app.goo.gl/P2kHYjBBgQNWG4GAA

REMOTE SENSING STUDIES OF THE ENVIRONMENT AND BIODIVERSITY

Sakshi Walker[1] and Jai Kumar*[2]

[1]Department of Botany,

St. John's College, Agra, Uttar Pradesh, India

[2]Centre for Geospatial Technologies, VIAET,

SHUATS, Prayagraj, Uttar Pradesh, India

*Corresponding authors E-mail: jaikumar0070@gmail.com

Abstract:

Remote sensing (RS) is used to locate, measure, and analyse properties of objects of interest without having to come into direct touch with them, while GIS is used to store, manipulate, and retrieve geographically referenced data. When used together, RS and GIS provide the capacity to collect, analyse, and integrate data quickly, as well as show the findings in geographic-referenced maps and reports. Satellite remote sensing techniques may provide a reliable and cost-effective way of obtaining timely data for natural resource development and management. Only RS and GIS can give a comprehensive approach to studying the environment and the interrelationships that exist among the many biophysical components. In agriculture, integrated RS and GIS technology offers enormous prospects for cost-effective treatment of crop stress sources. Its use in the field of hydrogeology and water resources development is fast expanding, and it can quickly explore urban landscapes, physiognomy, lakes, flora, points of interest, land consumption, building, and population distribution. It provides a robust technological foundation for agricultural statistics in agriculture. Agriculturalists can track variations in growth rate due to stress factors such as salt, drought, and temperature. Because of its great data acquisition capability and resource management, it may also be used for coastal environment management and monitoring. Satellite remote sensing is an important technology for environmental impact assessment and is a potential technique for conservation challenges. Plant disease detection in crop stands can be tracked owing to environmental and epidemiological variables. The knowledge on the location and quantity of cyanobacteria in interior water bodies has been used to help risk assessment and management, which has benefited human health. To supply frequency with high particular resolution, it is now possible

ISBN: 978-81-953600-3-1

to combine a lesser resolution geostationary ocean colour sensor with a high resolution polar orbiting sensor in a synergistic manner. In the framework of remote sensing data processing and GIS, this article presents a summary of applications and current theoretical breakthroughs.

Keywords: Remote sensing, geographic information systems, illness, stress, health, and agriculture.

Introduction:

In recent years, remote sensing (RS) has shown to be quite useful in gathering data for optimal resource management. In addition, the use of a Geographic Information System (GIS) in assessing trends and estimating changes in many topics aids in the management decision-making process. GIS and remote sensing technologies, in particular, provide the ability to collect data quickly, analyse and integrate data and information, and present outcomes in geographically linked maps and reports.

The area of RS has grown thrilling and exciting in recent years, with rapidly expanding potential. For natural hazard risk management, a GIS-based integrated strategy might be employed [1]. Remote sensing technology may now be utilised to swiftly explore urban terrain, physiognomy, lakes, flora, views, transportation, land use, construction, and population distribution. Remote sensing is vital in obtaining concrete information on conservation management and biodiversity. As it helps, collecting measures to provide us a wide assessment of everything happen around us.

Remote sensing is a world-accepted means of obtaining and evaluating Earth data in which judgments are made regarding our beloved planet's ongoing and future status. Through sensors (remote sensors) that record transmitted and emitted energy, these data are examined. Biodiversity talks about a region's amount and variety of plant and animal species. There's no life without biodiversity, it dictates our breath, the food we consume and our daily lives. Due of its functions, researchers have been seeking strategies to conserve biodiversity on planet earth in recent years.

1. Biodiversity boosts earth's resistance to disasters
2. Biodiversity promotes production among Earth's species
3. Biodiversity offers stability between plants and animals and thus continuation of life
4. Without sustaining biodiversity, living among ourselves, plants and animals would not exist.

In fact, using remote sensing to biodiversity will be a big stride so we can know the state of our climate, temperate zones, flora, and others.

Remote sensing will provide us easy means to discover biodiversity concerns and how we may tackle them using photos collected from satellites, smart cameras or sensors.Because of its enormous scope and qualities, it is virtually hard to collect measurements and use methods on how to preserve biodiversity, but due to improved technology, we can now use many biodiversity strategies. Below, I'll provide you a few remote sensing steps on biodiversity conservation.

Fire detection and monitoring:

Biodiversity, as we know, covers a vast amount of the earth's surface therefore finding vegetation fires will be quite difficult unless remote sensing. Remote sensing provides accurate identification and location of a vegetation fire that would be difficult for conventional detection methods. The land and vegetation area must be preserved for healthy ecosystem stability, hence any fire incidences throughout the globe should be identified and monitored quickly. While those tropical vegetation regions that are always prone to fire are continually monitored. To be specific, in December 1999, NASA revealed a high-class device called MODIS (Moderate Imaging Spectroradiometer), which promptly monitors and identifies smokes of active fires.

Landscape and land-cover changes:

This particular use of remote sensing helps to foresee or know in one instance the changes in the land. Remote sensing gives data or information on daily land-based ecological, meteorological, and biochemical changes. Ecosystem sustainability is the concern of man, and land covering a substantial percentage of biodiversity should be a primary focus for them. Remote sensors also detect and monitor changes in the ecosystem.

Habitat/distribution of species:

There are many species out there that are homeless or covered mostly because deforestation or bush fire ruined their habitats. Through geographic information systems (GIS) and remotely sensed pictures plotted, animals without a house will be found, establishing a new refuge. There is a system that makes habitat preference known for each animal; therefore it won't be difficult to create a new protected environment.

Sometimes finding free area to place these diverse types of animals may be tough, but using Gap analysis, a high-quality habitat would be constructed to fit that particular species.

ISBN: 978-81-953600-3-1

This habitat might be placed in towns or cities, but well-created and set up to make the animal feel at home or comfortable and free from poaching.

Forest observation:

Though, detecting and estimating parameters that would be more difficult if done manually or with other ways might be huge and laborious, but undoubtedly an easy way. Remote sensing techniques are used for information such as tree height, bole height, density, volume, and other critical metrics. Remote forest sensing would also assist avoid fire threat, pest and disease infestation .These alterations can be observed promptly by high-resolution aerial satellites. High-resolution pictures with sensitive photos can also assist determine whether a tree is under insect assault and then take severe action [15].

Ocean temperature/ice breakup:

In recent years, some microwave sensors have shown to feel salt and marine difficulties like ice destruction and others. Special sensor microwave/imager (SSM/I) is an example of such a remote sensor that delivers images to find ice fracture in the sea that may cause ships much devastation. With this, sea navigators may simply and rapidly identify ice to avoid ships collapsing from diverting or moving away from that path. There are also instances when the water would not be beneficial to sailing on maybe due to temperature and pressure at the moment when viewed and recognised with remote sensors, it is suggested not to sail. Many sensors show you the sea currents, and when it's really high, all ships will be prevented from sailing to avoid mishaps on the route that might lead to death.

In the disease:

Schistosomiasis and malaria are two parasite illnesses spread through water that impact millions of people globally, primarily in subtropical and tropical areas. Malaria is present in 72 of the Philippines' 78 provinces, whereas schistosomiasis is present in 24 of them. The Oncomelania snail and the Anopheles mosquito, which are involved in the transmission of these illnesses, rely on reliable ecological variables to keep them alive. The application of RS and GIS can be quite useful in determining how environmental variables impact the geographic distribution of these two illnesses. Geo-referenced data makes it easier to see predominant data in connection to physical maps, making it easier to measure illness conditions. The most prevalent factors that define high predominance areas for schistosomiasis were identified as

proximity to irrigation networks, snail breeding sites, and the highly agricultural character of the barangays, confirming the information that environments that support snail populations will also favour the occurrence of the disease. Humidity, temperature, soil type, presence of cultivated regions, preponderance of reproductive bush, distance from traditional water supply, and deep wells were all factors in malaria prediction models, which were all influenced by the factor of elevation. [2]

Papaya is an economically important plant in Thailand, both for domestic use and for export. Nonetheless, papaya is extremely susceptible to the papaya ring spot virus, which causes illness. Using GIS and RS data, the dispersal of papaya pollen in the wind was examined. Pollen traps were positioned in eight different orientations around a papaya crop. Remote sensing and near-range approaches have shown to be more effective in identifying illnesses and analysing crop stands for polluted plant sub-areas. Plant illnesses have a sporadic distribution in the field since they are based on definite epidemiological and environmental disorders. [3]

Imaging technologies are preferred over non-imaging technologies for plant disease monitoring and detection. A multi-disciplinary strategy including engineering, plant pathology, and informatics is necessary to fully leverage the promise of these sophisticated, pioneering technologies and high-dimensional, complicated data for precision crop protection. These innovative technologies may be used to improve plant phenotyping for resistance breeding or fungicide selection, in addition to precise crop protection.4

Plant stress:

By combining remote sensing and geographic information systems (GIS) technology, farmers may more effectively control the causes of crop stress at a lower cost5. One of the most significant constraints on agricultural output is salinity [6] [14]. Nutrients and sufficient water are required for successful crop production. Plants, particularly in arid and semi-arid parts of the world, are subjected to severe environmental stress such as salt in the water and soil, which restricts growth [7]. Furthermore, plants with nutrient deficiency symptoms differ from healthy plants because changes in cell structure alter the amount of reflected light in the infrared spectrum [8].

Human health:

The presence of large colonies of harmful cyanobacteria in recreational waterways can pose a major threat to human health. As a result, information on the abundance and distribution of cyanobacteria is needed to aid management and risk assessment. The employment of RS

ISBN: 978-81-953600-3-1

reconnaissance in combination with in situ monitoring methodologies, it is believed, will substantially aid in the evaluation of cyanobacterial dangers in inland waterways and increase our capacity to safeguard human health [9]. Cyanobacteria blooms are becoming more common in nutrient-polluted inland rivers across the world. Because many species are capable of creating a variety of very strong poisons, these mass populations, which can appear as blooms, scums, or biofilms, can pose major health concerns to animals and humans (so-called cyanotoxins) [10] [11].

Cholera outbreaks have long been known to occur when Vibrio cholerae, the bacterium that causes cholera, is present in sufficient quantities in drinking water to create an infective dose if consumed by people. Outbreaks connected to swimming and drinking contaminated river or brackish water may be caused by factors such as nutrient content, water temperature, and plankton generation, all of which may encourage the bacterium's development and reproduction. The available data sets are scarce and sporadic, despite the fact that certain environmental characteristics have been systematically assessed using water samples gathered aboard research ships. Furthermore, obtaining shipboard data is both time-consuming and costly. Interpolation to provincial scales might be difficult as well. Although the bacteria, V. cholerae cannot be detected directly, it can be inferred using remotely sensed data [12].

Agriculture:

Agriculture is the backbone of the Indian economy. The fast advancement of RS technology provides a strong technological foundation for the comprehensive use of Indian agriculture statistics. Satellite RS methods can provide resource managers with a reliable and cost-effective method of obtaining timely data for the management and development of our natural resources. RS data collected often across agricultural land aids in crop identification and mapping, as well as crop vitality assessment. Access to geographical information on a planetary scale is possible because to RS and its related image analysis technologies.

New detectors and imaging technologies are enhancing RS's ability to efficiently acquire digital spatial information at very fine resolutions. In a short amount of time, advanced information about the earth's properties and processes may be derived.

The main issue of remote sensing applications in agriculture is crop identification and production forecasting. Different spectral bands for vegetation sensitivity have been examined by researchers. RS has the ability to detect crop types, as well as estimate crop area and yield [13].

The evaluation of the links between yield and vegetation indices has been researched several times over the years and has consistently demonstrated to be favourable for yield prediction [13].

Conclusion:

In the areas of environmental and biodiversity conservation, remote sensing and geographic information systems (GIS) provide a wide range of applications and benefits. Together, RS and GIS present the facility with a proficient and cost-effective means of acquiring timely data for the advancement and management of natural resources. It provides farmers with significant options to manage agricultural stress, such as salt and drought, at a low cost. Satellite remote sensing can help with environmental impact assessment and conservation challenges. A multidisciplinary approach is necessary to fully exploit the promise of these extremely complex and novel systems.

In conclusion, remote sensing is a vital part of conserving biodiversity, since I don't see any other easy and efficient approach out there without it. Continuous remote sensing will stabilise our ecosystem (terrestrial and marine).

Acknowledgements:

The authors are thankful to the authorities of Department of Botany, St. John’s College, Agra and Centre for Geospatial Technologies, VIAET, SHUATS, Prayagraj for their support and encouragement to conduct this study.

References:

1. Chen K, Blong R, Jacobson C. Towards an Integrated Approach to Natural Hazards Risk Assessment Using GIS: With Reference to Bushfires. Environmental Management. 2003;31(4):546–560.
2. Leonardo LR, Rivera PT, Crisostomo BA, et al. A studies of the environmental determinants of malaria and schistosomiasis in the Philippines using remote sensing and geographic information systems. Parassitologia. 2005;47(1):105–114.
3. Sritakae A, Praseartkul P, Cheunban W, et al. Mapping airborne pollen of papaya (Carica papaya L.) and its distribution related to land use using GIS and remote sensing. Aerobiol. 2011;47(1):291–300.
4. Mahlein AK, Oerke EC, Steiner U, et al. Recent advances in sensing plant diseases for precision crop protection. Eur J Plant Pathol. 2012;133(1):197–209.

