

ISBN: 978-93-91768-16-4

Frontiers in Life Science

Volume IX

Editors

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First Edition: 2022

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Bhumi Publishing

2022

First Edition: June, 2022

ISBN: 978-93-91768-16-4



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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

*Life Sciences have always been a fundamental area of science. The exponential increase in the quantity of scientific information and the rate, at which new discoveries are made, require very elaborate, interdisciplinary and up-to-date information and their understanding. Enhanced understanding of biological phenomenon incorporated with interdisciplinary approaches has resulted in major breakthrough products for betterment of society. To keep the view in mind we are delighted to publish our book entitled "**Frontiers in Life Science Volume IX**". This book is the compilation of esteemed articles of acknowledged experts in the fields of basic and applied life science.*

This book is published in the hopes of sharing the new research and findings in the field of life science subjects. Life science can help us unlock the mysteries of our universe, but beyond that, conquering it can be personally satisfying. We developed this digital book with the goal of helping people achieve that feeling of accomplishment.

The articles in the book have been contributed by eminent scientists, academicians. Our special thanks and appreciation goes to experts and research workers whose contributions have enriched this book. We thank our publisher Bhumi Publishing, India for taking pains in bringing out the book.

Finally, we will always remain a debtor to all our well-wishers for their blessings, without which this book would not have come into existence.

Editors

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WEBS: DIVERSITY, STRUCTURE AND FUNCTION OF SELECTED SPIDERS IN WESTERN GHATS OF KARNATAKA, INDIA

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Abstract:

Web building has been such a highly successful innovative behaviour among spiders that the vast majority of spiders are web builders using silk. The architecture of spider webs varies to significant extent between species to species and web building has been completely lost in some clades. Spiders form different web patterns including orb web - the typical circular web with radii and spirals orb webs of orb weaving spiders like Araneidae, Tetragnathidae, Uloboridae, which have vertically orientation towards the ground or horizontal, sheet webs – appears like a thin two dimensional sheet with vertical lines found in Cyatholipidae, Linyphidae and cobwebs – three dimensional structure found in Nesticidae, Theridiidae, which consist of multi-dimensional meshwork and ascending sticky threads for holding up and capturing the prey. The building of webs varies even within species. This may be a significance of: (i) spiders habituate to their web architect to the natural world & its habitat; e.g., Family Nephilidae orb webs are constructed in areas where more open place is found, (ii) spiders modify their webs to performance their capabilities, e.g., when these individuals are disclosed to its kill or enemies, or (iii) silk expression or appearance, e.g., when on diets lacking certain nutrients. We have reviewed the literature globally, focusing on contributions from the regional level of Western Ghats of Karnataka, showing individual webs vary in function and structure at many zones and so must be considered a dynamics & variable. Webs accordingly represent the foraging, mating and defensive strategies, and physiological status, of the spider.

Keywords: Spiders, Web, Mangalore, Western Ghats, Karnataka.

Introduction:

Spiders belong to the class Arachnida of the phylum Arthropoda, animals that possess jointed appendages and a chitinous exoskeleton. The members of the class Arachnida are generally characterised by two body regions, the cephalothorax having four pairs of segmented legs attached to it, and the abdomen. Unlike insects, arachnids do not have antennae. The class Arachnida comprises the orders Scorpiones (scorpions), Schizomida (schizomids), Amblypygi

(tailless whip-scorpions), Uropygi (uropygids or whipscorpions), Opiliones (opiliones, harvestmen or daddy-long-legs), Pseudoscorpiones (pseudo-scorpions or false scorpions), Palpigradi (palpigrades or microwhipscorpions), Solifugae (windscorpions, sun spiders or solifugids), Ricinulei (ricinuleids), Acari (mites and ticks) and Araneae (spiders).



Figure 1.1: Spider & their relatives

**a.Spider, b. Scorpion, c. Whip Scorpion, d. Pseudoscorpion,
e. Tick, f. Harvestman. g. Camel Spider**

Suborder Araneomorphae Spiders are often misunderstood (because not all members build the web) and are called web builders or true spiders (Turnbull, 1973), comprise all most 90% of all extant spider species, and thus represent by far the most diverse spider group. The webs of Araneomorph spiders are highly distinguishable and found in almost every ecosystem on Earth.

The primarily function of spider webs is to capture its prey. However, they can perform other functions, including as a sensory system, a courtship and/or mating platform, a thermoregulatory platform, and a barrier against predators for survival. Although they are ubiquitous in all environments, the structural and ecological importance of spider webs in ecosystems is still poorly understood. This is partly because the ecological, evolutionary, and biophysical aspects of spider webs for individual spiders, populations and species are largely unexplored.

Probably the most easily recognizable form of spider web is the orb web. New molecular evidence has nonetheless suggested two alternative scenarios (Bond *et al.*, 2014; Fernandez *et al.*, 2014): (1) that the orb web evolved earlier than originally postulated and may represent the ancestral form of all spider webs, or (2) the orb web has had multiple independent origins.

Araneomorph diversity in Western Ghats of Dakshina Kannada, Karnataka

One among the 18 biodiversity hot spots (Myers *et al.*, 1990; Gadgil *et al.*, 1996) of the world, the Western Ghats, together with the West Coast, forms an important ecological region. Starting from the Arabian coast to the mountains with elevations above 2000m and rainfall from

less than 1000mm to more than 6000mm, the landscape here is very heterogeneous with different habitats (Myers *et al.*, 1990; Gadgil *et al.*, 1996).

Study site

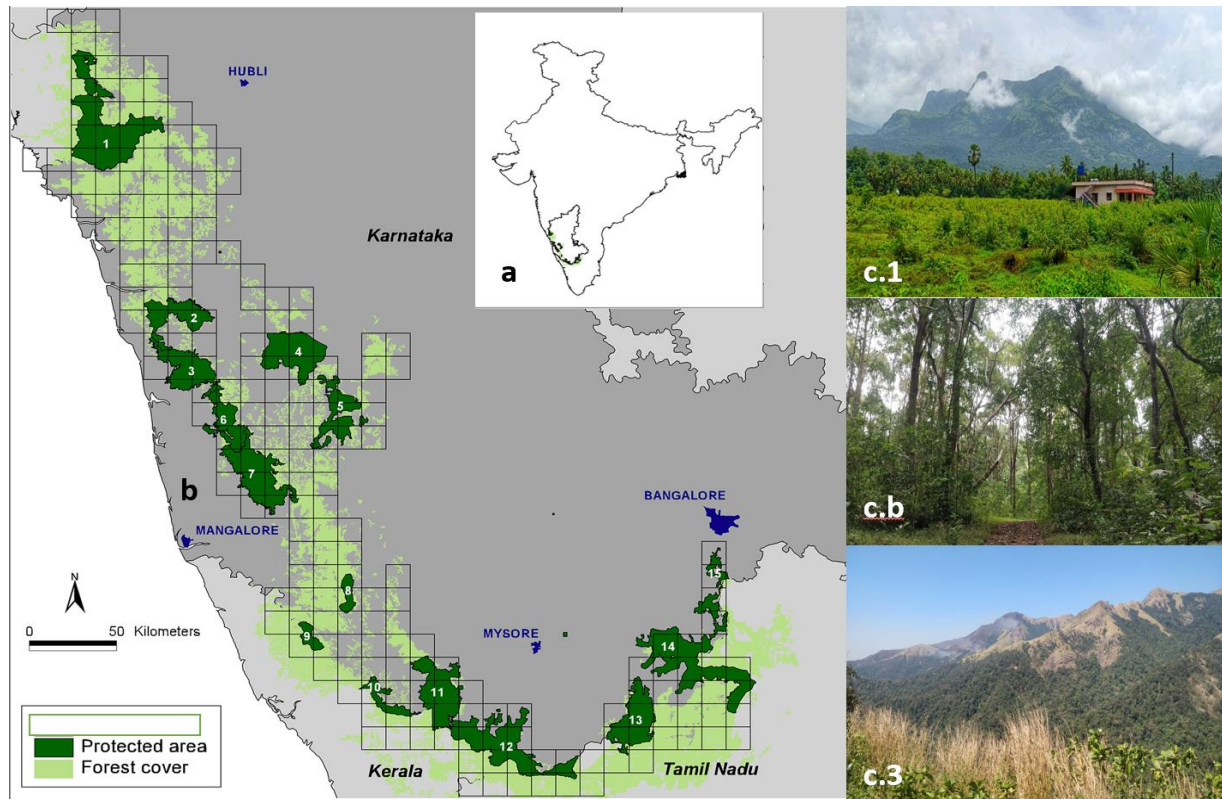


Figure 1.2: Map showing the current study site

a. Sate representation b. Regional location

c.1, c.2 & c.3 showing different heterogenous habitats in Western Ghats

There are about 47,099 globally described spider species in 4,073 genera and 113 families (World Spider Catalogue 2021) Spiders are one of the most diverse groups of creatures available in India. There are 1,520 spider species belonging to 377 genera of 60 families have been recorded in this country. Of these 1,520 species, 1,002 are endemic to Indian mainland, 71 species are endemic to Andaman and Nicobar Islands and one species is endemic to Lakshadweep (Keswani *et al.*, 2012).

Most of the genera of orb-web spiders are found in the Western Ghats. India alone is said to have the greatest diversity of web-building spiders in the world, with 56 of the 112 known Araneomorph families (World Spider Catalogue, 2021). Such an impressive diversity qualifies the Western Ghat as a spider biodiversity hotspot and highlights the importance of on-going research in the region.

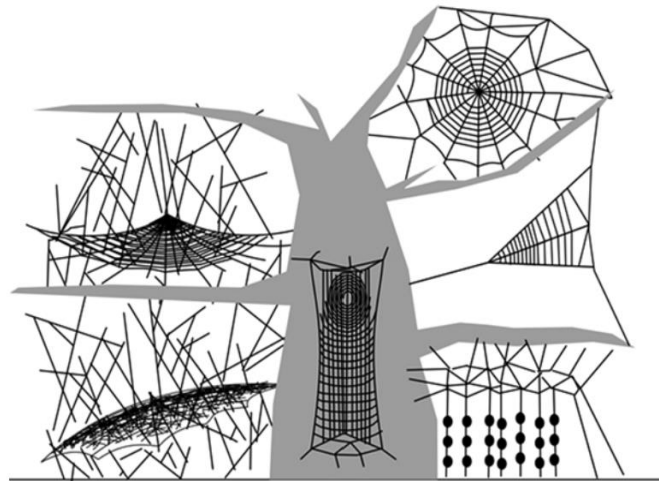


Figure 1.3: Examples of the diversity of Western Ghat spider web building, showing (clockwise from the upper left-hand side) two-dimensional Orb web of Araneids, planar horizontal orb webs, an example of a derived orb web, Gumfooted threads of Theridiid cobwebs, three-dimensional sheet webs and Cyrtophora three-dimensional orb webs. An example of a ‘ladder web’ is shown on the trunk of the tree

Orb weaver spiders are a common group of spiders globally. The ones most easily seen are more common in late summer and fall before frost. The classic orb web is structured as illustrated below:

The sticky spiral is the prey-catching structure, so measurements of it that differ between species are likely to have a direct effect on use of food resources. If those characteristics are similar then differences in height of web could make use of a different part of the habitat.

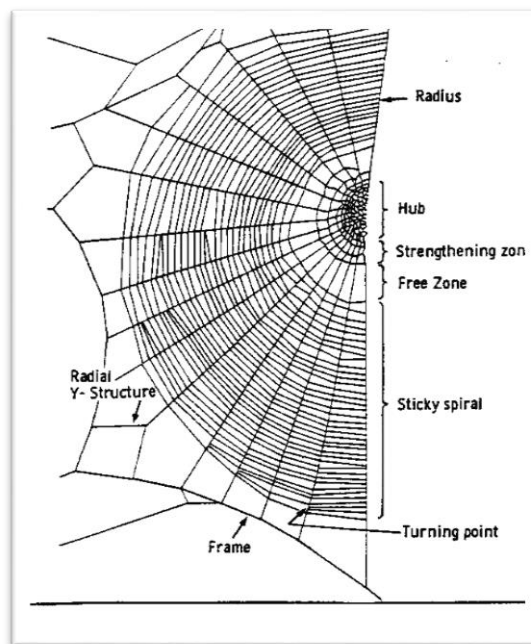


Figure 1.4: Web Orientation Horizontally & Vertically

Argiope

This is usually the most obvious orb-weaver and known as the common garden spider. It is black and yellow or black and silver on its oval abdomen and typically forms a zigzag pattern in the hub of its web. The spider usually sits in the center of its web with its long legs forming an X. They are common in fields of goldenrod.

Neoscona

These are brown spiders with round abdomens varying in size from .5-1cm. On the ventral side of the abdomen there are 2 light spots. These spiders like brushy areas or under overhangs (guardrails, playgrounds) as well as old fields. Unlike garden spiders, they are usually found in the vegetation on the edge of the web and appear if the web is vibrated.

Araneus

Spiders (of Charlotte's web fame) have very large, light colored, often orange to yellow round abdomens 1-2cm in diameter. They form a curled-leaf hiding spot on the web's edge. Typically, their webs are higher off the ground and anchored on more sturdy vegetation than *Argiope*.

Tetragnatha

These spiders forms horizontal webs usually low in the grass or near streams. These spiders have long, thin bodies and hold their legs next to them so that they resemble a greenish stick when sitting in the center or to the side of their web.

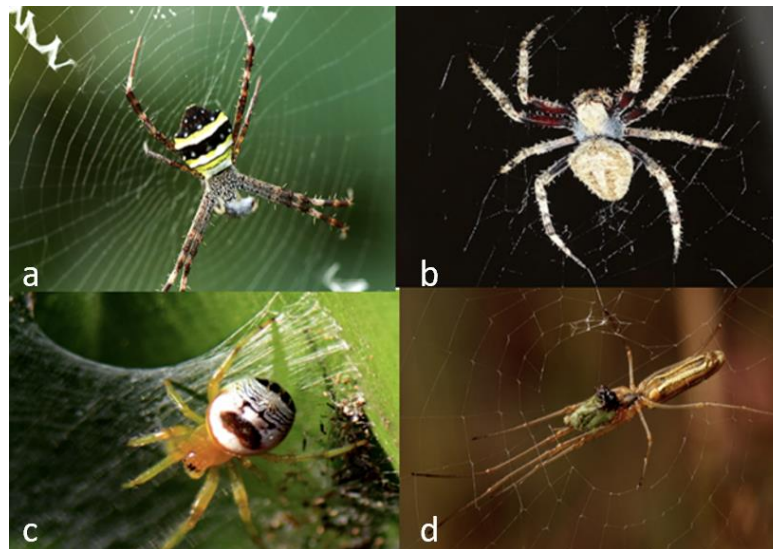


Figure 1.5: Spiders and their web arrangement

a. Argiope anasuja; b. Neoscona crucifera; c. Araneus mitificus; d. Tetragnatha straita

Web function

The primary function of spider webs is to catch flying insect prey. All webs have antimicrobial activities. Prey capture by a spider web involves three actions — prey interception,

stopping and retention (Eberhard, 2014; Zaera *et al.*, 2014), with different silk patterns are constructed or modified to perform one or more activities in the silk material of web (Blackledge and Hayashi, 2006; Blamires *et al.*, 2017). The constructing materials of all orb webs are two-dimensional webs: (i) with the help of surface area they capture their prey, (ii) the wide area between the spaces of the spirals makes meshwork or network & (iii) the length and width along with different patterns of any arrangements added to the web.