ISBN: 978-81-953600-3-1

5. Nutter Jr FW, Tylka GL, Guan J, et al. Use of remote sensing to detect soybean cyst nematode–induced plant stress. J Nematol. 2002;34(3):222–231.
6. Turhan H, Genc L, Smith SE, et al. Assessment of the effect of salinity on the early growth stage of the common sunflower (Sanjay cultivar) using spectral discrimination techniques. African J Biotechnol. 2008;7(6):750–756.
7. Vina AA, Gitelson A, Rundquist DC, et al. Monitoring maize (Zea mays L.) phenology with remote sensing. Agron J. 2004;96:1139–1147.
8. Hunter PD, Tyler AN, Gilvear DJ, et al. Using remote sensing to aid the assessment of human health risks from blooms of potentially toxic cyanobacteria. Environ Sci Technol. 2009;43(7):2627–2633.
9. Rao PVL, Gupta N, Bhaskar ASB, et al. Toxins and bioactive compounds from cyanobacteria and their implications on human health. J Environ Biol. 2002;23(2):215–224.
10. Carmichael WW, Azevedo SMFO, An JS, et al. Human fatalities from cyanobacteria: Chemical and biological evidence for cyanotoxins. Environ Health Perspect. 2001;109(7):663–668.
11. Lobitz B, Beck L, Huq A, et al. Climate and infectious disease: Use of remote sensing for detection of Vibrio cholerae by indirect measurement. Proc Natl Acad Sci U S A. 2000;97(4):1438–1443.
12. Gopperundevi M, Kannan V. Paddy yield estimation using remote sensing and geographical information system. J Mod Biotechnol. 2012;1(1):26–30.
13. Ehlers M, Edward G, Bedard Y. Integration of remote sensing with geographic information systems: A necessary evolution. Photogramm. Engg Remote Sens. 1989;55(1):1619–1627.
14. Walker, S.; Kumar, J.; Biswas, B. Assessment of different indices (vegetation, salinity) and salt effected areatrend analysis using shannon entropy approach–a case study in a semi-arid region of india using rs/gis.Plant Arch.2019,19, 3457–3466.
15. Walker S., B. Biswas and J. Kumar, (2018). Sustainablemanagement of environmental resources of a semi-aridregion of India using RS/GIS Volume 5, Issue 4 (XII), 28-38.

WHITE POLLUTION OF MARINE ECOSYSTEM: A GLOBAL TRAGEDY FOR OUR OCEANS AND SEA LIFE

Adhira M Nayar[1]*, Asha V. G.[1] and Deepasree M. I.[2]

[1]Postgraduate Department of Zoology and Research Centre,
Mahatma Gandhi College, Trivandrum, Kerala

[2]Department of Zoology, Iqbal College,
Peringamala, Trivandrum, Kerala

*Corresponding authors E-mail: adhira.m.nayar@gmail.com

Introduction:

White pollution and plastosphere are the new terminologies now being used to indicate the catastrophic effects of plastic pollution in the environment. Littering beaches by plastic bags, styrofoams, etc., has become a global crisis. Tons and tons of plastic debris comprise about 40 percent of the world's oceans. In the current scenario, plastic is expected to replace the entire marine organisms by 2050.

Plastics are found each and everywhere in our planet earth. It is used in wrapping the food, commonly in our houses, and also in the technology we use. It is a efficient substance that has been used to make medical devices, thus bringing a high advancement in the field of health care. But, improper disposable of these plastics has end up, in announcing their presence in land-fills, landscapes, and polluting the marine ecosystem. Unfortunately, plastic is so durable that the EPA reports “every bit of plastic ever made still exists.” According to the United nations Joint Group of Experts on Scientific aspects of Marine pollution (GESAMP), terrestrial disposal of plastic wastes account for 80% of the marine pollution and 60 to 95 % of the waste being plastic debris. It is considered as a severe anthropogenic issue affecting the ecosystems especially coastal and marine ecosystems. The contaminants cause direct or indirect havoc by interrupting the structure and function of ecosystems.

We're surrounded entirely by plastics. The first plastic polymer, Bakelite was synthesised in 1907. Whatever plastic materials that has been synthesised since 1907 is still in our ecosystems due to their high life span. Every year, pounds of plastic end up in the world's oceans. Studies indicate that there are approximately 15–51 trillion tonnes of plastics in various forms in the oceans of the world extending from the equator to the poles, from Antarctic ice sheets to the deepest ocean trenches. Sadly, not even a single area in the ocean is free of plastic.

ISBN: 978-81-953600-3-1

The problem is now growing into a great crisis. The fossil fuel industry plans to increase plastic production by 40 percent over the next decade. This means more toxic air pollution and plastic in our oceans. We need urgent action to address the global plastic pollution epidemic.

Marine plastic pollution:

We are turning our beautiful oceans into a plastic soup. The five of the Earth's major ocean gyres are filled with plastic debris, with the Great Pacific Garbage Patch as the largest one. The Great Pacific Garbage Patch, located in the north-central Pacific Ocean, is where the largest quantity of plastic debris accumulates.

Each year, about 10 million tonnes of plastic enters our marine ecosystem. Overuse of plastics and the mismanagement in their waste disposal are a danger to countless marine birds and animals, leading them to death either by entanglement or poisoning. This also leads to the chemical contamination of the fish that we eat, thus returning back to us. The large amounts of plastic wastes seen on the sea surface represent only just the tip of an iceberg. But what lies beneath is highly dangerous one. The microbeads and plastic debris are minute ones and is impossible to remove them. These particles are easily ingested by marine organisms. Once we take in this marine organism as food, these plastics enter our own body and cause graet havoc inside us. An urgent and stringent call should be made immediately to restore our marine ecosystem combining, creating awareness among the people about reducing the use of plastic commodities and to massively improve the plastic waste disposal. An efficient way to reduce plastics from reaching the oceans is us to say no to plastic commodities, or if at all, just reduce the use of plastics.

Sadly, the plastic is the main cause of the deterioration of marine ecosystem. Plastic pens and plastic straws are the most wretched example of our current use and throw culture. A large amount these single use plastic commodities each the oceans every single day, which is comparable to twice the size of the world. Each year, 192 countries that share border with the major oceans like, Atlantic, Pacific and Indian oceans, are seen to dump millions and millions of tonnes of these plastic waste into these oceans. Approximately, 250 million tonnes of this waste are plastic wastes disposed after use by people in the land, and 32 million tonnes of the mismanaged plastic waste from the coasts. The demands for plastic commodities are still on the increase and studies have shown that the plastic consumption is expected to be around 500 million tonnes/year by 2025.

It is high time to act wisely to avoid and reduce the rate of plastic flow into the ocean. If it goes unchecked, a great danger is awaiting us, in such a way that the oceans would contain 1 kg of plastic for every 3 kg of fish by 2025, and the amount of plastics will be more than the

marine organisms by the year 2050. Inadequate waste management is a significant challenge in the developing world, Due to unplanned and inadequate waste management, countries including India, with rapidly growing populations in coastal areas, are estimated to account for as much as 50% of the plastic waste entering into the marine ecosystems. Research shows that our major rivers, Ganga and Yamuna, located in heavily populated are where littering is common and waste management is poor, carry more than 50% of the global output of plastics that finally gets drained off into the seas and ocean. 95% of the plastic trash in seen in the ocean beds whereas only 5% are seen floating on the ocean surface. Plastic trash has been detected even in the Arctic and the Antarctic regions, thus revealing the sad truth that no place in the earth and Ocean is plastic free.

The majority of plastic waste in our oceans includes the billions of minute plastic particles, called microbeads, which are being added to toothpaste, face wash, floor cleaners etc. The microbeads are so small in size that they can easily pass through the filters in the sewage treatment plants, finally making their way to oceans and polluting them. Microplastics reach the oceans via the waste water drained off after the washing the synthetic fabrics, like nylon, in the washing machines. Microplastics have a size as less than 5 mm. Studies indicate that even our table salt that we use in cooking and the water that we drink contains microplastics. Switching over to the use of bio-degradable plastic, is a good initiative, but it is not apt in the natural environment, specially not in the oceans, mainly because are energy intensive and expensive. Furthermore, even in ideal conditions, biodegradability does not resolve critical issues such as entanglement, or ingestion of plastic materials by marine animals. Banning the use of plastic bags, entirely in the society would account for only 1% reduction in plastic waste.

Consequences of plastic pollution– heavy toll on marine wildlife:

Presence of plastics in our oceans has a very large impact on ocean wildlife. Plastic debris is being eaten by marine organisms like fishes, whales, turtles and seabirds, which often results in their death. Carcasses of whales were found with their bellies full of plastic. Plastic debris possess great danger to marine animals and sea birds, which, when entangled in the plastic debris, cause serious injuries and finally death. Seals and turtles are the major organisms that are greatly affected by the plastic debris. They are unable to distinguish the plastic debris as uneatable and most of the time gets caught in plastic debris like plastic bags, torn seines and lines. Moreover, plastic materials are non biodegradable, but it breaks down into minute particles, the microplastics, that is ingested by fishes, shell fishes and finally enter into the food chain.

ISBN: 978-81-953600-3-1

Fishes ingest tons of plastic each year that causes damages and injuries in their intestines and finally death. They also transfer these plastic debris up in the food chain to a bigger fishes, which then continue to large marine mammals and finally to human beings. Plastic debris contains toxic chemicals which are used for their manufacture, Most of these chemicals such as polychlorinated biphenyls or PCBs and phthalates are found to be cause endocrine disruption and some are even carcinogenic. The peculiarity of plastic pollution is that it is nearly impossible to remove it completely from the environment or trace back to its origin. Lots of scientific researches have shown that these harmful substances can enter the tissue of fishes and finally reach back to us, on consumption.

Sea turtles feed on floating plastic bags, mistaking it as edible food. They get entangled, choked, sustain internal injury and may even die or sometimes even starve by getting these plastics fill their stomachs. Sea turtles are the major marine organism that possesses greatest danger from the plastic pollution of marine ecosystem. They are the major organisms that ingest the plastic debris floating the oceans. Researchers have shown that the plastic littering of the beaches and shores have a negative troll on their reproductive cycle.

Thousands of seabirds are in great danger of extinction by ingesting plastic every year. Intake of plastics in their diet reduces their stomach volume, finally causing starvation. Carcasses of seabirds are seen with stomachs filled with plastic debris from tha land, which gives an insight about the large amount of plastic garbage in our oceans which has risen rapidly in the past 40 years.

Action plans undertaken globally:

There is growing awareness an the impact of plastic on our marine ecosystems. It has led to increase in constituting action plans both nationally and internationally. As a first resort, the UN, in 2017, has launched a campaign program, “Clean Seas”, to curb plastic wastes from reaching the oceans. European Union has started an action plan against plastic waste, to clean up the existing plastic wastes as possible and ensures each and every piece of plastics produced in Europe is either reusable or recyclable by the year 2030. G7 leaders, in June 2018, adopted the “Oceans Plastics Charter”. In the G20 Summit held in Osaka in June, 2019, Japan has advised the adoption of an obligatory action framework to reduce and tackle the plastic marine pollution. Many countries have even banned the use of plastic bags and use of microbeads in cosmetics and detergents.

Business economy has also taken part in reducing the use of plastics. As part of the New Plastics Economy Global Commitment, industries that are produce 20% of plastic packing products have taken pledge that they shall eradicate plastic waste and reduce plastic pollution by

2050. Many countries have taken up initiatives to reduce plastics from reaching the marine ecosystems, by organising beach clean-up programmes with local support. NGOs have created many public awareness short films about the plastic pollution and their impacts on the ecosystem, thus helping get the message out of the bottle.

What needs to happen?

In order to prevent the entry of plastics into the ocean will require a combined action, which includes mainly the reduction in the use of plastics, proper waste disposal and waste collection of used plastics, and expand recycling facilities. We should change our view from a linear economy, viz. manufacture, use, dispose, to circular economy where resources, are used, and reused over and over again, instead of heading directly to the landfill. The most efficient way to reduce the inflow of plastics into the oceans is to reduce its use.

Corporate action:

We need to urgently reduce single-use plastic, and while companies' support for initiatives that help recycle and clean-up plastics are important, the key solution is for them to urgently introduce alternative sustainable packaging.

Government leadership:

Efficients efforts are required to be taken by governments to take leadership on environmental policy in order to tackle this global havoc. An immediate measure needed to reduce the barriers to finance better waste management in order to reduce the plastics from reaching the ocean.

Personal responsibility:

Each and every citizen has to personally responsible for the mindful use of plastic commodities. In order to limit the use of plastics, as a first step, we use a reusable water bottle or tea cup, take our own cloth bag or reusable bag for shopping, cut down the consumption of single use plastic commodities such as use and throw pens, plastic food packaging, plastic straws and make sure that whatever plastic materials we use, are recyclable.

Education and public awareness:

Some governments have incorporated education about plastics, waste management and recycling into their school curriculums. This is a helpful strategy and public education efforts should be amplified. But they also need to go further, faster to incentivize businesses to change and to also adopt procurement policies that reduce their plastic footprint.

Fighting ocean plastic pollution – in a nutshell:

1. Switch to reusable products (non plastic preferred)

ISBN: 978-81-953600-3-1

2. Use of paper bags and biodegradable bags
3. Reusable water bottles
4. Switch to natural fibres rather than synthetic ones
5. Refuse plastic straw, when you are offered with a drink-
6. Ask restaurants for compostable packing.
7. Recycle and Reuse and refuse
8. Ban problematic consumer products like polystyrene/Styrofoam in foodware and packing
9. Ban cosmetics that contains microbeads
10. Clean up initiatives
11. Organise beach and river cleanups
12. Recover the lost fishing gears

And Help the earth to rejuvenate….

References:

Jambeck J. R., B.D. Hardesty, A. Brooks, T. Friend, K. Teleki, J.Fabres, Y. Beaudouin, A. Bamba, J.Francis, A. Ribbink, T. Baleta, H. Bouwman, J. Knox, C.Wilcox (2017): Challenges and emerging solutions to the land-based plastic waste issue in Africa. Marine Policy.

Jambeck J. R., R. Geyer, C. Wilcox, T. R. Siegler, M. Perryman, A. Andrady, R. Narayan, and K. L. Law (2015): Plastic waste inputs from land into the ocean, Science, Volume 347, pp 6223

McKinsey Center for Business and Environment and Ocean Conservancy (2015): Stemming the tide: land-based strategies for a plastic-free ocean.

Schmidt C, T. Krauth, S. Wagner (2017): Export of Plastic Debris by Rivers into the Sea, Environmental Science and Technology, vol.51, pp 12246-12253.

UNEP (2015): Biodegradable Plastics and Marine Litter, Misconceptions, concerns and impoacts on Marine Environments, United Nations Environment Programme.

UNEP (2018): Single-use plastics: A roadmap to Sustainability.

World Economic Forum (2016): Ellen MacArthur Foundation and McKinsey & Company, The New Plastics Economy: Rethinking the future of plastics.

BIOTECHNOLOGY FOR INNOVATIONS IN SUSTAINABLE AQUACULTURE AND FISHERY

J. P. Sarwade[1], R. V. Kshirsagar[2] and S. J. Mankar[2]*

[1]Post Graduate Department of Zoology,
Arts, Science and Commerce College,
Indapur, Maharashtra, India

[2]Research Centre in Zoology,
Modern College of Arts, Science and Commerce,
Ganeshkhind, Pune, Maharashtra, India

*Corresponding authors E-mail: saritahupare24@gmail.com

Abstract:

Aquaculture is the fastest emerging food sector in the world as the consumption of seafood is increasing worldwide with its increasing role in the economy and safe food strategy of countries. Because of the constant depletion of fish stocks, and aquaculture, fortunately, there have been a lot of innovations and technology developed recently geared towards the longevity and sustainability of consumed aquatic products. Biotechnology options appear to represent good potentials for increasing aquaculture productivity, food security and environmental quality around the world. Valuable options such as gene and genome mapping, protein expression, microsatellite, DNA chips, DNA vaccines, proteomics, embryonic stem cells and transgenic technology, etc. are offered by biotechnology. This technology provides molecular cloning, genetic manipulation, transgenic diagnostics, embryo manipulation, and immunoprotective agents. All of these applications can help improve the selective breeding, hybridization, productivity, health, growth, nutrition, cryopreservation, and conservation of genetic resources in aquaculture stock for the benefit of mankind (Muhammet *et al.*, 2012).

Keywords: Transgenic technology, embryo manipulation, selective breeding, hybridization,

Introduction:

Modern biotechnology in aquaculture has provided many opportunities to increase production and improve the quality of fresh and processed farmed species. In addition, cultured species are now being developed for disease resistance, and this will reduce losses and allow for increased production in the same area, thus bringing potential benefits to rural areas (Muhammet *et al.*, 2012). Finally, biotechnology can make a significant contribution to the aquaculture

ISBN: 978-81-953600-3-1

industry, for example by helping to achieve more diversification into farmed species that will be more attractive to consumers. Modern biotechnology will be instrumental in the genetic improvement of cultured aquatic species and the protection and management of wild aquatic populations.

The biotechnology can make a significant contribution to improving the yield of aquaculture and thus aquaculture can help to meet the growing demand. Aquaculture animals are particularly suitable for research in biotechnology. Experimentation is facilitated by the availability of large numbers of gametes (germ cells), the use of external fertilization, and the ease of raising embryos in the laboratory. In addition, during development many aquatic animals can be treated with hormones to induce functional sex reversal or infertility and therefore simplify experimental methods. The agenda of modern biotechnology in aquaculture appears to be very similar to that of livestock and agriculture. In the recent past years, remarkable achievements have been made in increasing livestock and production of aquaculture through biological and genetic technological tools.

Potential areas of biotechnology in aquaculture include the use of synthetic hormones in inducible reproduction, monosex production, uniparental and polyploidy production, molecular biology, transgenic fish, gene storage, improved feed and health management and the development of natural products from marine organisms**.** Marine biotechnology can be defined as the use of marine biological resources as a target or source for biotechnology applications. This includes marine organisms, or parts thereof, used as feedstock, for example as food, fuel, or bioactive substances or compounds OECD, Marine biotechnology (2014). Moreover, the term "marine biotechnology" has been used extensively in the international literature to also include fishing and fish processing activities (Karlsen and Isaksen, 2011). Biotechnology is required to cultivate and isolate the unique bioactive compounds regardless the wide possibilities of utilization of marine organisms in the food industry (Rasmussen and Morrissey, 2007). In this article, we discuss recent developments and upcoming research on aquaculture and fishery by using tools and techniques in biotechnology.

Techniques:

1. Genetically modified species (GMO)

As a major application of modern transgenic biotechnology, there are new species of species bred by cross-breeding of two strains in order to transfer the desired traits from each to the new variety to improve the genetic traits of the species used in aquaculture. The genetically modified species involve the selective transfer of one or more genes for desirable traits such as

improved growth rates, larger size, and more efficient conversion of forage to muscle and control of sexual maturity from one cultivar to another. Growth hormone genes from human or animal sources have been successfully introduced into many fish species such as salmon, trout and tilapia, resulting in growth many times faster than their natural counterparts.

This is a more accurate and rapid method of breeding new varieties. For example, better tolerance to environmental stresses and increased resistance to harsh environments are important applications for establishing unique properties and producing a valuable biological product such as antifreeze protein (AFP) gene transfer in fish for adaptation to a freezing environment (Hew *et al.*, 1992). The intensification and sustainable development of aquaculture will depend on disease prevention, thus biotechnology is an essential means of greater resistance to pathogens and improvement of health of farmed species through selection for disease resistance (Al-Zaeem and Asim, 2004). There are two most important methods of transmitting genetic material in fish. These are ICSI (injection of genetic material into newly fertilized fish eggs) and electroporation (transfer DNA into embryos or directly into tissues through the use of an electric current).

Risk:

However recent concerns about genetically modified species and food products derived from them may curtail their widespread use. GMOs have not gained worldwide acceptance. Horizontal gene transfer from transgenic plants to microbes has been demonstrated in a previous study that genes from transgenic crops move rapidly to the herbaceouspopulation which may limit their widespread use. GMOs have not gained universal acceptance. Horizontal gene transfer from transgenic plants to microorganisms has been demonstrated in a previous study that genes from transgenic crops are rapidly transferred to the herbaceous (Snow *et. al.*, 1999).

2. Hormonal applications

Biotechnology tools can be used to induce early development of aquatic species and fish breeding. Gonadotropin-releasing hormone (GnRH) is now the most widely used in induced reproductive processes of fish and is commercially differentiated worldwide (Alok *et al.*, 2000). Hormone therapy is applied to improve and control reproductive cycles during domestication and may provide techniques to improve reproductive success and survival of endangered species, thus helping to conserve biodiversity in the wild. The high growth of species in the aquaculture sector is one of the main objectives of the farmers, and therefore different types of growth hormone are used in fish and other aquatic animals.

Risks:

Hormones are issues of great public concern because they pose a serious threat to humans and the environment. Hormone therapy also induces triploidy and infertility.

ISBN: 978-81-953600-3-1

3. Chromosome set manipulation/Chromosome Engineering:

Chromosome manipulation has been widely applied in fish farming improvement for gonad sterilization and control of sex and reproduction. An important practical method in cultured fish species is to induce polyploidy and monoparous chromosomes. There are two types of uniparental inheritance: generation which is the process of development with the inheritance of the mother and the process of formation of masculinity that takes place through the father (Muhammet *et al.,* 2012). Polyploidy is used to induce the production of sterile strains, and this technique can also be used to generate homozygous strains in fish such as tilapia, carbonate and salmon.

Sex chromosome manipulation techniques to induce polyploidy (triploidy and tetraploidy) and uniparental chromosome inheritance (androgenesis and gynogenesis) have been widely applied in farmed fish species (Lakra and Das, 1998; Pandian and Koteeswaran, 1999). These techniques are important in improving fish farming because they provide speed. The methods used to induce manipulation of triploids and other types of chromosome sets in fish, and the applications of these biotechnologies in aquaculture and fisheries management are well described (Purdom, 1983; Chourrout, 1987; Allendorf and Thorgaard, 1984; Pandian and Koteeswaran, 1999).

These techniques are important in improving fish farming because they provide a rapid approach to sterilizing the gonads, controlling sex, and improving hybrid viability and reproduction. Tetraploid breeding lines are of potential interest to aquaculture, by providing a convenient method to produce large numbers of sterile triploid fish through simple hybridization between tetraploid and diploid (Chourrout *et al.,* 1986; Guo *et al.,* 1996). Although tetraploidy is induced in many species of fin fish, the viability of tetraploids was low in most cases (Rothbard *et al.,* 2006).

4. Cryopreservation:

Cryopreservation is a process in which biological materials are preserved over the long term by cooling to low temperatures typically at -196°C in liquid nitrogen. Since any physiological activities and biochemical reactions are effectively calmed and stopped at these lower temperatures, which makes it possible to keep them viable for a long time. Anti-freeze solutions are used in the process to prevent the preserved cells from being damaged by freezing during the cooling and thawing process. The development of cryopreservation technology

provides short-term and long-term storage of gametes, thus the technology has been adapted for the cryopreservation of spermatozoa and semen of fish.

5. Aquatic species health and vaccines:

Biotechnology tools such as genetic probes and polymerase chain reaction (PCR) have shown great potential in the health of aquatic animals. Genetically modified (DNA) vaccines are also being developed to protect fish from pathogens. In general, DNA vaccines contain only the genes of the pathogen that produce the antigen. The cost of this technique is low compared to the production of weakened organisms. These vaccines are more stable at normal temperatures and have been adopted for aquaculture to improve the health and well-being of farmed aquatic organisms. The development of new vaccines helps living organisms to recognize and combat diseases prevents the use of chemicals in aquaculture, and means prevents chemical pollution in the environment and potentially dangerous effects on human health.

Risk:

However, genetically modified vaccines carry a great deal of unpredictability and a number of potential and inherent harmful risks such as potential risks around the vaccine DNA to invade the host genome and possibly induce genes related to tumor development.

6. Hybridization:

The hybridization between the haploid genomes of two different parental species belonging either to the different genera (intergenerational) or same genus (interspecific) and even between two lineages of the same species (unspecified) to produce hybrid species is called as hybridization. Crossbreeding generally brings together potentially desirable positive traits like better growth, disease resistance, meat quality, early or later maturity, better fecundity, etc. in its offspring.

7. Genetic Manipulations:

Genetic manipulation is a modern approach to producing inbred strains and improving individuals through male reproduction and polyploidy. These are the most viable tools for producing highly homozygous strains of a monosex individual.

8. Surrogacy:

The alternative brood stock technology or spawning flock technique consists of producing donor-derived gametes in an surrogate fish by transplanting germ cells from a donor

ISBN: 978-81-953600-3-1

into a recipient of different strain or species (Yoshizaki and Yazawa, 2019). This technique can be facilitated by transplantation of a cell suspension from testis or ovary containing germ line stem cells, which will finally become sperm or eggs, respectively, into larvae immediately after hatching. After implantation into the intraperitoneal cavity by a fine glass pipette, they spontaneously migrate to the immature testis and ovary, where they fuse and initiate spermatogenesis and formation Oocytes, respectively (Fig. 1). The implantation of germ line cells involves a very simple microscopic process using a stereomicroscope and a coarse motion microprocessor.

Figure 1: Microinjection of donor-derived germ line stem cells into abdominal cavity of rainbow trout hatchling

9. Neo female technology:

Neo-female is a technique of obtaining females through a male gender reversal that produces all male offspring (Jena *et al.*, 2017). In these techniques, the sex of young males is changed through microscopic removal of androgenic gene silencing (RNA interference method) of females called “neo females”. In India, this neo female technology project was implemented by RGCA, Tamil Nadu.

10. Genetically modified plants as a fish feed

Genetic modification has been successfully used to introduce new traits into crop plants with novel complement DNA through non-homologous final linkage. The novel GM protein can affect the efficiency of animal consumption of plants. Various types of GM plants such as GM soybean (RR1), GM, and transgenic cotton have been successfully evaluated in the diets of Atlantic salmon and zebra fish, respectively.

Significance:

Biotechnology in aquaculture is now developing very rapidly and contributes significantly to the development of this sector. In general, technology promises huge gains in food security, economic issues and environmental protection. Efforts are directed towards:

Improving the quality of fish feed; Genetic engineering in order to improve the protein value of fish etc. Biotechnology is an important key to aquaculture, but it may be too early to judge the future impact of biotechnology on its sustainable development.

Referances:

Allendorf and Thorgaard, (1984): F.W. Allendorf, G.H. Thorgaard, Tetraploidy and the evolution of salmonid fishes, B.J. Turner (Ed.), Evolutionary genetics of fishes, Plenum Press, New York , pp. 1-53

Alok D., Talwar G.P.,Garg L.C.(2000): In vivo activity of salmon gonadotropin releasing hormone (GnRH), its agonists with structural modifications at positions 6 and 9, mammalian GnRH agonists and native GnRH-II on the spawning of an Indian catfish,Aquaculture International 7, 383-392.

Alok Kumar Jena, Soibam Khogen Singh, Pradyut Biswas (2017): Advanced Biotechnological Innovations in Aquaculture for Intensification (50).

Al-ZaeemS.Y., Aseem S.S.(2004): Application of biotechnology in fish breeding: 1 – production of highly immune genetically modified Nile, tilapia Orechromisniloticus with accelerated growth by direct injection of Shark

Amir, S. (2011): Monosex culture of prawns through androgenic gene silencing. INFOFISH International.22-24.

Ayyappan, S. (2011): Hand book of fisheries and Aquaculture. Indian council of Agricultural Research, New Delhi.

Chourrout, D, (1987): Genetic manipulation in fish:review of method. In k Tiews (ed), selection, hybridization and genetic Engineering in aquaculture Bordeaux Heenemann , Berlin, 2:111-126

Fernandez, (2015): “Surrogate Technology: The Future of Aquaculture.” Atlas of Science-another view on science.

Francis, T., Devi, C. A. and Selvamagheswaran, M. (2013): lndian Journal of Science and Technology.ISSN: 9Zz}-68q6; Vol 6 (5)

Guo, G.A. DeBrosse, S.K. Allen,(1996): All-triploid Pacific oysters (Crassostreagigas Thunberg) produced by mating tetraploids and diploids. Aquaculture, 142 (3–4) pp. 149-161

HEW. C.L. AND FLECHERE-(Eds) (1992): Transgenic Fish.World Scientific Publishing Co.Singapore.

Karlsen J, Isaksen A, Spilling (2011): The challenge of constructing regional advantages in peripheral areas: the case of marine biotechnology in Tromsø, Norway.EntrepRegDev;

23:235–57.

Lakra, W. S. and Das P., (1998): Genetic engineering in aquaculture, Indian J. Anim. Sci., 68(8): 873-879.

Liao, I.C. and N.H. ChaO. (1997): Developments in aquaculture biotechnology in Taiwan. 1. Mar. Biotechnol. 5:16-z.