Some examples of well-known Araneomorph group

Deinopid net-casting webs

The net-casting spiders *Deinopis spinosa* and *D. longipes* (Deinopidae) produces cribellate capture threads, and they have a unique prey-capturing method. They position themselves on their habitat and spin a rectangular-shaped net to capture, net of cribellate silk releasing the net as an insect moves beneath. The net surrounds the insect, which becomes entangled in the woolly cribellate silk (Opell Getty and Coyler 1996). Large flying insects, such as moths, are caught by flicking the cribellate silk backwards (Getty and Coyler 1996). The silk reflects ultraviolet light (Craig *et al.*, 1994) which might be utilized to lure prey toward the net.

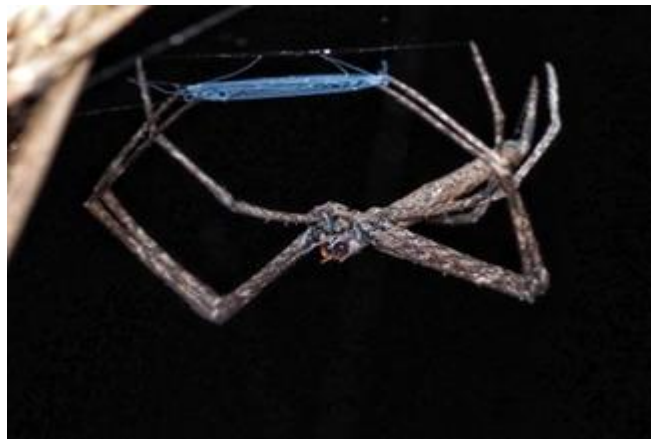


Figure 1.6: Deinopid Net-Casting Spider

Uloborid orb webs

Uloborid orb webs tend to be aligned horizontal to the ground. The reduced webs of *Miagrammopes* spp. and *Hyptiotes* spp., however, they are aligned to the ground vertically, which might be helpful in enabled by their greater cribellate thread adhesiveness (Opell, 1994a, b). Recently (Santos and Gonzaga, 2017) a new Uloborid genus was discovered and described (*Uaitemuri*) from Brazil which builds orb webs vertically. The parallel positioning of webs seems to lowers the prey capture rates of webs (Bishop and Connolly, 1992), it may be employed out of requirement to hold prey in their web. Uloborids, often combines their webs or architect their webs in close propinquity to the webs of other spiders (Finke, 1981). When in close proximity multiple webs are found, insects get stuck or reduce their flying velocity, thereby that makes easy to catch their prey by a nearby web (Uetz, 1989; Yip *et al.*, 2008; Blamires *et al.*,

2010). The more webs in the locality, the more likely it is that an insect will eventually get caught in web. This phenomenon is described as the ‘ricochet effect’ and has been projected as having an evolutionary benefit connected with spider web accumulations (Uetz, 1989)



Figure 1.7: Uloborid Orb Web Spider

Theridiid cobwebs

The gumfoot threads of a cobweb extend downward from the tangled retreat. The gumfoot adhesive dewes at the thread base stick to prey creeping along the ground, and when the prey struggles, the thread is released from the substrate. Upon release from its pyriform attachment, a gumfoot thread transmits vibrational stimuli toward the cobweb so that the spider, prey has been caught in its web (Peters 1987). Viscid beads have been found within the twisted retreat of *Achaeranea* webs (Barrantes and Weng 2006). Nevertheless, the function of these globules remains unclear. Their small size suggests that they are of little value in prey retention, but this function should not be ruled out (Benjamin *et al.*, 2002; Barrantes and Weng 2006).



Figure 1.8: Theridiid Cobweb Spider

Araneidae Orb webs

Orb webs aligned vertical to the ground, such as those spun by *Neoscona*, *Argiope*, *Nephila* and *Araneus*, appear to be adapted for the capture of high-kinetic energy prey (Kohler and Vollrath, 1995; Harmer *et al.*, 2011; Sensenig *et al.*, 2012). The greatest role in stopping

prey, as the energy absorption capacity in radiated threads of their silks is an order of scale greater than that of the viscid silks (Vollrath, 1994). The preliminary smoothness and eventual durability of radiated threads provides the absorption inelasticity for high-kinetic-energy interception (Denny, 1976; Craig 1987; Harmer *et al.*, 2011; Sensenig *et al.*, 2012). The energy absorbed depends where on the web's surface area, the force applied, and the angle of interception (Craig, 1987). Covering the flagelliform webs with aqueous collective glue causes the flagelliform silk to elasticize and become highly stretchable. This enables the kinetic energy of affecting prey to be reported onto the web, lowering the possibility of the prey escaping through the web (Boutry and Blackledge, 2013).



Figure 1.9: Araneidae Orb web Spider (Nephila)

Conclusion:

The diversity of spider webs and web-building spiders from the Western Ghats of regional area of Mangalore has been well described owing to over a century of detailed observations. We have given an overview of some examples of the multitude of different web forms found in the Western Ghats of Karnataka, highlighting some striking web forms and their structural and functional variability. Since the Western Ghats contains up to 56 genera of spiders including 112 species, including Orb weavers, Jumping Spiders with different spider web architect and the various alterations thereof described herein will be a close representation of overall spider web diversity.

A spider's web is an extended phenotype depicting its hunting, pairing, and defensive strategies, and physiological status. Spiders exhibiting web plasticity can continue to build functional webs across highly variable environments. The seasonality and geography of the Western Ghats region, and the array of spider predators and prey that can be found there, have undoubtedly shaped the unique diversity of the region's spiders.

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IDENTIFICATION OF IMMUNOTOXIC EFFECTS OF FUNGICIDE MANEB BY COMPUTATIONAL APPROACH

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Abstract:

Maneb is a hormonally active fungicide widely used for the protection of fruits, vegetables, field crops and to increase their production. Humans get exposed to this contaminant either directly through the farm or indirectly by consumption of contaminated food. Maneb exposure can have serious health consequences including neurological disorders, reproductive problems, and carcinogenic impacts. However, very little is known about its immune toxic effects. In this context, we selected putative genes involved in immune system diseases from the comparative toxicogenomics database in response to the Maneb exposure. Further, pathway analysis was done for targeted disorders using PANTHER. An initial database search shows the involvement of Maneb in 136 different immune system diseases. Among them, three diseases including Arthritis, Asthma, multiple myeloma, were targeted. Pathway analysis for these disorders shows the involvement of several pathways from which significant pathways were selected by statistical overrepresentation test analysis. The finding shows that Inflammation mediated by chemokine and cytokine signaling pathway was most expected in arthritis and asthma. In contrast, the CCKR signaling map pathway was found to be most expected in multiple myeloma. In addition, we also analyzed genes present in most affected pathways and found Bcl-2 and IL-2 were highly affected genes by maneb toxicity responsible for immune system disorders.