Muhammet, Altunok PekerZerife,SerezliRamazan,TekinayAhmetAdem,KizakVolkan,(2012): Biotechnology and Aquaculture in Sustainable Development,Conference: 3rd International Symposium on Sustainable Development (ISSD2012). Sarajevo, Bosnia and Herzegovina.

OECD. Marine biotechnology: enabling solutions for ocean productivity and sustainability. OECD,Paris,publishing;http://dx.doi.org/10.1787/9789264194243-en[accessed 04.10.14].

Pandian T. J., R. Koteeswaran,(1999): Natural occurrence of monoploids and polyploids in the Indian catfish, Heteropneustesfossilis, Currentence, 76 (8), pp. 1134-1137

Pandian, T.J.and S,G.Sheela (1995): Hormonal induction of sex reversal aquaculture,138:1-22.

Rasmussen, R.S. and Morrissey, M.T. (2007): Marine Biotechnology for Production of Food Ingredients. In: Taylor, S.L., Ed., Advances in Food and Nutrition Research, 52, Elsevier, New York, 237-292.

Rothbard S. (2006): A review of ploidy manipulations in aquaculture: The Israeli experience, The Israeli Journal of Aquaculture = Bamidgeh, 58 (4) ,pp. 266-279

Sissener *et al.* (2011): Genetically modified plants as fish feed ingredients. Canadian Journal of Fisheries and Aquatic Sciences (Can. 1.Fish.Aquot.Sci.) 68, 56

Snow, A.A, Andersen, B, Jørgensen, R.(1999): Costs of transgenic herbicide resistance introgressed from Brassica napus into weedy B. rapa. Molecular Ecology 8:605–615.

Wikimedia Foundation Inc. Encyclopedia on-line. Available from https:// en.wikipedia.org/wiki/AquAdvantage salmon.

World Fish. (2004): GIFT Technology Manual: An Aid to Tilapia Selective Breeding. Penang, Malaysia: World Fish.

Yoshizaki, Goro and Ryosuke Yazawa (2019): Application of surrogate brood stock technology in aquaculture,Fisheries Science ,85:429–437

TAXONOMIC WORK OF THE GALL MIDGES IN INDIA

K. A. Ahad Najam

Department of Zoology,

Shankarrao Chavan Mahavidyalaya,

Ardhapur, Dist. Nanded 431704, MS, India

Corresponding authors E-mail: dr.kan09@gmail.com

Studies of taxonomy of the gall midges were initiated in India by Schiner in 1886, when he described *Lasioptera byyoniae*. Later on wood masion (1881) recoreded well know silver shoot gall Midges of paddy under the name Cecidomyia oryzae from kharagpur, Kieffer (1907) describe some Bengal species and later a large number of the midges from India. He has also described of the few species of Ceylon in 1912. Rao (1917) midge made extensive collections of the midge flies and made observation of the food habits of the numerous grass infesting midges. Brunetti (1929) published a list of the Indian gall midges known up to 1920. Felt (1921) described 31 Indian midge species from 21 different grasses. Senior White (1928) compiled available information on the gall midges described from the subcontinent.

The major contribution on Cecidomyiidae was started in 1926 when Dr.M.S.Mani began work on this group. Trained in Taxonomic work with Dr. H.S. Pruthi and inspired by worker like kieffer, Felt and Barnes, he made significant contributions to taxonomic study of the galls and the gall formers especially Cecidomiids. He worked on this group for nearly 40 years. Mani (1934 to 1974) erected seven new genera and described sixty one new gall midge species collected from different host plants. Mani (1946) published the key to genera of midges from oriental region to replace the earlier keys prepared by Kieffer (1913) and Felt (1925) along with the text figures. He erected the genus *Amrodiplosis* in 1947 and enlisted 16 midges infesting mango, out of which 13 were identified. He explored the midge fauna of south India, high altitudes of Himalaya and also described pusa collections. He reported many economically importance midges on different cultivated plants.

Nayar (1944, 1945, 1947, 1948, 1949) erected two genera and described 15 species mostly from south india. Rao (1971) described four new genera 43 new species. He (1955) published a catalogue on oriental Itonodidae to replace earlier catalogue of Brunetti (1 920) and

ISBN: 978-81-953600-3-1

senior White (1928) Rao and his associate workers like Adwant, Deshpande, Sharma, and shivpuje, explored gall midge fauna of Marathwada region of Maharashtra state.

Rao and Gerover (1959) reported two new species of the gall midges. Rao and adwant (1970, 1971 and 1975) described one allotype of *Lestremia Sancti –Johanni* Roa, nd three new species from Aurangabad. Rao and Sharma (1977, 1978, and 1981) described one new genus and 5 new species from Marathwada. Grover P. (1961, 1962, 1963, 1964, 1965, 1966, 1967, 1968, 1970, 1971, 1972, 1973, 1975, 1979) described four new genera 67 new species on different host plants. She (1975) published the key to the genera of the gall midges from the oriental region and revised tribes *Lestremiini*, *Laseopterini Porriycondylini*, *Oligotrophini* and *Aspondylini* and *Cecedomyiini*. Grover (1981) prepared catalogue for gall midges for India. Grover and Bakshi (1977-78) erected a new genus and 31 new species. They reported new gens *Chanchudiplosis*.

Deshpande *et a.*, (2002) reported one wnew species and two new genera *Garudiplosis Kitella* beside four new species of Anaretella (Enderlein from Maharashtra. He also described a new gall on Garuga pennata, a forest tree from Bhokar forest of naned district (M.S).

Gagne (1973, 1975, 2004) published the catalogue of family Cecidomyiidae of the oriental region. He reported 92 genera and 332 species as valid species and 70 unplaced species. He synonymiesed the genera prolasioptera Rubsaamen and Bimba Grover with *Lasioptera* Meigen; genus oresolla Kieffer and Massalango; genus Amraemyia Rao with *Amrodiplosis* Mani; indodiplosis Felt with procontarinia Kieffer and Cocconi; genus *Vanchidiplosis* Nayar withStomatosema Kieffer. Sharma and Rao (1978, 1979, 1980, 1981) described one new gens and 9 species from Marathwada. They (1981) published checklist of gall midges of the marathwada region.

Sharma R.M. (1980, 1984, 1986, 1987, 1988, 1993) worked on gall midge fauna of Marathwda. (1989) published a paper on midge gall of Andaman Islanand. This paper deals with the exomorphic description of 35 midge galls with 24 plates. These 35 galls are collected from 25 different localities of Andaman Island of these 35 galls , 27 are new to the India. Key and gall index are provided in the paper. This is the first report of galls from Andaman Island. He has also reported many new gall midges from pune, Westrenghat, Raigad, and Melghat reservoir (Maharshtra), Nilgiri reservoir (Karnataka) and Hissar (Haryana). He published a check list of plants gall of India (2003) that occurs in Maharashtra. He has reported the occourance of 379 species from 138 genera from India (2006)

Deshpande *et al.* (2002, 2003) reported two new genera *Garugadiplosis and Kitella* Nanded (Maharashtra). They also reported revision of genus *Anaretella Enderilen*. A new midge

gall occurring on *Garuga pinneta* Roxb was reported from Bhokar forest of Nanded (Maharashtra), which is the first midge gall on the host tree.

References:

Barnes, H. F. and Nayar, K. K. (1954): The Black Medick or "Trefoil" gall midge. Plant Pathology. 3 (2), pp. 51-54.

Brunetti, E., (1920): "Catalogue of Oriental and South Asiatic Nemocera" ,Reo. Ind. Mus., XVII, pp. 8-18, 267-271.

Deshpande V. D., T. H. Shaikh and R. M. Sharma (2002): A new genus a]~d new species of gall midge [cecidomyiidae : diptera] infesting garuga pinnata roxb., [burseraceae] from india. Rec. Zoo. Surv. India: 100 (Part 3-4): 111-115.

Deshpande V. D., T. H. Shaikh and R. M. Sharma (2005): A New Genus And New Species Of Gall Midge [Cecidomyiidae : Diptera] From India. Rec. Zoo. Surv. India: 104 (Part 1-2) : 79-83.

Felt, E. P. (1929): Gall midges or gall gnats of the orient Itonididae or Cecidomyiidae. Lin. Sci. J., 7 : 413-474.

Felt, E.P. (1921). Indian grass gall midges. Mem. Dept. Agric. India, Entomol. 7(3): 15-22.

Gagné R. J. (2004): A Catalog of the Cecidomyiidae (Diptera) of the World. Memoirs of the Entomological Society of Washington No. 25. pp 408.

Grover P, Bakhshi M. On the study of one new genus and thirty-one new species (Cecidomyiidae: Diptera) from India. Cecidologia Indica. 1978; 12-13:5-267.

Grover P. (1981): Studies on gall-midges of India XLIV: A catalogue of Indian gall-midges. Cecidologia Internationale. 2:63-108.

Grover, P. and Prasad, S.N. (1976). Bionomics of 'silver shoot' gall fly , Pachydiplosis oryzae (Wood-Mason), a pest of rice in India. Final Technical Report of PL480 Project No. A7-Ent-97; Dept. of Zoology, Univ. of Allahabad, Allahabad, India, 78 + 3pp. and xxv Tables + xix Plates.

Grover.P. Studies on gall midge of India. 36 A revision of India Lasiopterini. Cecid. Indica,1972, 7, 123-133.

Jaschhof, M. (1998): Revision der "Lestremiinae" (Diptera,Cecidomyiidae) der Holarktis. – Studia Dipterologica, Suppl. 4: 1–552.

Kieffer, J. J., (1907): Eine neue endoparasite Cecidomyide. Zeitschrift für systematische Hymenopterologie und Dipterologie, 7: 129-130.

ISBN: 978-81-953600-3-1

Mani, M. S. (1946): Studies on Indian Itonididae [Cecidomyiidae : Diptera] Key to the genera from the oriental region. Indian J. Ent., 7(1+2) : 189-235.

Mani, M. S. (1953): Descriptions of three gall midges (Itonididae: Diptera) from India. Indian Journal of Entomology 15(2): 119-125.

Mani. M.S. (1934): Studies on Indian Itonididae (Cecidomyiidae:Diptera). Rec. Indian Mus., 1934, 36, 371-452.

Panelius, S. (1965): A revision of the European gall midges of the subfamily Porricondylinae (Diptera: Itonididae). –Acta Zoologica Fennica 113: 1–157.

Rao, Y.R. (1917). Notes on some South Indian Cecidomyiidae causing galls in grasses. J. Asiat. Soc. Bengal 13: 299-306.

Rao, Y.S., Israel, P., Yadava, C.P. and Roy, J.K. (1971). Nature of gall midge resistance in rice. Curro Sci. 40: 497-498.

Senior-White, R., (1928): "Ceoidomyidae" Cat. Ind. Ins., pt. 15, pp. 1-23.

Sharma R. M. (2009): Checklist of Indian Gall midges (Diptera: Cecidomyiidae). http://www.zsi.gov.in/zoologicalsurvey of india /zsidata/checklist.

COMPARATIVE STUDY OF MICRO ALGAE IN PRODUCTION OF BIODIESEL AT LABORATORY CONDITION

Y. K. Gawai[1] and S. T. Naphade[2]*

[1]Department of Biochemistry,

SBES College of Science, Aurangabad (M. S.) India.

[2]Department of Zoology,

Yeshwantrao Chavan College, Sillod, Dist. Aurangabad (M. S.) India

*Corresponding authors E-mail: drsudhirn11@gmail.com

Abstracts:

Presently the world's energy needs are met through non-renewable resources such as petroleum, natural gas and coal. Since the demand and cost of petroleum based fuel is growing rapidly, and if the present pattern of consumption continues, these resources will be depleted in few years. Hence, efforts are being made to explore alternative source of energy. Biodiesel is biodegradable, less CO_2 and NO_2 emissions. Continuous use of petroleum sourced fuels is now widely known as unsustainable due to depleting supplies and therefore the contribution of those fuels to the buildup of CO_2 within the environment. Renewable, carbon neutral, transport fuels are necessary for environmental and economic sustainability. Algae have emerged together of the foremost promising sources for biodiesel production. It can be inferred that algae grown in CO_2 enriched air can be converted to oily substances. Such an approach can contribute to unravel major problems of pollution resulting from CO_2 evolution and future crisis thanks to a shortage of energy sources. This study was undertaken to understand the right transesterification, amount of biodiesel production (ester) and physical properties of biodiesel. In this study we used common species Oedogonium and Spirogyra to match the quantity of biodiesel production. Algal oil and biodiesel (ester) production was higher in Spirogyra sp than Oedogonium. However, biomass (after oil extraction) was higher in *Spirogyra* than *Oedogonium* sp. Sediments (glycerine, water and pigments) was higher in *Spirogyra* than *Oedogonium* sp. There was no difference of pH between *Spirogyra* and *Oedogonium* sp. These results indicate that biodiesel can be produced species is better source *Spirogyra* sp.

Keywords: Algal oil, biodiesel, transesterification, glycerine

ISBN: 978-81-953600-3-1

Introduction:

The necessity of energy is increasing constantly, due to increase in industrialization and population explosion. The Basic sources of energy are fossil fuels (petroleum, coal and natural gas), hydro and nuclear however, fossil sources are limited and will be exhausted by near future. Biodiesel may be a biofuel consisting of monoalkyl esters that are derived from organic oils, plant or animal, through the method of transesterification. It is also biodegradable, nontoxic and has low emission profile as compared to petroleum diesel. In fact algae are the very best yielding feedstock for biodiesel. It can produce 250 times quite the quantity of oil per acre as soybeans. In fact biodiesel from algae could also be the sole thanks to produce enough automobile fuels to exchange current gasoline usage. Algae produce 7 to 31 time greater oil than vegetable oil. The best algae for biodiesel would be microalgae. Microalgae have far more oil than macroalgae and it's much faster and easier to grow and harvest. The use of microalgae are often an appropriate alternative because algae are the foremost efficient biological producer of oil on the earth and a flexible biomass source and should soon be one among the Earth's most vital renewable fuel crops. Higher photosynthetic efficiency, higher biomass production, a faster growth rate than higher plants, highest CO_2 fixation and O_2 production, growing in liquid medium which can be handled easily make the algae to face high ahead of other oil seed crops. Their production isn't seasonal and may be harvested throughout the year. As a matter of fact, average oil yield from microalgae are often 10 to twenty times above the yield obtained from oleaginous seeds and/or vegetable oils. Different types of biofuel can be derived from microalgae. These include methane produced by microalgae oil and photo-biologically produced bio-hydrogen.

Bioenergy is one among the foremost important components to mitigate greenhouse emission emissions and Substitute of fossil fuels. The need of energy is increasing continuously, because of increases in industrialization and population. The basic sources of this energy are petroleum, natural gas, and coal, hydro and nuclear. The major disadvantage of using petroleum based fuels is atmospheric pollution created by the use of petroleum diesel. Petroleum diesel combustion is major source of greenhouse gas (GHG). Apart from these emissions, petroleum diesel is also major source of other air contaminants including NO_2, SO_2, CO_2, particulate matter and volatile organic compounds. Biomass is one among the higher sources of energy. Large-scale introduction of biomass energy could contribute to sustainable development on several fronts, environmentally, socially and economic. Biodiesel (monoalkyl esters) is one among such alternative fuel, which is obtained by the transesterification of triglyceride oil with monohydric alcohols. It has been well-reported that biodiesel obtained from canola and soybean, palm,

sunflower-seed oil, algal oil as a diesel oil substitute. Biodiesel may be a nontoxic and biodegradable alternative fuel that's obtained from renewable sources. Biodiesel fuel are often prepared from waste vegetable oil , like palm, soybean, canola, rice bran, sunflower, coconut, corn oil, fish oil, chicken fat and algae which might partly decrease the dependency on petroleum-based fuel. The burning of an enormous amount of fossil fuel has increased global warming. Biomass has been focused on as an alternative energy source, since it is a renewable resource and it fixes CO_2 in the atmosphere through its combustion has no impact on the CO_2 balance in the atmosphere, because the CO_2 emitted by the burning of biomass is offset by the CO_2 fixed by photosynthesis.

Algae:

Algae are organisms that are like plant and vegetables. They are commonly found living within the sea, rivers, lakes or ponds. All algae make energy from the sun. There are two different types of algae and they are called “Macroalgae” and “Microalgae”. Macroalgae is commonly known as “seaweed”. The Word “macro “means big so you'll consider an enormous plant that lives within the sea. Microalgae are often called “phytoplankton”. They are so small that you simply are going to be unable to ascertain them within the water together with your eyes. They are normally viewed under a microscope. Macroalgae (Seaweed) is a multicellular organism. This means that they contain many cells that let the macroalgae function. The main parts of the macroalgae are “the holdfast”, “the blade”, “the frond”, “the stipe”, “the thallus”, “the mid-rib “and “the air-bladders”.The blade is like the leaf of a tree and the stipe is like the stem of a flower, if present. This is Algae who will be giving us information on macroalgae. This is Mike who are going to be giving us information on microalgae. The stipe transports nutrients to the holdfast. A collection of blades are known as a frond. The body of the macroalgae is known as the thallus. Some macroalgae have air-bladders to assist them float. The air-bladders contain gas and help lift the macroalgae to the surface so that the organisms can get sun light. Other macroalgae that do not have air-bladders have long stipes to help them float to the surface. Some macro algae have midribs which are located in the centre of the fronds. Macroalgae are divided by their colour into brown, green and red macroalgae. The colors of the macroalgae are due to the different pigments within the organisms. There are brown, green and red pigments within the various macroalgae. All macroalgae have green pigments in order that they can make energy from the sun. Maerl is another type of marine alga. It has a red colour and forms a tough crust. Maerl is small in size and you could hold it in your hand. Unlike other macroalgae they are not attached by a holdfast but live on the sea bed with the sand. Microalgae are small floating organisms that contain one cell and so are called “Unicellular” organisms. The cell is surrounded

ISBN: 978-81-953600-3-1

by a cell wall. Microalgae can make their own energy and store their energy in the cell Microalgae are different in their size, shape and colour. They are very small size, usually one quarter of a mille meter.The colour of the microalgae cell depends on their pigments. They can either appear blue-green, yellow, brown or orange. The two main sorts of microalgae are "Diatoms" and "Dinoflagellates". Diatoms are a type of microalgae. They have different shapes. Some are shaped like spheres, elliptical (shaped like a rugby ball), triangles and other diatoms may be shaped like stars. They contain tiny amounts of oil within their cell. The oil helps them move within the water to seek out their food and nutrients. They are weak swimmers therefore the water currents help them to maneuver. A diatom cell is surrounded by a silica shell which makes the cell wall. The silica seems like glass and is employed to guard the cells. The cell membrane within the diatom is sort of a box with an overlapping lid. They cannot move themselves so they float free. The second sort of microalgae is named dinoflagellates. Most dinoflagellates have two "flagella" which appear as if short tails that help them move through the water. One flagellum is wrapped round the cell and therefore the other is visible and helps the microalgae move. They can also use the oil within their cell to sink or swim. Dinoflagellates are surrounded by shell although which they use for protection. Certain sorts of dinoflagellates glow within the dark in the dark when disturbed. Diatoms and dinoflagellates grow very quickly and enormous amounts are called algal bloom. Algal blooms can cause problems for the environment. Both of these microalgae have the ability to cause food poisoning. Shell fish may eat the microalgae as their food and if humans then eat the shellfish they'll get sick. Some algal blooms are very beneficial to the environment making huge amounts of food for fish. Macroalgae and microalgae produce oxygen.Marcoalgae are large algae and they look like plants. They are multicellular as they contain many cells. They contain a "holdfast" which may be attached to sand, boats or rocks. They contain a "stipe" similar to a stem of plants. They contain "blades" similar to leaves of a plant and a collection of blades are called "fronds". Some macroalgae have "air-bladders" this helps them float to the surface to catch the sun. Other macroalgae haven't any "air-bladders" just long flexible stipes.

Microalgae are usually microscopic, prokaryotic or eukaryotic, and uni- or pluri-cellular organisms. Among the photosynthetic organisms, microalgae are the most efficient in the absorption of CO_2 and their growth is directly related to the reduction of GHGs, since they require large quantities of CO_2 as carbon source.Macroalgae are divided into brown, green and red macroalgae. The different colors are due to the pigments brown, green and red pigments within the macroalgae. There is another type of marine algae which is called "Maerl". These are

very small and are unattached to rocks so they live on the sea bed. They have a hard calcium crust and are red or brown in colour. Whenever they die they lose their colour and turn grey.Microalgae are much smaller organisms. They can only be seen under a microscope. They are like floating plant and they are unattached. There are two common types of microalgae. Diatoms are one variety and that they have oil within their bodies. This helps them to float. Dinoflagelletes are another sort of microalgae which have "flagella". The flagella help the din flagellates to swim. Certain sorts of din flagellates glow within the dark in the dark when disturbed, are unicellular which means they have only one cell.

***Spirogyra*:**

It is a genus of filament green algae of the order, zygnametales named for the helical or spiral arrangement of the chloroplasts that is diagnostic of the genus. It is commonly found in freshwater areas, and there are quite 400 species of *Spirogyra* within the world. *Spirogyra* measures approximately 10 to 100μm in breadth and should stretch centimeters long.

General Characteristics:

Spirogyra is unbranched with cells connected end to end in long male reproductive system filaments. This genus of green algae undergoes a haploid-dominant life cycle. The cell wall has two layers: the outer wall is composed of pectin that dissolves in water to make the filament slimy to touch while the inner wall is of cellulose. The cytoplasm forms a skinny lining between the cell membrane and therefore the large vacuole it surrounds. Chloroplasts are embedded within the peripheral cytoplasm; their numbers are variable (as few as one). The chloroplasts are ribbon shaped, serrated or scalloped, and spirally arranged, leading to the prominent and characteristic green spiral on each filament. Each chloroplast contains several pyrenoids centers for the production of starches, appearing as small round bodies.*Spirogyra* is very common in relatively clean eutrophic water, developing slimy filamentous green masses. In spring *Spirogyra* grows under water, but when there is enough sunlight and warmth they produce large amounts of oxygen, adhering as bubbles between the tangled filaments. The filamentous masses come to the surface and become visible as slimy green mats. Mougeotia and zygnima are often found tangled together.

Reproduction:

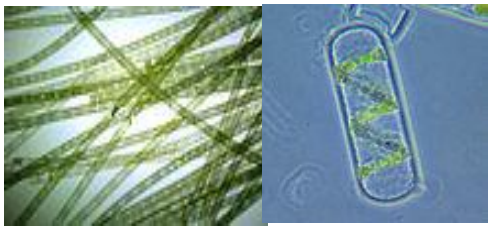
Spirogyra can reproduce both sexually and rarely asexually. In vegetative reproduction, fragmentation takes place, and *Spirogyra* simply undergoes the intercalary mitosis to form new filaments.

ISBN: 978-81-953600-3-1

Sexual Reproduction is of two types:

1. Scalariform conjugation requires association of two different filaments lined side by side either partially or throughout their length. One cell each from opposite lined filaments emits tubular protuberances known as conjugation tubes, which elongate and fuse, to make a passage called the conjugation canal. The cytoplasm of the cell acting as the male travels through this tube and fuses with the female cytoplasm, and the gametes fuse to form a zygospore.

2. In lateral conjugation, gametes are formed in a single filament. Two adjoining cells near the common transverse wall give out protuberances referred to as conjugation tubes, which further form the conjugation canal upon contact. The male cytoplasm migrates through the conjugation canal, fusing with the feminine. The rest of the method proceeds as in scalariform conjugation.The essential difference is that scalariform conjugation occur between two filaments and lateral conjugation occurs between two adjacent cells on the same filament.



Nutrition:

Spirogyra Longata perform photosynthesis to receive its nutrients. This algae contains special cells called stomata that open and close so the organism can take in carbon dioxide and release oxygen during the chemical reaction in photosynthesis. It is through the chlorophyll within the chloroplasts that *Spirogyra* Longata absorbs light. Chlorophyll reflects all wavelengths of sunshine apart from green, which is why it appears green to us. When the organism absorbed light energy (carbon dioxide), chlorophyll causes the reaction to occur which makes ATP and NADPH. During this reaction water molecules get split, releasing oxygen into the air. In the case of this organism, a lot of it is released. Algae use this energy for numerous functions in its cells. It has also been found that algae also use physics to become efficient. *Spirogyra* Longata uses this process of photosynthesis to receive its nutrients. This organism can easily produce many oxygen and energy due to its constant submergence in water and readily available chloroplasts winding throughout its cells.

***Oedogonium*:**

It may be a genus of filamentous chlorophyte, with unbranched filaments that are one cell thick. *Oedogonium* can be free-floating, though it is usually attached to aquatic plants by a holdfast. It appears greenish and inhabits calm, water.

Asexual Reproduction:

Oedogonium can reproduce asexually by fragmentation of the filaments, through some other types of non-motile spores, and also through zoospores, which have many flagella. These develop in a zoosporangium cell, one zoospore per zoosporangium. After settling and losing its flagella, a zoospore grows into a filament.

Sexual Reproduction:

The life cycle of *Oedogonium* is haplontic, i.e., meioses are zygotic. Antheridia which produce sperm, and oogonia which produce an egg, release the sperm and egg. The egg and sperm then fuse and form a zygote which is diploid (2n). The zygote then produces the filamentous green alga which is haploid (1n).

Species:

Species of *Oedogonium* are divided into two major groups on the basis of the distribution of the sex organs:

Macrandous Species: In these species, antheridia are borne on filaments of normal size. This group is further subdivided into:

Macrandous Monoecious: In these species, antheridia and oogonia are found on an equivalent filament. E.g.: O. nodulosum and O. fragile

Macrandous Dioecious: In these species, antheridia and oogonia are borne on different filaments. Although filaments bearing antheridia and oogonia are morphologically similar, they differ physiologically. E.g.: O. crassum and O. aquaticum

Nannandrous Species: In nannandrous species, filaments bearing antheridia and oogonia show morphological distinction. The male filament, which is much smaller than the female filament, is called a dwarf male or nannandrium. Nannandrous species are always dioecious, i.e., antheridia and oogonia are borne on different filaments. The small male filaments are likely

Diesel:

The word "diesel" is derived from the family name of German inventor Rudolf Diesel who in 1892 invented the compression-ignition Diesel engines are a type of. Internal combustion Rudolf Diesel originally designed the diesel engine to use coal dust as a fuel. He also experimented with various oils, including some vegetable oils, such as Peanut oil, which was used to power the engines which he exhibited at the 1900Paries Exposition and the 1911 World's Fair in Paris Petroleum diesel, also called petro diesel or fossil diesel is produced from the fractional distillation of Crude oil between 200 °C and 350 °C at atmospheric pressure, resulting in a mixture of carbon chains that typically contain between 8 and 21 carban atoms per molecule.

ISBN: 978-81-953600-3-1

Chemical composition:

Diesel does not mix with water. Petroleum-derived diesel is composed about 75%, Saturated, hydrocarbons, (primarly, paraffins including, iso, and cycloparaffins), and 25% aromatic hydrocarbons (including naphthalene and alkyl benzenes). The average chemical formula for common diesel fuel is $C_{12}H_{23}$, ranging approximately from $C_{10}H_{20}$ to $C_{15}H_{28}$.

Sources of biodiesel:

Sources of biodiesel was categorically divided into four main classes thus, Edible sources, Non-edible Sources, Animals source and other sources such as Algae, Bacteria, Fungi

Difference between Diesel and Biodiesel:

Today there are great debates going, on the possibility of replacing diesel with an environment friendly fuel called biodiesel. Biodiesel is obtained from vegetable oils through a process called as trans-esterification. It differs from diesel in many ways.