Keywords: Maneb, Immune system diseases, comparative toxicogenomics database, panther pathway, IL-2

Introduction:

The maneb is a broad-spectrum pesticide comes under the class of fungicide. Chemically it is belonging to thylene bis-dithiocarbamate fungicides (DTCs) family. Maneb is widely used alone or in combination with other pesticides for the protection of a variety of food crops (Richard, 2015). First time it was registered in the 1962 at United States and then broadly used worldwide (EPA, 2005). However, later application of maneb was restricted or banned by different organization including the Swedish Chemicals Agency SCA (2008), the European Parliament EP (2009), and the United States Environmental Protection Agency (EPA) and EFSA

(EFSA 2013) due to its potential toxic effects on mammals. Several studies reported the carcinogenic, genotoxic, reproductive and neurotoxic effects of the maneb (Belpoggi *et al.*, 2002; Cecconi *et al.*, 2007; Costello *et al.*, 2009). It is also classified as an endocrine disruptor that negatively impact the female reproductive system by disrupting normal ovarian function (Dinisri *et al.*, 2021). As for immune system toxicity in general, very few studies are available that showed the immune system toxicity of maneb. Reported studies showed the effect of maneb alone or in combination on T cell regulatory mechanism and natural killer cell function (Bonner, 2005; Li *et al.*, 2015). However, in the present era with the advent of technology, numerous animal-free methods are available for determining the toxicological impact of maneb like compounds. This method is known as in silico approach or computational system analysis. It can predict the toxicity of any chemical without the need of any animal or cell model. New methodological philosophies (NAMs) that include computational models, in vitro high throughput screening (HTS), omics considerations and the 3S methodology (orderly, systemic, and frameworks science and toxicology) are just a few examples (Hartung *et al.*, 2017; Smirnova *et al.*, 2018; Andersen *et al.*, 2019). Because of the wide use of maneb for domestic and industrial purposes, and evaluation of immunotoxic effect of maneb is a major concern for public health. With this background, current work is designed to explore the pathways involved maneb induced immune system disorders of maneb with the help of comparative toxicogenomics database and panther pathway analysis.

Material and Methods:

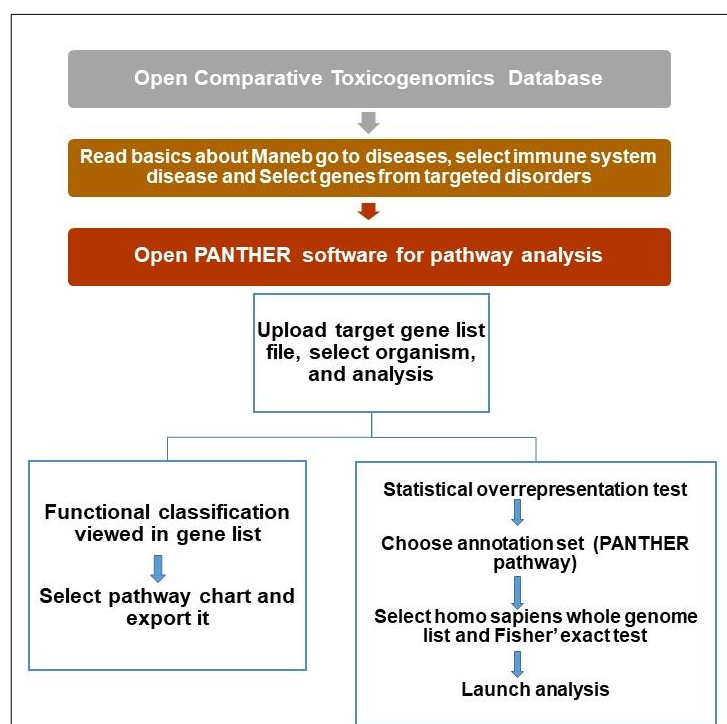


Figure 1: Flowchart of the *in-silico* approach to analyze pathways involve in the adverse effects of maneb on immune system

To perform this study, initially comparative toxicogenomics database (CTD) was screened for the maneb. General toxicity information of maneb was recorded in terms of gene interactions induced and affected pathways. Then, specific information about maneb induced immune system diseases was filtered. CTD is a publicly accessible largest database aiming to increase the knowledge of environmental chemicals, their exposure route and consequently adverse effect on human health. Based on database, we targeted three top immune system diseases based on the inference score. Maneb interacting genes found to be involved in these diseases were extracted. Further, these genes were analyzed by PANTHER software selecting *Homo sapiens* organism. Functional classification and statistical overrepresentation test were performed using panther pathway annotation. From this, we obtained significant pathways and genes for most affected pathways. A schematic representation of workflow used to conduct this study is given in the form of flowchart in Fig.1.

Results:

Extraction of general toxic information data of maneb from comparative toxicogenomics database

General toxicity analysis of maneb at molecular level shows that it interacts with different kinds of genes in various organism (<http://ctdbase.org/detail.go?type=chem&acc=D008344>). Top 10 interacting genes are given in Table 1. Basic toxicity characteristic predicted by CTD in terms of gene interactions, diseases, pathways and exposure studies were checked and presented in the Table 1.

Table 1: Basic molecular characteristic of the maneb inferred by database

Sr. No.	Parameters	Statement
1	CAS Registry Number	12427-38-2
2	Top interacting genes	SNCA, NOS-2, CYP2E1, TNF, GSTA4, IL-1B, TH, LRRK2, TRP53, BAX
3	Total gene interactions	990
4	Total affected genes	458
5	Diseases	1788
6	Pathways	486
7	Exposure studies	16
8	References	154

Extraction of maneb interacting genes involve in immune system diseases

Based on database, we targeted three top immune system diseases based on the inference score. The targeted immune system diseases include asthma, rheumatoid arthritis and multiple

myeloma. Genes predicted to be involved in these diseases were extracted and analyzed by PANTHER software in *Homo sapiens* organism for panther pathway analysis (<http://pantherdb.org/>). Genes found to be involved in targeted immune system diseases are given in Table 2.

Table 2: Genes involved in Immunotoxin diseases of maneb retrieved from CTD

Sr. No.	Diseases	Genes	Inference score	Reference
1	Asthma	ALDH2 ARG1 BCL2 CAT CCL2 CYP21 GSTP1 HMOX1 HSD11B2 ICAM1 IL1B IL6 NOS2 NQO1 SOD1 STAT4 TGFB1 TNF VEGFA	33.36	29
2	Arthritis, Rheumatoid	BCL2A1 BMP4 CAT CCL21 DDIT4 ENO1 FKBP5 IFNG IL1B IL6 MTHFR NCF1 SOD2 STAT4 TNF TNFAIP3 TRAF6 VEGFA	30.99	20
3	Multiple Myeloma	BCL2 BCL2L1 BNIP3 CCL2 CCND1 CDKN2A FGFR3 IL6 MCL1 NQO1 SOD2	27.44	7

Analysis from Protein Analysis through Evolutionary Relationships (PANTHER) Classification system

The PANTHER program was used to analyze pathways regulated by these genes based on genes collected from a comparative database.

Pathway analysis in Asthma

Maneb has an effect on 22 genes in asthma, affecting 19 pathways in total. Maneb has an effect on 22 genes in asthma, affecting 19 pathways in total. Inflammation mediated by chemokine and signaling cytokine signaling pathway (15.8%), apoptotic pathway (10.5%), and Interleukin signaling pathway (10.5%) were the most expected pathways (10.5%). Bcl-2, TNF, and Il-2 genes are all involved in these pathways, according to a gene analysis. However, a test of overrepresentation pathways revealed that none of the expected pathways were significant (Fig.2)

Pathway analysis in Rheumatoid Arthritis

In rheumatoid arthritis, a total of 20 genes were involved in 22 pathways. The most anticipated pathways were inflammation driven by chemokine and signaling cytokine signaling pathway (18.2%), apoptotic signaling (9.1%), interleukin signaling network (9.1%), and toll receptor signaling pathway (9.1%). The Chemokine, IL-2, and IF- were discovered to be engaged in the highly expected pathways (Fig.3).

Pathway analysis in Multiple Myeloma

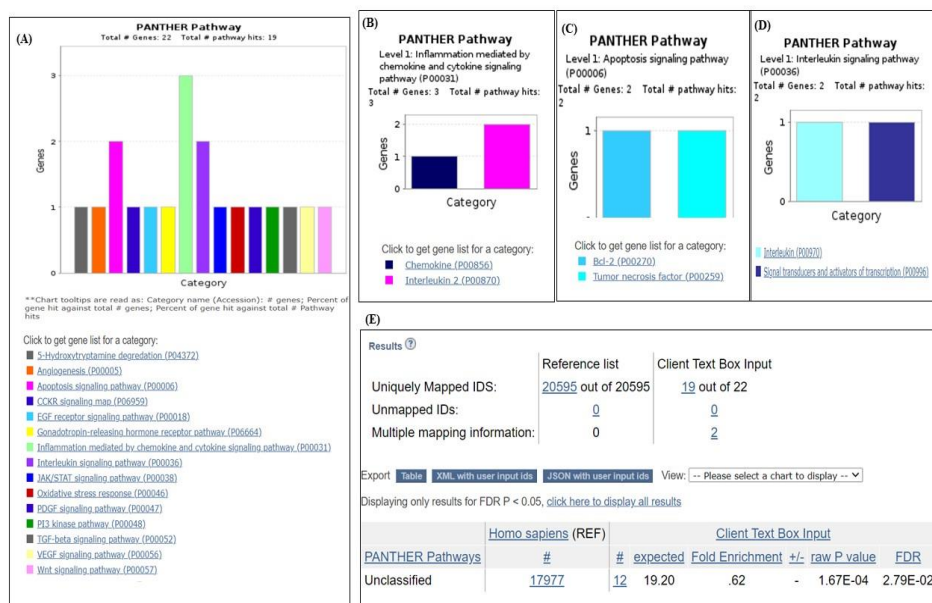


Figure 2: Panther pathway analysis of genes involved in asthma disorder: (A) Total pathways hits by selected genes (B) Genes of highly expected inflammation pathway, (C) Genes of Apoptosis signaling pathway, (D) Genes of Interleukin signaling pathway, (E) Results of statistical overrepresentation test analysis

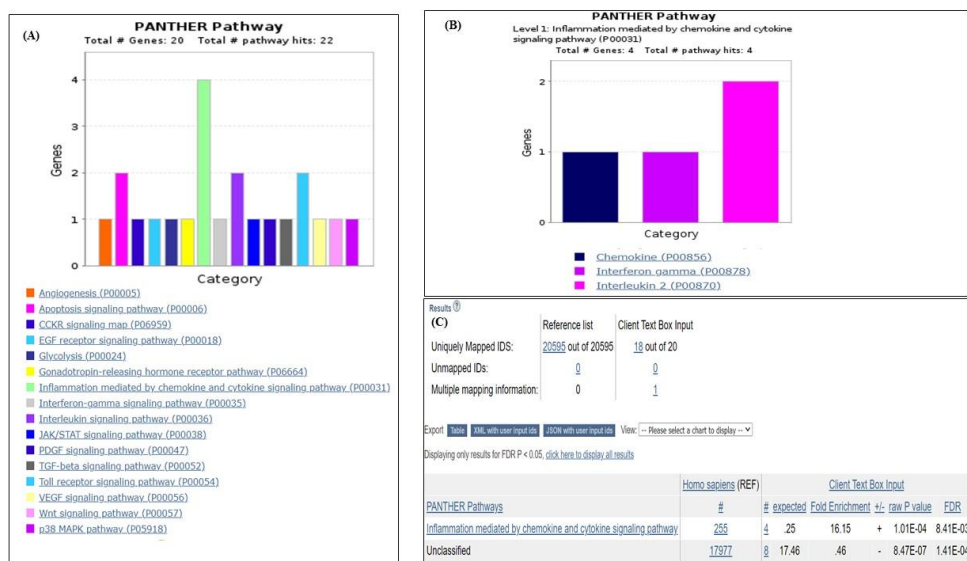


Figure 3: Panther pathway analysis of genes involved in rheumatoid arthritis: (A) Total pathways hits by selected genes (B) Genes of highly expected inflammation pathway, (C) Results of statistical overrepresentation test analysis

In multiple myeloma, 11 genes were shown to be involved in a total of 18 different pathways. The CCKR signaling (22.2%), apoptotic signaling (16.7%), inflammation driven by chemokine and cytokine signaling (11.1%), and the p53 pathway were the most commonly predicted pathways (11.1 %). The Bcl-2, CCND1, and MCL-1 genes were found to be involved in these pathways after gene analysis (Fig. 4). Furthermore, statistical overrepresentation test study revealed that three pathways were significant at P Value=0.05 across all pathways. Fig.5 shows an overlaid area chart of difference for significant pathways.