Manufacturing process:

Diesel fuel is manufactured or made from crude oil through a process called as fractional distillation. Crude oil extracted from underneath the earth contains different hydrocarbon compounds each with different boiling point. Using the difference in boiling point hydrocarbon compounds is separated. When the distillation chamber reaches a temperature of 200 °C to 350 °C, diesel is separated from crude oil. The distilled substrate is then purified to what we use as fuel in our cars and trucks.Biodiesel on the other hand is obtained from vegetable oils like Soya, Jatropa, Rice bran etc. by chemically altering it through a process called as “transesterification”. The process starts by heating the oil to a temperature of about 55°C and while stirring a titrated mixture of Methanol and potassium hydroxide is added to it. The mixture is allowed to cool. The fuel is separated, with fatty acids at the top and Biodiesel down at the bottom.

Properties:

Biodiesel is more viscous than diesel and has a higher boiling point of 315°C to 400 °C. The flash point of diesel (60°C to 80°C) is less than that biodiesel which in the range of 100°C to 138°C, which make biodiesel easier and safe fuel to store.

Usage:

Diesel can be directly used from gas stations to fill your tanks whereas biodiesel is not widely and when used are blended with diesel to make it work properly. There basically 6 different types of proportions B5, B10, B20, B40, B60, B80, where ‘B’ indicates biodiesel and the numeric indicated the percentage of biodiesel in the mixture. A B5 mixture would contain 5% Bio-diesel and the remaining is diesel.

Advantages:

Biodiesel provides lot advantages over diesel fuel. The main advantage is the impact on the environment; biodiesel uses vegetable oil or recycled oil for producing and biodiesel powered vehicle has less pollution thus reducing the emission of greenhouse gases. Another difference is that biodiesel production is scalable. Small and large scale business, private consumers and co-operatives have made biodiesel from the feed stock available locally and thus helping the local economy. Even though biodiesel is available in some retail outlets it has not lived up to the expectation of being the alternate fuel. The main reason could be attributed to the properties of the fuel and its adaptability in diesel engines.

Transesterification and biodiesel production:

The most common process of converting oil extracted from algae to biodiesel is Transesterification also called alcoholysis, this process convert oil in to biodiesel in the presence of catalyst.Trans-esterification (also called alcoholysis) is that the reaction of a fat or oil with an alcohol to make esters and glycerol,

Biodiesel production:

Biodiesel is a mixture of fatty acid alkyl esters obtained by transesterification (ester exchange reaction) of vegetable oils animal fats. These lipid feedstock are composed by 90–98% (Weight) of triglycerides and little amounts of mono and triglycerides, free fatty acids (1–5%), and residual amounts of phospholipids, phosphatides, carotenes, tocopherols, sulphur compounds, and traces of water. Transesterification may be a multiple step reaction, including three reversible steps serial, where triglycerides are converted to triglycerides, then triglycerides are converted to monoglycerides, and monoglycerides are then converted to esters (biodiesel) and glycerol (by-product). The overall transesterification reaction is that the radicals R1, R2, R3 represent long chain hydrocarbons, referred to as fatty acids.For the transesterification reaction oil or fat and a short chain alcohols (usually methanol) are used as reagents in the presence of a catalyst (usually NaOH). Although the alcohol: oil theoretical molar ratio is 3:1, the molar ratio of 6:1 is generally used to complete the reaction accurately. The relationship between the feedstock mass input and biodiesel mass output is about, which suggests that theoretically, 1 kg of oil leads to about 1 kg of biodiesel.

A homogeneous or heterogeneous, acid or basic catalyst are often wont to enhance the transesterification reaction rate, although for a few processes using supercritical fluids (methanol or ethanol) it's going to not be necessary to use a catalyst.The extracted oil was evaporated under vacuum to release the solvent mixture solutions using rotary evaporator at 40- 45 °C. Then, the oil produced from each algal species was mixed with a mix of catalyst (0.25g NaOH) and 24 ml

ISBN: 978-81-953600-3-1

methanol, a process called transesterification with stirring properly for 20 min. The Mixture was kept for 3hrs in electric shaker at 3000 rpm. After shaking the solution was kept for 16 hrs to settle the biodiesel and the sediment layers clearly. The biodiesel layer was separated from sedimentation by flask separator carefully.Quantity of sediments (glycerin, pigments, etc) was measured. Biodiesel was washed by 5% water repeatedly until it becomes clear then Biodiesel was dried by using dryer and eventually kept under the running fan for 12 h. the produced biodiesel was measured (using measuring cylinder), pH was recorded and stored for analysis.

Transesterification:

The process of converting vegetable & plant oils into biodiesel fuel is called transesterification, and is fortunately much less complex than it sounds. Transesterification refers to a reaction between an ester of one alcohol and a second alcohol to form an ester of the second alcohol and an alcohol from the original ester, as that of methyl acetate and ethyl alcohol to form ethyl acetate and methyl alcohol see also interesterification. Chemically, transesterification means taking a triglyceride molecule or a posh carboxylic acid, neutralizing the free fatty acids, removing the glycerin and creating an alcohol ester. This is accomplished by mixing methanol with sodium hydroxide to make sodium methoxide. This liquid is then mixed into oil. The entire mixture then settles. Glycerin is left on rock bottom and methyl esters, or biodiesel, is left on top. The glycerin are often wont to make soap and therefore the methyl esters is washed and filtered. Transesterification is not a new process. Scientists E. Duy and J. Patrick conducted it as early as 1853. One of the first uses of transesterified vegetable oil was powering heavy-duty vehicles in South Africa before World War II.Transesterification of Algal Oil into Biodiesel: Transesterification of algal oil is normally done with Ethanol and sodium ethanolate serving as the catalyst. Sodium ethanolate are often produced by reacting ethanol with sodium. Thus, with sodium ethanolate as the catalyst, ethanol is reacted with the algal oil (the triglyceride) to produce bio-diesel and glycerol. The end products of this reaction are hence biodiesel, sodium ethanolate and glycerol.

This end-mixture is separated as follows: Ether and salt water are added to the mixture and mixed well. After sometime, the whole mixture would have separated into two layers, with rock bottom layer containing a mix of ether and biodiesel. This layer is separated. Biodiesel is in turn separated from ether by a vaporizer under a high vacuum. As the ether vaporizes first, the Biodiesel will remain.

Significance of algal biodiesel:

There are many advantages to using algae-based biofuel. Algae-based fuel is has a lot less of an impact on the environment. When the algae are growing, it actually requires co a reactant to grow instead of the huge output of CO2 produced by the burning of petroleum products, plus algae have the highest rate of consumption of CO2 among the plants. It is easy to mention that growing more algae will have better impact to the environment because it will reduce the CO2.The algae growing facilities might be situated around power plants and the CO2that is being produced routed directly to the algae so that it can grow and produce oxygen. A coal power-plant flue gas, which contains about 10 to 30 times as much carbon dioxide as normal air, can be cleaned by this method.

Sample collection:

Algae *Spirogyra* sp and *Oedogonium*sp 20.0 g were collected from the Panwadod River 'Jui'

Oil extraction:

Separation of biodiesel from algae Extraction of oil: Harvest the algae from its growth medium and extract the oil out of it. Extraction can be broadly categorized into two thus; Mechanical and Chemical Methods:

Mechanical methods:

The simplest method of extracting oil from algae is mechanical crushing. Usually mechanical crushing is used in conjunction with chemicals.

Chemical methods:

Algal oil can be extracted using chemicals such as Benzene and Ether, algal oil can also be separated by hexane extraction, Hexane solvent extraction can be used in isolation or it are often used along side the oil press/expeller method. After the oil has been extracted using an expeller, the remaining pulp are often mixed with cyclohexane to extract the remaining oil content. The oil dissolves within the cyclohexane, and therefore the pulp is filtered out from the answer. The oil and cyclo-hexane are separated by means of distillation. These two stages (cold press and hexane solvent) together will be able to derive more than 95% of the total oil present in the algae.

Biomass collection:

The biomass was collected after filtration and weighted.

Evaporation:

The extracted oil was evaporated in vacuum to release hexane and ether solutions using rotary evaporator.

ISBN: 978-81-953600-3-1

Mixing of catalyst and methanol:

0.25 g NaOH was mixed with 24 ml methanol and stirred properly for 20 min.

Biodiesel production:

The mixture of catalyst and methanol was poured into the algal oil in a conical flask.

Transesterification:

The conical flask containing solution was shaken for 3 h by electric shaker at 300rpm.

Shetteling:

After shaking the answer was kept for 16 h to settle the biodiesel and sediment layers clearly.

Results and Discussions:

Comparison of *Oedogonium* sp. and *Spirogyra* sp. shows that *Spirogyra* sp. produce higher quantity of biodiesel than *Oedogonium* sp. and extracted oil in *Oedogonium* sp. was also higher than the *Spirogyra* sp. Biomass (after oil extraction) of *Spirogyra* sp., was higher than the comparing species whereas sediment quantity was greater in *Oedogonium* sp., No prominent difference of biodiesel pH was found between the algal specimens such as *Spirogyra* sp. and *Oedogonium* sp. Production of alternative fuel has attracted wide attention during the past few years, due to the diminishing petroleum reserves and environmental consequences of exhaust gases from fossil diesel. In this context, biodiesel which is characterized as a renewable, biodegradable, andEnvironment-friendly fuel is becoming a blooming area of high concern. Biodiesel can be produced from macroalgae because it contain considerable amount of lipid contents In addition in heterotrophic condition lipid content can be more in algae investigated that lipids of some macroalgae (seaweeds) was reported to be very high, up to 51% of total fatty acids.

The majority of biodiesel today is produced by alkali catalyzed (e.g., NaOH, KOH) transesterification with methanol, which results in a relatively short reaction time examined different biodiesel sources (edible and nonedible), virgin oil versus waste oil, algae-based biodiesel that is gaining increasing importance, role of different catalysts including enzyme catalysts, and the current state-of-the-art in biodiesel production.The biodiesel esters were characterized for their physical and fuel properties including density, viscosity, iodine value, acid value, cloud value, pure point, gross heat of combustion and volatility and fuel produces slightly lower power and torque, and higher fuel consumption than No. 2 diesel oil Biodiesel is best than diesel oil in terms of sulfur content, flash point, aromatic content and biodegradability

Conclusion:

Algae are an economical choice for biodiesel production, because of its availability and Environmental friendly properties. Considerable amount of biodiesel can be produced from macroalgae. Due to greater biomass and sediments *Spirogyra* sp. proves, a better choice for biodiesel production than *Oedogonium* sp. Biomass after oil extraction may be used for livestock, ethanol production and also in paper making.

References:

Bajhaiya, A.K., S.K. Mandotra, M.R. Suseela, K. Toppo and S. Ranade (2010): Algal Biodiesel: the next generation biofuel for India. Asian J. Exp. Biol. Sci., 1(4): 728-739

Bala, B.K. (2006): Studies on biodiesel from transformation of vegetable oils for diesel engines. Energy Educ. Sci.Technol., 15: 1-45.

Bansal, B.K. and M.P. Sharma. (2005): Prospects of biodiesel production from vegetable oils in India. Renew. Sustain.Energy. Rev., 9: 363-378.

Chisti, Y. (2007): Biodiesel from microalgae. Biotech.,25: 294-306.

Chisti, Y. (2008): Biodiesel from microalgae beats bioethanol.Trends Biotech. 26: 126-131.

Demirbas, A. and M.F. Demirbas. (2011): Importance of algae oil as a source of biodiesel. Energy Conver. andManag., 52:163-170.

Demirbas. A. (2008): Biofuel sources, biofuel policy, biofuel economy and global biofuel projections. Energy Conver.and Manage., 49: 2106-2116.

Demirbas. A. (20090: Production of biodiesel from algae oils. Energy Sources, 31:163-168.

Freedman, B., E.H. Pryde and T.L. Mounts. 1984. Variables affectingthe yields of fatty esters from transesterified vegetable oils. J. Am. Oil Chem. Soc., 61: 1638-1643.

ISBN: 978-81-953600-3-1

Guiry, M.D.; Guiry, G.M. (2008): "Oedogonium". Alga Base. World-wide electronic publication, National University of Ireland, Galway.

Hossain, A.B.M.S., A. Salleh, A.N. Boyce, P. Chowdhury and M. Naqiuddin. (2008): Biodiesel fuel production from algae as renewable energy. Am. J. of Biochem. Biotech. 4(3): 250-254.

Janaun, J. and E. Naoko. (2010): Perspectives on biodiesel as a sustainable fuel. Renew. Sustain. Energy. Rev., 14: 1312- 1320.

Kulkarni, M.G. and A.K. Dalai, (2006): Waste cooking oil-an economical source for biodiesel: A review. Ind. Eng. Chem.Res., 45: 2901-2913.

Melting, F.B. (1996): Biodiversity and application of microalgae. J. Ind. Microbiol., 17: 477-489.

Patil, V., K.Q. Tran and H.R. Giselrød. (2008): towards sustainable production of biofuels from microalgae. Int. J.Mol. Sci., 9: 1188-1195.

Pohl, P. and F. Zurheide. (1979): Fatty acids and lipids of marine algae and the control of their biosynthesis by environmental factors. In: Marine Algae in PharmaceuticalScience. (Eds.): H.A. Hoppe, T. Levring and Y. Tanaka, Walter de Gruyter, Berlin, pp: 473-524.

Renaud, S. and J. Luong-Van. (2006): Seasonal variation in the chemical composition of tropical Australian marine macroalgae. J. Applied Phycol., 18: 381-387.

Schenk, P.M., S.R. Thomas-Hall, E. Stephens, U.C. Marx, J.H.Mussgnug, C. Posten. O. Kruse and B. Hankamer. (2008): Second generation biofuels: High-Efficiency microalgae for biodiesel production. Bioenerg. Res., 1: 20-43.

Spolaore, P., C. Joannis-Cassan, E. Duran and A. Isambert. (2006): Commercial applications of microalgae. J. Biosci.Bioeng. 101: 87-96.

Vasudevan, P. T. and M. Briggs. (2008): Biodiesel production current state of the art and challenges. J. Ind. Microbiol.Biotech. 35: 421-430. (Received for publication 20 October 2010).