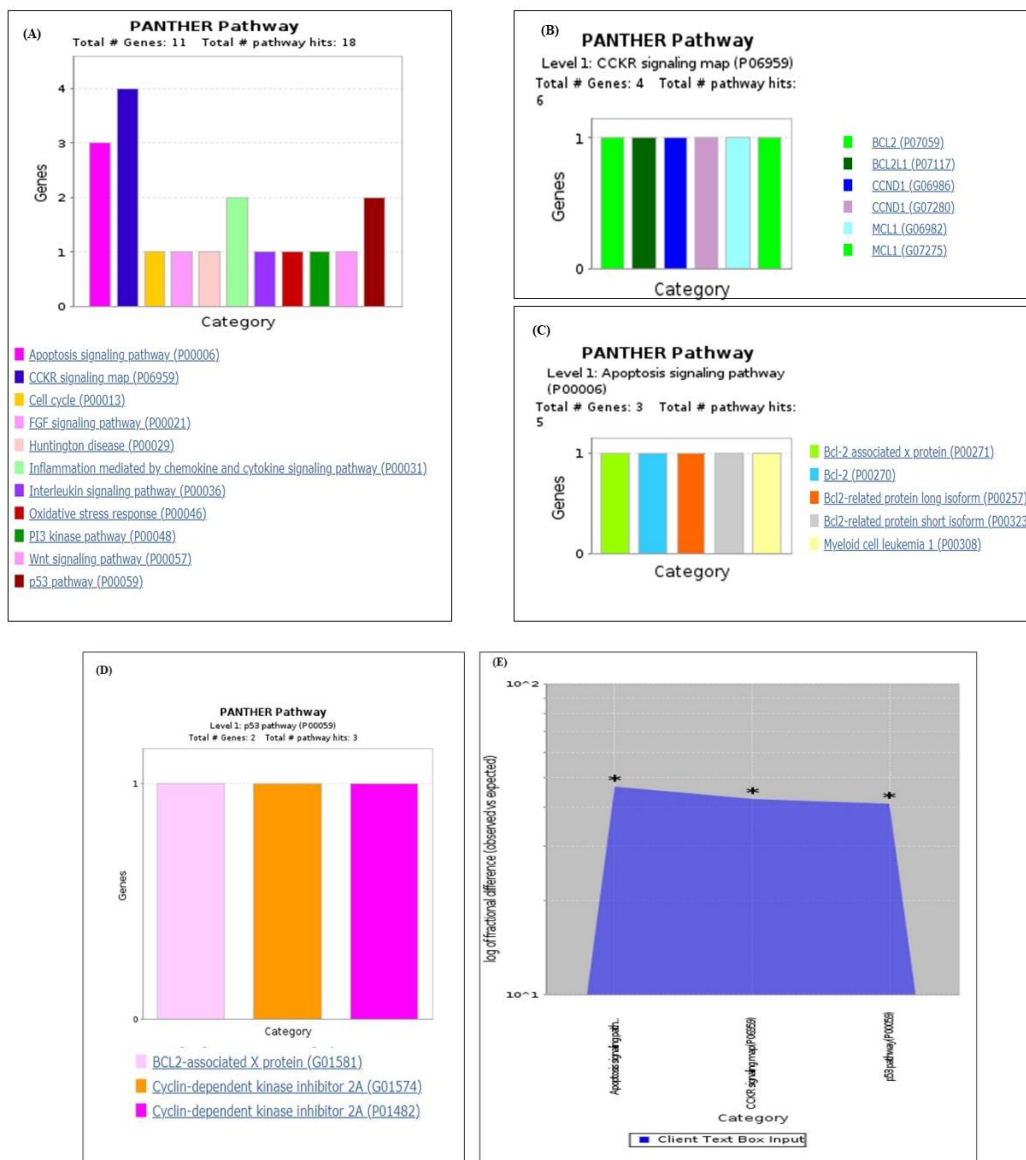


Figure 4: Panther pathway analysis of genes involved in multiple myeloma: (A) Total pathways hits by selected genes (B) Genes of highly expected CCKR signaling pathway, (B) Genes of Apoptosis signaling pathway, (D) Genes of p53 signaling pathway, (E) Results of statistical overrepresentation test analysis presented in the form of a chart of significant pathways (P<0.05)

Discussion:

The immune system is an organization of cells and molecules, and its primary function is to defend the body from invading pathogens (Parham, 2014). Immune cells must be able to detect and attack external threats while also tolerating body-borne structures in order to do so. The delicate balance between immune system defense and tolerance is managed by a sophisticated immune system regulatory network includes different cells and molecules (Parkin and Cohen, 2001). However, this immune system regulatory network could be disrupted and eventually leads to the development of chronic inflammation, such as allergies, autoimmune responses, and infections. Environmental contaminants can create such disruptions by changing immune cell function in such a manner that they react to allergens and the body's own molecule, or that they are no longer able to defense body. This indirect effect is also known as adjuvant effect and well known for pesticides (Lehmann, 2017). One of such pesticide is fungicide maneb, mainly known for its neurotoxic effects (Zhang *et al.*, 2003; Costello *et al.*, 2009). For the present study, robust comparative toxicogenomics database (CTD) was screened and specific information regarding effect of maneb on immune system disease was extracted. This database is very useful for high throughput studies, provides information about chemical-gene or protein interactions and chemical phenotype relationships in several organisms (Mattingly *et al.*, 2006). Likewise, in a different study mental toxicity of organochlorine insecticide endosulfan was predicted using same approach (Sharma *et al.*, 2021). Several previous studies also reported the screening of databases ToxCast and CompTox for the prediction of chemical toxicity (Richard *et al.*, 2016; Williams *et al.*, 2017). We targeted top 3 diseases based on the inference score includes, asthma, rheumatoid arthritis, and multiple myeloma. Further, this CTD information was used for the prediction of biological functional pathway to understand the toxicity mechanism of maneb in targeted immune system disorders with the help of PANTHER software. The PANTHER (Protein Analysis through Evolutionary Relationships) is a high-throughput analysis system classified proteins and their encoding genes into several functions (Muruganujan and Thomas, 2012). In asthma disease, most expected pathway was inflammation mediated by chemokine and signaling pathway followed by apoptosis and interleukin pathway. Role of inflammation pathway in asthma disease is well established by the contribution of Toll-like-receptors (TLRs) and NF- κ B (Mishra *et al.*, 2018). In case of rheumatoid arthritis, the inflammation pathway was found to be highly expected pathway. The RA is an autoimmune disease involve systemic inflammation that mainly affects the joints (Lee and Weinblatt, 2001; Ngian, 2010; Charles *et al.*, 2013). It is believed that environmental contaminants exposure triggers this disease, however very few data is available that support the role of pesticides in the RA (Salliot *et al.*, 2020). Findings of present study also showed the role of maneb in multiple myeloma disorder. Multiple myeloma (MM) is a type of blood cancer that accounts for approximately 10% of all hematologic cancers (Kyle and Rajkumar, 2009; Rajkumar *et al.*,

2007). It was found that maneb induced the multiple myeloma mainly by CCKR pathway. Though, there is no available study that established the role of this pathway in multiple myeloma disease. Other than CCKR, apoptosis and p53 pathway was found to be significantly ($P \leq 0.05$) affected by maneb in multiple myeloma. Additionally, most affected genes were found to be TP53 and Bcl-2. It is evident that tumor suppressor gene P53 (TP53) mutation is responsible for multiple myeloma (Walker *et al.*, 2015; Lodé *et al.*, 2010). Further, previous studies showed that organophosphate pesticide exposure increased the TP53 gene expression and ultimately DNA damage responsible for cancer disease (Hreljac *et al.*, 2008; Lane, 1994; Bumroongkit *et al.*, 2008). Overall, present study shed light on the untouched pathways of immune system altered by maneb toxicity by the use of bioinformatics tool. Likewise, a novel tool based on artificial intelligence, AOP-help Finder, uses text mining to screen abstracts of published articles to identify co-mentioned terms, which could be a chemical and an AOP event (Carvaille *et al.*, 2019; Rugard *et al.*, 2019).

Conclusion:

The current study provides a unique *in silico* approach that can be used to identify the potential targets, in a systemic manner, from already known and published information. It is very helpful animal-free method which can give insights into the unknown pathways regulated by chemical. Though it cannot replace the importance of *in vitro* or *in vivo* testing for chemical toxicity analysis. But it can give specific genes, pathways and functions which can be targeted for wet lab studies.