IMPORTANCE OF MUSHROOM

S. S. Patil

Department of Botany,

Sharadchandra Arts, Commerce and Science College,

Naigaon Dist- Nanded, MS, India

Corresponding authors E-mail: shyampatilss1975@gmail.com

Mushrooms are in general term applied to the fruiting bodies of the fleshy macro fungi of variable shape and size. Many have cap and stalk but some varieties are devoid of stalk. The large number of mushroom species growing wild in nature, while many are edible, some are highly poisonous. Generally they grow saprophytically, sometimes symbolically with roots of trees called mycorrhizal mushrooms (e. g. *Russula* species). In this case, the mushroom mycelium encircles the root and changes the shape of root. The mushroom absorbs water and minerals for the tree, but in return the tree gives the mushroom nutrients, too.

The most abundant renewable biomass on earth consists of cellulose. Much of cellulose in nature is bound physico-chemically with lignin. Because lignin is highly resistant, it protects cellulose against attack by most of microbes, and it must be degraded by chemical or biological means before the cellulose can be utilized. Mushrooms can degrade lignin, cellulose and convert them directly into fungal protein suitable for human consumption.

The waste, which is left after harvesting of (fruit bodies) mushroom, is called as spent straw or spent compost. The spent straw from mushroom growing (industry) can also be recycled for use of animal feed, soil conditioning and fertilizers. Some of the spent straw is used as casing material as a feed, as a manure containing appreciable quantities of N, P and K. The spent straw is also used as a cultivation substrate for same or different mushroom.

The agricultural waste generated in the form of residues, form prime source of live stock feed. The digestibilities of these residues are affected by low protein and high lignin content over green feed. The mushroom cultivation can improve the straw nutritionally by reducing lignin, cellulose, hemicelluloses, tannin, crude fiber content and increase in protein content of straw. This spent straw becomes protein enriched and more digestible substance, suitable for use as good animal feed (Khalon and Das, 1987).

ISBN: 978-81-953600-3-1

The problem of malnutrition is become more severe in the country. Mushroom production has gained the acceptance as the most efficient and economic food production technology, to satisfy the demands of increasing global population. Indian diet is primarily cereal based and in most cases per capita protein intake is highly inadequate and declining with time, which is the main dietary defect in the country. This results into wide spread protein malnutrition especially in children and women of vulnerable groups. In order to meet the demands of required protein and other chemical components to vulnerable people, the cultivation of edible mushroom is an urgent need of nation.

To solve the problem of protein hunger, FAO has been recommended the edible mushroom as a food. They are excellent source of proteins, vitamins, minerals and are popularly called as vegetarians meat. Mushroom contains 20-35 % of protein (Kadam *et al.,* 2009), which is higher than in most vegetables, fruits and is of superior quality. Mushroom protein is intermediate between that of animals and vegetables (Kurtzman, 1976). Mushroom contains all essential amino acids but rich in lycine and tryptophan that are deficient in cereals. .They are good source of vitamin C and B complex (thiamine, riboflavine, niacin, folic acid and pantothenic acid). They have low quantities of fats, carbohydrates and without starch and cholesterol. Due to the least quantities of fat and carbohydrates, the mushroom becomes an ideal food for diabetic patients. Mushroom acts as best food material for the persons suffering from hypertension and heart diseases because it contains high potassium-sodium ratio (Rai *et al.,* 1998). Mushroom shows certain medicinal properties, as they have antifungal, antibacterial property and enhances defense mechanism against viral infections. The mushrooms are valuable source of dietary fibers. Water extract from mushroom are useful against eye problems. In India the mushroom has not become popular as a food among the common people because of lack of awareness about its cultivation method and non-availability at low prices for common man. Hence it is necessary that a large number of people are made aware of the simple methods required for successful cultivation of edible mushroom.

As per the regional environmental conditions, mainly there types of edible mushrooms are cultivated in India on commercial scale. They are white button mushroom (*Agaricus bisporus*), oyster mushroom (*Pleurotus sp*.) and paddy straw mushroom (*Volvariella* sp.). The production of button mushroom is dominant in cooler, hilly regions of North India due to its low temperature requirement. The cultivation of button mushroom need a lot of technical skill, specific growing conditions, specially prepared compost and proper care during all stages of growth.

Pleurotus is an efficient lignin degrading mushroom. They can grow well on different types of ligno-cellulose materials. The cultivation of oyster mushroom becoming more popular in Indian plains because of the availability oflarge quantities of agro-waste along with tropical to sub tropical weather in large part of country. *Pleurotus* species, generally grows on various substrates after boiling in water (80 °C for 1 - 2 hr.) or after chemical sterilization. This mushroom has very simple, low cost technology and give consistent and higher biological efficiency. The different species of *Pleurotus* can grow in variable temperature conditions. Hence they are ideally suited for cultivation at different times for the year in various regions of our country.

References:

Kadam, R. M., Patil, S.S. and Jadhav, B. S. (2009) Production of Protein by fungi, *Pleurotus* species from different agricultural wastes. *Int.J.Plant Sci*. 1(2), 45-47.

Khalon, S. S. and Das, S. K. (1987) Biological Conversion of Paddy Straw into feed. Biol. Waste. 22 (1): 11-21.

Kurtzman, R. H. (1976) Nitration of Pleurotus sapidus effects of lipid. Mycologia. 68: 268-295.

Rai, R. D.; Ahlawat, O. P. and Verm, R. N. (1998) Nutritional value and post harvest technology of mushrooms. In: Recent advances in cultivation technology of edible mushroom, NRCM, Solan (HP). (Eds. R. D. Rai; B. L. Dhar and R. N. Verma) pp. 241-242.

ISBN: 978-81-953600-3-1

CHECKLIST OF BIRDS DIVERSITY OF SHIVRAJ COLLEGE CAMPUS GADHINGLAJ, DISTRICT KOLHAPUR, MAHARASHTRA, INDIA

K. J. Adate*, V. M. Deshmukh and S. N. Asode

Department of Zoology,

Shivraj College of Arts, Commerce and D. S. Kadam Science,

Gadhinglaj, Dist. Kolhapur, Maharashtra 416 502, India

*Corresponding authors E-mail: kjadate@gmail.com

Abstract:

Diversity of avian fauna plays a very significant role in determining the health of an ecosystem. The present study was carried out to prepare proper checklist of terrestrial birds of Shivraj college of Arts, Commerce and D. S. Kadam Science, Gadhinglaj Dist. Kolhapur Maharashtra 416502 campus, over a period of 12 months from January to December 2020. As a result 61 bird species were recorded belonging to 12 orders and 34families. The Order Passeriformes was recorded as the most dominating order representing 30species of birds. There are 7 species to Columbiformes, Cuculiformes 3 species and 2 species each to Accipitriformes, Bucerotiformes, Charadriiformes, Galliformesand Strigiformes.Apodiformes and Piciformeseach showed only one species.

Keywords: Passeriformes, Columbiformes, Cuculiformes, Gadhinglaj

Introduction:

Birds are present in every part of the world. They are generally easy to observe and have been well-studied by humans. Birds are feathered clad, warm blooded, air breathing, oviparous, biped flying vertebrates and are called as masters of air.They are of scientific, environmental, cultural, economic, and artistic importance. Birds are acts as indicators of most of the places which are not detected by other groups of animals.Birds are helpful in dispersing seeds, pollinating plants, controlling pests, disposing of animal carcasses.They are also contributing a large to global economy through bird-watching (Whelan *et al.*, 2015). “They are helpful and help to provide rich food for mankind and are known to man since ages (Chitampally, 1993).” They are widely recognized as the bio-indicators of the ecosystem (Gill, 1994). Birds act as an early warning system for environmental pollutants or other destructive forces (Lovett *and* Fitzpatrick, 2016).”The list of bird’s species indicates species diversity of a particular area or habitat (Bibby

et al., 1992, 1998).Such studies would provide information on the diversity, abundance and ecological status of birds in a particular place.

Many birds are in the risk of extinction as a result of multiple human activities. A recent study found that forty percent (40%) of the world's bird species are in decline due to the activities like agriculture, deforestation; invasive species, hunting, development and climate change etc. (E360 Digest, 2018). Decreased population of birds including threatened species markedly affects components of food webs and ecosystem functions (Rosenberg *et al.*, 2019).

The present study is targeted not solely on preparing the checklist of birds, however also to find out their occurrence, status as well as to create the awareness for their conservation. In addition, this study will also provide the basic information of the avifauna for further studies related to campus biodiversity. It also helps in preparing a baseline data regarding bird diversity of the state; since there is no satisfactory report on this respect till date. Shivraj College campus is the only large green spot in the Gadhinglaj city where the number of terrestrial birds can get cover. Hence the area should be wisely used without disturbing the activity of the birds and listed maximum number of species.

Materials and Methods:

Study area:

The Shivraj college of Arts, Commerce and D. S. Kadam Science, Gadhinglaj is located in rural and hilly area of the Kolhapur districton Maharashtra-Karnataka border area ($16^0$13'45.0"N$74^0$20'43.1"E). The college was established on 26th June 1964. The college has got 33,300sq.m of eco-friendly premise of its own where the academic building, administrative building, the huge playground, multipurpose hall, Ladies hostel and Gymkhana along with well

ISBN: 978-81-953600-3-1

maintained garden issituated. According to latest survey of Department of Botany of our college our campus is having 402 plants of various types including trees, shrubs and medicinal plants.

Methodology:

The study was carried out for a period of one year from January 2020 to December 2021. The bird's survey was conducted in the morning and evening period within the campus and these observations was also done throughout the day. The bird species were observed with a field binocular (8*40). Photographs were taken with the help of Canon EOS 1300 D with 55-250 mm zoom lens. Bird identification is done by Ali and Ripley (1983), Coomber (1991), Ali and Ripley (1996), Ali (1996) and Grimmett *et al.* (1999). The birds were classified on the basis of standard field guides by, Ali (2002). Birds were spotted, counted, and identified by using direct count methods from walking within the college campus. A special care was taken to identify the birds that are breeding inside the college campus.Every possible area such as trees and buildings were searched individually for the nests of birds. The behaviour of bird is carefully observed; identified the breeding place and care was taken, not to disturb the activity on nest.

Results and Discussion:

Over the course of twelve months from Jan. to December 2020 we have recorded sixty one bird species belonging to twelve orders and thirty fourfamilies which are listed below. During this study it has been observed that some birds are seen frequently and these are;Indian Peafowl, Common Kingfisher, Green Bee-eater, Blue Rock Pigeon, Brahminy Starling, Indian Robin, Purple Sunbird, Black Drongo, House Crow, Spotted owlets, Red-vented Bulbul, Cattle Egret, and Common Myna etc. Some birds were sighted rarely such as Indian roller, Coppersmith Barbet, Indian Golden Oriole, Indian gray hornbill etc. Some migratory birds are also spotted during the study; these are Black Ibis, Yellow-footed Green-Pigeon etc.

Rich diversity of birds is attributed to habitat structure and geographical location of college campus. It shows that there is a need to protect habitat structure present in college campus because it makes the food niches of bird species. This study is a first kind attempt to prepare a checklist of birds at the Shivraj college campus and recorded a very good number of terrestrial birds at the college campus.

Sr. No.	Common Name	Scientific Name	Order	Family
1.	Black Kite	*Milvus migrans*	Accipitriformes	Accipitridae
2.	Black-shouldered Kite	*Elanus caeruleus*	Accipitriformes	Accipitridae
3.	Little Swift	*Apus affinis*	Apodiformes	Apodidae,
4.	Indian gray hornbill	*Ocyceros birostris*	Bucerotiformes	Bucerotidae
5.	Common Hoopoe	*Upupa epops*	Bucerotiformes	Upupidae
6.	Red-wattled Lapwing	*Vanellus indicus*	Charadriiformes	Charadriidae
7.	Yellow-wattled Lapwing	*Vanellus malabaricus*	Charadriiformes	Charadriidae
8.	Blue Rock Pigeon	*Columba livia*	Columbiformes	Columbidae
9.	Laughing Dove	*Streptopelia senegalensis*	Columbiformes	Columbidae
10.	Spotted Dove	*Streptopelia sinensis*	Columbiformes	Columbidae
11.	Ring Dove	*Streptopelia decaocto*	Columbiformes	Columbidae
12.	Yellow-footed Green-Pigeon	*Treron phoenicopterus*	Columbiformes	Columbidae
13.	White Dove	*Streptopelia risoria*	Columbiformes	Columbidae
14.	Spotted Dove	*Streptopelia sinensis*	Columbiformes	Columbidae
15.	Indian Roller.	*Coracias benghalensis*	Coraciiformes	Coraciidae
16.	Pied Kingfisher	*Cerylerudis*	Coraciiformes	Alcedinidae
17.	Common Kingfisher	*Alcedoatthis*	Coraciiformes	Alcedinidae
18.	White-throated Kingfisher	*Halcyon smyrnensis*	Coraciiformes	Alcedinidae
19.	Green Bee-eater	*Meropsorientalis*	Coraciiformes	Meropidae
20.	Asian Koel	*Eudynamys scolopacea*	Cuculiformes	Cuculidae
21.	Common Hawk-cuckoo	*Hierococcyx varius*	Cuculiformes	Cuculidae
22.	Greater Coucal	*Centropus sinensis*	Cuculiformes	Cuculidae
23.	Common Quail	*Coturnix coturnix*	Galliformes	Phasianidae
24.	Indian Peafowl	*Pavo cristatus*	Galliformes	Phasianidae
25.	Ashy-crowned Sparrow-lark	*Eremopteryx griseus*	Passeriformes	Alaudidae
26.	Wire-tailed Swallow	*Hirundo smithii*	Passeriformes	Hirundinidae
27.	Barn Swallow	*Hirundo rustica*	Passeriformes	Hirundinidae
28.	White-browed Wagtail	*Motacilla maderaspatensis*	Passeriformes	Motacillidae
29.	Red-whiskered Bulbul	*Pycnonotus jocosus*	Passeriformes	Pycnonotidae