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A BRIEF TALK ON “LACTEAL” -THE GUT LYMPHATICS

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Introduction:

Lymphatic vessels are a vital component of the cardiovascular system and play a central cardinal role in absorption, gut homeostasis, body fluid balance, hypertension, obesity, diabetes, inflammation, and cancer metastasis. In Gut the lymphatic system, more specifically lacteals in the intestinal villi are very much essential for the absorption and transport of lipids and fat-soluble nutrients, a distinctive function. Spontaneous contractile movement of lacteals is mediated by smooth muscle and controlled by the autonomic nervous system. The efficient drainage of absorbed molecules in small intestinal villus lacteals, act as active pumps through regulated manner.

However in the recent times with the advancement of science lacteal has gained lot of attentions of the researcher as the structure and functions and its various regulators are closely associated with patho physiology of gut and overall health.

The small intestine is the site of digestion of foods and absorption of nutrients. The mucosal surface of the small intestine is covered with villi. Enterocytes of villi absorbs the majority of digested nutrients, drugs, across the apical membrane and released into the lamina propria. The lacteal, located at the center of each villus, an essential pathway for the drainage of absorbed lipid, to the systemic circulation via messenteric lymphatics (Tammela, 2010; Alitalo, 2011; Dixon, 2010).

Genesis of lymphatic vascular system

The development of the lymphatic system begins after blood vascular system. During early embryonic life sub population of lymphatic endothelial progenitors at anterior cardinal vein expresses Prox1. After the differentiation of the lymphatic endothelial cell, the lymphatic endothelial progenitors shed off on influence of VEGF-C and migrate dorso laterally and forms chains of interconnected cells, subsequently the primary lymph sacs and superficial lymphatic vessels.

The development of lymphatic vascular network and its proliferation, sprouting, regulated by lymphangiogenic signals like VEGF-C/D-VEGFR-3 and Angiopoietin (Angpt)-TEK (Tie2) pathways etc. Alejandra (2021)

The structural and functional organization of lacteal -The Gut lymphatics

Lacteal are the fine lymphatic vasculature within the intestinal villi plays crucial roles in fluid homeostasis, immune surveillance and transport of lipid and lipid soluble particulates from the intestine to the blood. With the recent advancement of the imaging system and surface markers of LEC the structure and functional correlation of the lacteal has gained lot of attention in understanding the patho physiology of gut. Lymphatic system is a unidirectional system.

The lymphatic networks are blind-ended capillaries located in most tissues that joins into collecting vessels. Lymph from the gastrointestinal and lumbar region drains into the cisterna chyli posterior to thoracic duct. In the intestine, lymphatic capillaries are located in the villi in propria submucosa called lacteals. Lacteal length normally reaches 60%–70% of villus length across the tract. (Bernier *et al.*, 2015). Lacteals are surrounded by a highly organized network plexus like structure of arterial and venous capillaries and set of contractile smooth muscle fibers (Bernier *et al.*, 2015). The extracellular matrix proteins (integrin $\beta 1$, integrin $\alpha 9$) in the villi interact with LEC protein (integrins) and known to regulate lymphangiogenesis and lacteal function (Wiig *et al.*, 2010; Bernier *et al.*, 2015). Additionally tenascin C, periostin found in the villus plays important role in tissue stretching, injury, and inflammation, Mfge8 (milk fat globule epidermal growth factor-like 8), a ligand for integrins $\alpha v\beta 3$, $\alpha v\beta 5$, and $\alpha 8\beta 1$ has been reported to influence intestinal lipid absorption (Khalifeh-Soltani *et al.*, 2014, 2016). Lacteals tips are characterized by fine cytoplasmic, actin-rich cellular extension (filopodia) particularly in active form (Yu *et al.*, 2017; Wong, 2017).

Lymphatic epithelial cells use glycolysis and fatty acid β -oxidation (FAO) for energy production (Yu *et al.*, 2017; Wong, 2017). FAO generates acetyl-coenzyme A used by the histone acetyltransferase p300 for histone acetylation of key genes involved in lymphangiogenesis. LEC have higher expression of fatty acid binding protein FABP4 and FABP5, fatty acid transport proteins FATP3 and FATP4, and long-chain fatty acid translocase CD36 (Wong, 2017)

Lymphatic endothelial cells are connected by specialized cell junctions containing both adherens and tight junction proteins (vascular endothelial cadherin and claudin-5). Initial lymphatics are interconnected by discontinuous button-like junctions, whereas downstream the collecting lymphatics are characterized by continuous zipper-like junction with no openings (Baluk, 2007; Dejana *et al.*, 2009).

The flap-like openings between adjacent “buttons” allow access of interstitial fluid and immune cells into the lymphatic lumen. The collecting vessels with continuous cell-cell junctions are less permeable, which avoids lymph leakage during transport from capillaries to lymph nodes (Baluk, 2007).

Lacteals display a mix of continuous and discontinuous junctions, demonstrating features of both sprouting and quiescent lymphatic capillaries..Bernier-Latmani J, (2015) Lacteal junctions have been recently shown to regulate CM entry into the lacteals, Zhang F *et al.*, (2018)

Regulation of Lacteal functions:

There are numbers of regulators and factors associated with lacteal function which works together. Altered lacteal lymphangiogenesis hinders absorption of dietary lipid. Some important proteins and factors are closely associated with the lymphangiogenesis like prospero homeobox protein 1 (Prox1), (Harvey *et al.*, 2005), vascular endothelial growth factor (VEGF)-C, Nurmi H *et al.*, (2015) notch ligand, delta-like ligand 4 (DLL4),.(Bernier-Latmani *et al.*, 2015) and adrenomedullin, (Davis, 2017). During early embryonic life lymphangiogenesis is governed by VEGF-C via binding of its tyrosine kinase VEGF receptor 3 (VEGFR-3) highly expressed in LECs. In the absence of VEGF-C, the growth of lacteal vessels begins during early embryonic life. Subsequently the lacteal buds get branched and form plexus under the control of VEGFR-3 by VEGF-C, (Nurmi *et al.*, 2015) and lymphatic vessel endothelial hyaluronan receptor 1–positive macrophages and activation leads to protein kinase C dependent activation of ERK1/2, implicated in cell proliferation (Kim *et al.*, 2012). However interstitial flow is very essential in this regard.

The premature form of VEGF-C fails to activate the phosphorylation of VEGFR-3 or down stream signaling, kinase activity or NO synthase etc. so maturation of the VEGF-C is critical in lymphatic sprouting (Bouvree *et al.*, 2012).

The VEGFRs signaling pathways are again regulated by neuropilin 1 and 2 (NRP1 and NRP2), non–tyrosine kinase transmembrane proteins that bind VEGFs (Bouvree *et al.*, 2012)

Some important factors and regulators in lacteal structural and functional integrity:

Prox1

Prox1 is a transcription factor (Homeobox gene) critical for lymphatic vasculature regulation (Wigle *et al.*, 1999, 2002) disruption in the functionality and development of Prox1 may lead to development of defective lymphatic vascular integrity and subtle leakage of lymph in the visceral area, dysfunctional submucosal and mesenteric lymphatic system, obesity, high mesenteric fat deposition, pulmonary edema and even postnatal death (Wigle *et al.*, 1999; Escobedo *et al.*, 2016; Harvey *et al.*, 2005). Altered function and availability of Prox1 leads to loss of lymphatic vessel integrity and may be a leading cause of obesity.