ISBN: 978-81-953600-3-1

30.	Red-vented Bulbul.	*Pycnonotus cafer*	Passeriformes	Pycnonotidae
31.	Bay-backed Shrike	*Lanius vittatus*	Passeriformes	Laniidae
32.	Long-tailed Shrike	*Lanius schach*	Passeriformes	Laniidae
33.	Oriental Magpie-Robin	*Copsychus saularis*	Passeriformes	Muscicapidae
34.	Indian Robin.	*Saxicoloides fulicatus*	Passeriformes	Muscicapidae
35.	Indian Chat	*Ceromella fusca*	Passeriformes	Muscicapidae
36.	Large Grey Babbler	*Turdoides malcolmi*	Passeriformes	Leiothrichidae
37.	Jungle Babbler	*Turdoides striata*	Passeriformes	Leiothrichidae
38.	Yellow-eyed Babbler	*Chrysomma sinensis*	Passeriformes	Paradoxornithidae
39.	Ashy Prinia	*Prinia socialis*	Passeriformes	Cisticolidae
40.	Common Tailorbird	*Orthotomus sutorius*	Passeriformes	Cisticolidae
41.	Great Tit	*Parus cinereus*	Passeriformes	Paridae
42.	Purple Sunbird	*Cinnyris asiaticus*	Passeriformes	Nectariniidae
43.	Purple-rumped Sunbird	*Leptocoma zeylonica*	Passeriformes	Nectariniidae
44.	Red Avadavat	*Amandava amandava*	Passeriformes	Estrildidae
45.	Scaly-breasted Munia	*Lonchura punctulata*	Passeriformes	Estrildidae
46.	House Sparrow	*Passer domesticus*	Passeriformes	Passeridae
47.	Chestnut-shouldered Petronia	*Petronia xanthocollis*	Passeriformes	Passeridae
48.	Indian Baya Weaver	*Ploceus philippinus*	Passeriformes	Ploceidae
49.	Common Myna	*Acridotheres tristis*	Passeriformes	Sturnidae
50.	Brahminy Starling	*Sturnus pagodarum*	Passeriformes	Sturnidae
51.	Indian Golden Oriole	*Oriolus oriolus*	Passeriformes	Oriolidae
52.	Black Drongo	*Dicrurus macrocercus*	Passeriformes	Dicruridae
53.	House Crow	*Corvus splendens*	Passeriformes	Corvidae
54.	Large-billed Crow	*Corvus culminatus*	Passeriformes	Corvidae
55.	Cattle Egret	*Bubulcus ibis*	Pelecaniformes	Ardeidae
56.	Little Egret	*Egretta garzetta*	Pelecaniformes	Ardeidae
57.	Indian Pond Heron	*Ardeola grayi*	Pelecaniformes	Ardeidae
58.	Black Ibis	*Pseudibis papillosa*	Pelecaniformes	Threskiornithidae
59.	Coppersmith Barbet	*Megalaima haemacephala*	Piciformes	Megalaimidae
60.	Common Barn Owl	*Tyto alba*	Strigiformes	Tytonidae
61.	Spotted Owlet	*Athene brama*	Strigiformes	Strigidae

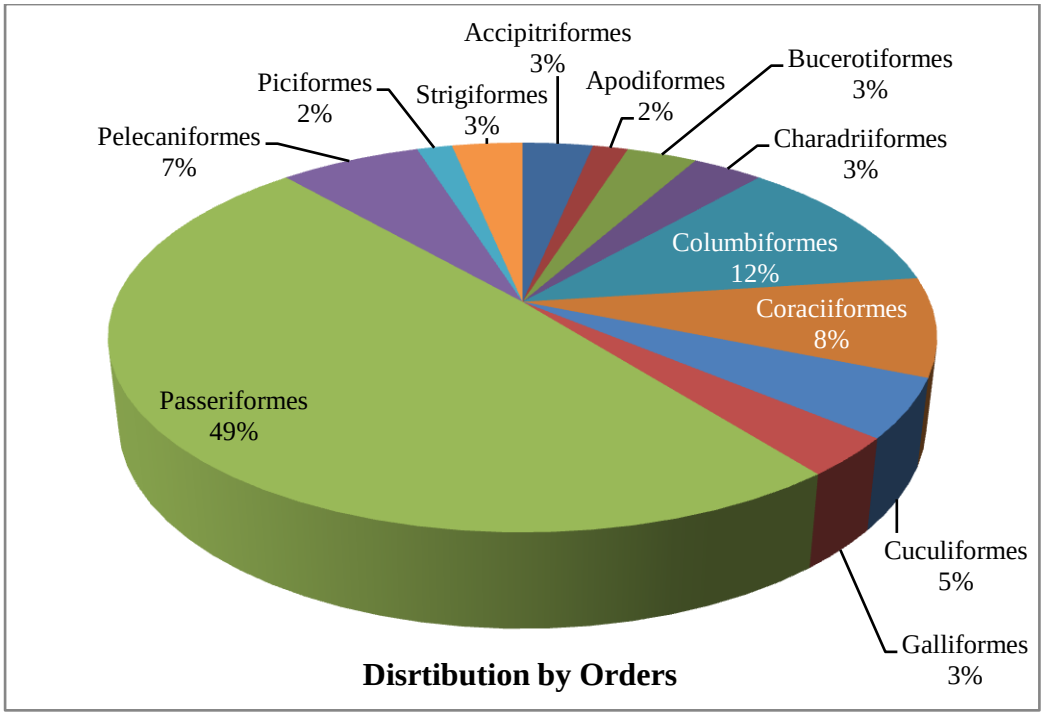


Figure 1: Order-wise number of birds recorded in Shivraj College Campus

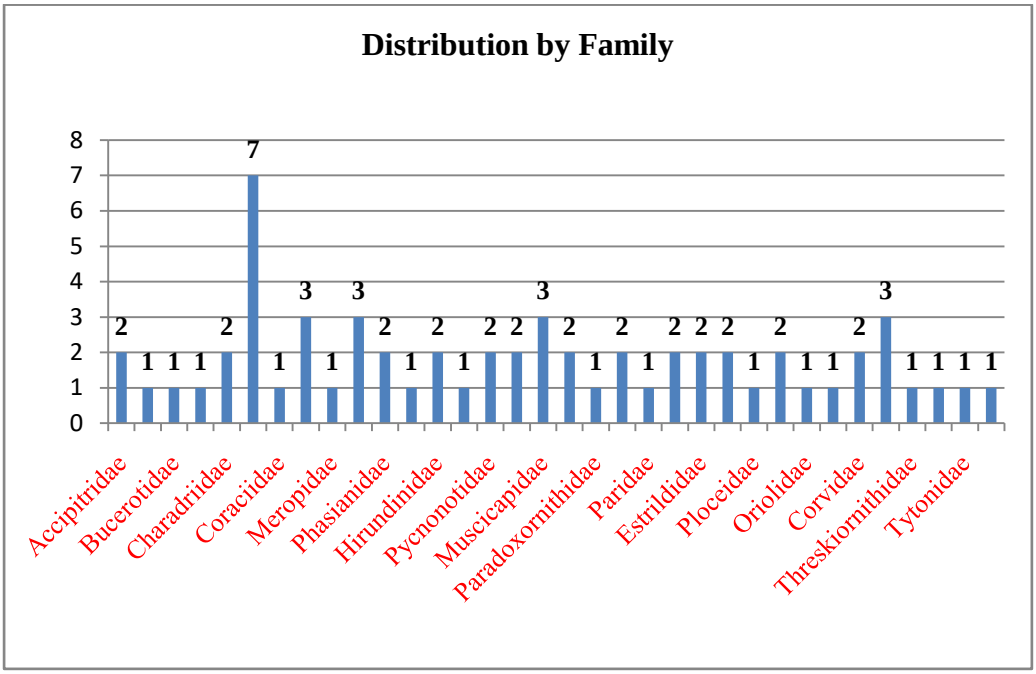


Figure 2: showing family-wise number of birds recorded in Shivraj College Campus

ISBN: 978-81-953600-3-1

Spotted Owlet

Prinia

Bee Eater

Red-Whiskered Bulbul

Purple Sunbird

White Eye

Long Tailed Shrike

Pied Bush Chat

Hill Pigeon

Crested Myna

House Sparrow -Male

House Sparrow-Female

Clamorous Reed Warbler

Black Drongo

Brahminy Starling

Pied Kingfisher

ISBN: 978-81-953600-3-1

Laughing Dove

Black-Shouldered Kite

Pond Heron

Red Wattled Lapwing

Oriental Magpie-Robin

Red- Naped Ibis

Common Kinhfisher

Red Rumped Swallow

Hoopoe

Indian Peafowl

House Crow

Pied Bush Chat

Coppersmit Barbet

Purple Rumped Sunbird

Indian Ring Nacked Parrot

Plum Headed Parakeet

ISBN: 978-81-953600-3-1

Acknowledgement:

The authors are very much thankful to the President, Secretary and Principal of Shivraj College of Arts, Commerce and D. S. Kadam Science, Gadhinglaj for their encouragement and inspiration during this work. Authors also thankful to B. Sc. II Zoology students and all laboratory staff for their help during field visit, celebration of 'World Sparrow Day' on 20th March, preparation and keeping 'Bamboo Nests' on the trees and at various places in the college campus.

References:

Ali, S (1996). The Book of Indian Birds. 12th Edition. Oxford University place, Delhi, 140pp.

Ali, S and S. D. Ripley (1983). A Handbook of the birds of India and Pakistan together with those of Bangladesh, Nepal, Bhutan and Sri Lanka. Compact edition. Oxford University place, New Delhi.

Ali, S and S. D. Ripley (1996). A pictorial guide to the Birds of the Indian subcontinent. 2nd Edition (update). Oxford University place,Mumbai,150pp.

Bibby, C. Jones, M. and Marsdden, S. (1998). Expedition Field Techniques: Bird Surveys expedition Advisory centre. Royal Geographical Soceity, London p78.

Bibby, C.J. Collar, N. B. Crosby, M. J. Health, M. F. Imboden, C. Jonston, T.H. Long, A.J. Satterfield, A.J. and Thirgood, S.J. (1992). Putting Biodiversity in the Map: Priority areas for Global conservation. Barrington press, Cambridge.

Chittampalli, M. and B.P.N. Bhatkhande. 1993. Hansdev's Mrigpakeshi Shastra. M.S. Board of literature and culture, Mumbai Pp 1-279.

E360 Digest. "Forty Percent of the World's Bird Populations Are in Decline, New Study Finds [Blog post]." Yale Environment 360, April 23, 2018. Retrieved from: https://e360.yale.edu/digest/forty-percent-of-the-worlds-bird-populations-are-in-eclinenew-study-finds/

Gill, F.B. (1994). Ornithology, 2nd edition. New York . Harney, N.V. Dhamani, A.A. and Andrew, R.J (2011). Studies on avifaunal diversity of three water bodies near Bhadrawati, District. Chandrapur (MS). Ind. Streams Res.J.1(6): 40-51.

Grimmett, R., C. Inskipp and T. Inskipp (1999). Pocket Guide to the Birds of the Indian Subcontinent. Oxford University Press, New Delhi. 384pp.

Lovette, Irby J. and Fitzpatrick, John W. Eds., Cornell Lab of Ornithology. Handbook of Bird Biology. United Kingdom: Wiley-Blackwell, 2016.

Rosenberg, Kenneth V., Dokter, Adriaan M., Blancher, Peter J., et al. "Decline of the North American avifauna." Science, Oct 4, 2019, pp. 120-124.

Whelan, C.J., Sekercioglu, C.H. and Wenny, D.G. "Why birds matter: from economic ornithology to ecosystem services." Journal of Ornithology, 156 (Supplement 1), 2015, pp. 227-238.

Frontiers In Life Science Volume III

ISBN: 978-81-953600-3-1

About Editors

Dr. Babita Rana is a Plant Tissue Culturist and Assistant Professor at G. N. Khalsa College Matunga, Mumbai. She has completed her Ph.D. from the University of Mumbai, Kalina. Her research interest is Plant Tissue culture and Micro-algal culture. She has worked on in-vitro propagation of indigenous medicinal plants and culturing of blue-green algae. She has many research articles published in national and international journals to her credit. She has presented her research work in various symposia and conferences. She has completed two minor research projects funded by the University of Mumbai.

Dr. Ninad S. Dharkar (M.Sc., Ph. D., F.B.F.I., F.I.S.C.A.) is working as Assistant Professor in Botany at S. P. M. Science and Gilani Arts, Commerce College, Ghatanji, Dist. Yavatmal (MS). India. His research area is Mycology. He has 18 years of teaching experience Dr. Dharkar published 34 research papers in various National and International Journals. He was awarded with Dr. A. P. J. Abdul Kalam Young Scientist award by Marina R&D Lab, Chennai two times in the year 2015 and 2019. He also received Distinguish Researcher award in the International Conference organized by Shri. Shivaji Science College, Nagpur in 2017.

Dr. Mrs. Swati Nilesh Zodpe is Assistant Professor in Microbiology at Shri. Shivaji College of Arts, Commerce and Science, Akola, Maharashtra. She has 11 years of teaching and research experience. Her research interests are Agricultural Microbiology, Environmental Microbiology, Actinomycetes. She has published 27 research papers in national and international journals. She has attended and presented her research work in 46 conferences and symposia. Dr. Zodpe published five book chapters and also wrote and published popular science articles in newspapers. She is recognized research guide of SGB Amaravati University. Under her guidance 29 students completed their master's degree projects. She received Excellence Award with Silver Medal in RIO + 22 UN Sustainable Energy for All India Programme, 2015.

Dr. Ganesh C. Nikalje obtained MSc (Botany) and Doctorate in Botany from Savitribai Phule Pune University and Bhabha Atomic Research Centre, Mumbai, India. He is an Assistant Professor in Botany at Seva Sadan's R. K. Talreja College of Arts, Science and Commerce, Affiliated to University of Mumbai. He is also serving as a member of Board of Studies at Tuljaram Chaturchand College, Baramati, India. He has two independent research projects funded by University of Mumbai, received research fellowship from Department of Atomic Energy, M.S. Swaminathan Research Excellence Award and Young Plant Scientist award 2021 from Association of Plant Science Researchers (APSR), Plantica Foundation, Dehradun, India. He is working as editor and reviewer for several International Journals. He has been invited as a resource person in different conferences/symposia, guest lecturer at Botanical Survey of India, Pune. Till date he has published 15 research/review papers in International journals and 10 book chapters published by international publishers with 448 citations and H-index 9.

Bhumi Publishing

ISBN 978-81-953600-3-1
9 788195 360031