VEGF-C and VEGFR-3/VEGFR-2

In the intestine, VEGF-C is secreted by smooth muscle around the smooth muscle in the villus and surrounding the lacteal within the intestinal wall 22.44 Lamina propria macrophages and fibroblast also produce VEGF-C (Cursiefen *et al.*, 2004; Pollina, 2008) Disruption or absence of this factor leads to defective lipid absorption and excretion of cholesterol and free

fatty acid in faeces in higher amount with lacteal regression Defective VEGFR-3 tyrosine kinase motif may leads to retention of triglycerides (TGs) in the enterocytes of the small intestine and increased excretion of lipids into the stools. VEGFR is also essential in chylomicron mobilization in blood stream (Choe *et al.*, 2015; Nurmi, 2015).

Delta-like ligand 4

DLL4, a Notch ligand is mainly associated with the regeneration of the lymphatics in lacteal. DLL4 was highly expressed in tip cells of sprouting lacteal filopodia and had a lower expression in stalk cells. DLL4, is also essential for maintainance of lymphatic junctions and chylomicron uptake and transport (Bernier-Latmani *et al.*, 2015).

Adrenomedullin

Adrenomedullin expression on LECs is regulated by Pox and functions for normal development of the lymphatic system. Adrenomedullin promotes endothelial cell growth and survival, lymphatic permeability via ZO-1 and endothelial cadherin in cell membrane (Caron *et al.*, 2007; Dunworth *et al.*, 2008).

CD36

CD36 is a trans membrane protein plays role in intestinal lipid absorption and transport and chylo micron drainage . CD36 is vital for vascular homeostasis and survival functions of LEC (Love-Gregory *et al.*, 2011, 2016; Masuda *et al.*, 2009).

VEGF-A is a key player in chylomicron uptake via lacteal by regulating Cell-Cell Junctions (Zhang *et al.*, 2018).

Higher VEGF-A induces button-to-zipper junction transformation in the lacteals, which inhibits CM entry into the lymphatic capillaries causing lipid malabsorption and reduced weight gain (Vincenza Cifarelli *et al.*, 2018).

The entry of chylomicron into lacteals actively, regulated by the transcription factor **pleomorphic adenoma gene-like 2** (Plagl2) (Frederik Van Dyck *et al.*, 2007).

KOH Gou Young at the Center for Vascular Research, within the Institute for Basic Science (IBS, South Korea) have identified new subsets of gut connective cells, crucial for lymphatic growth which secretes VEGF-C. They reported that **regulatory proteins YAP/TAZ** in villi's connective cells, stromal cells, play a vital role in the growth lacteals. The mechanical stimulation within gut activates YAP/TAZ in the intestinal stromal cells, which in turn release VEGF-C (Hong *et al.*, 2020).

Mechanism of lipid absorption through lacteal:

The lipids are generally diffused through the interstitial space of villi. Long chain fatty acids stay longer in the enterocytes to be converted into triglycerides and assembled into lipoproteins, while shorter chain fatty acids diffuse across the enterocytes rapidly (Porter *et al.*, 2007). The short chain fatty acids are more hydrophilic and smaller so they are more quickly

cleared out through the blood vessels in lamina propria, rather than through the lacteals. Lacteals have much higher permeability. Piston-like retraction and extension of intestinal villus and lateral contraction of lacteals in orchastre aided by smooth muscles facilitate the absorptive functions. Structurally lacteal exhibit mixed functions of initial (discontinuous button-like junctions and lack of pericyte coverage). The enhanced absorptive ability of lacteal is due to synchronistic interaction between the lymphatic endothelial cell and surrounding smooth muscle movement, under the regulations of the the autonomic nervous system (Choe *et al.*, 2015).

The parasympathetic and sympathetic nerve stimulation can increase and decrease lacteal contraction. The sympathetic nerve stimulation can decrease blood flow, which can change the intestinal villus motility and can also affect the lacteal contraction. Contraction of the mesenteric collecting lymphatics, may affect the lacteal contraction. The Enteric nervous system can control the lacteal contraction (Womack *et al.*, 1987, 1988).

Absorption of dietary lipid and lipid soluble particulate in the gastrointestinal tract is a complex process. The free fatty acids and monoglycerides generated by the hydrolysis of neutral lipids, transfer through the apical membrane of enterocytes (Mansbach *et al.*, 2010; Tso *et al.*, 1986; Porter *et al.*, 2007)

In the enterocytes enzymes re-esterify them into triglycerides and TGs together with cholesterol, cholesteryl esters, phospholipids are assembled into CMs and are released from the enterocyte's basolateral membrane and enters the lacteals (Mansbach *et al.*, 2010; Porter *et al.*, 2007).

Dynamics of lymphatic flow during lipid absorption:

Orchestrate contraction of the lacteal under the influence of autonomic nervous system mediate the lipid drainage. An intrinsic pumping activity by collagen vessels and smooth muscle initiated by intra luminal pressure and release of vasoactive substance is also involved in drainage through gut lymphatics (Hargens *et al.*, 1977; Dixon *et al.*, 2006; Scallan, 2016)

Lacteal a potential target for preventing obesity:

In detail understanding on the lacteal structure and functions researcher have addressed the problem of obesity due to more lipid intake. Scientific investigations are initiated to prevent the chylomicron uptake and to blood through intestinal through lacteals by genetic alterations of endothelial Neuropilin1 (Nrp1) and Vascular endothelial growth factor receptor 1 and increasing VEGF-A bio availability. Transition of lacteal cell junctions may be used in therapeutics targets for preventing dietary fat uptake (Feng zhang *et al.*, 2018).

Gut microbiota and lacteal integrity

Small intestinal is rich in variety of microbial population which have co-evolved with the host play vital role in local tissue homeostasis, immunity, digestion, metabolism and energy balance gut vascular development etc. Gut microbes are also found to play an interplay in lacteal

integrity. The VEGF-C derived from macrophages in gut upon recognition of microbes is a key in gut microbiota-mediated maintenance of lacteal integrity. Gut microbiota is crucial for lacteal integrity for providing unique micro environment and regulating villus macrophages in small intestine (Sang Heon *et al.*, 2019).

Conclusion:

Multiple and dynamic regulators are required to preserve the unique structure and function of lacteals to maintain the lipid metabolism and gut health. In the recent times the role of lacteal is no more restricted to lipid metabolism. Studies have been extended to understand regulation of lipid uptake, modulation of lacteal permeability and proliferation and how to cope up with obesity. It is very essential to explore the link between gut lymphatics and systemic metabolism which will evolve in understanding physiological and pathophysiological conditions. Though many pathophysiological relationship like obesity, gut inflammation, irritable bowel syndrome, chylomicron transport, but the exact specific mechanisms if established it will open many therapeutic prospects. How exactly the lacteal stimulation can mobilize the lipid and chyle uptake it is to be answered.

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ROLE OF CALCIUM SIGNALLING AND A TRANSLATION INITIATION FACTOR IN RIBOSOME BIOGENESIS: A UNIQUE PARADIGM

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Abstract:

Eukaryotic translation initiation factor 6 (eIF6), a highly conserved protein from yeast to mammals, does not act just as a canonical initiation factor. Rather, it is essential for 60S ribosome biogenesis, which is mostly a nucleolar function. eIF6 is also needed to escort nascent pre-60S ribosomal particles from the nucleus to the cytoplasm for their final maturation. The protein is a limiting component and has to be released and recycled back to the nucleus for continued 60S biosynthesis. This nucleo-cytoplasmic shuttling of eIF6 is essential and well regulated. Phosphorylation of eIF6 at conserved Ser-174 and Ser-175 residues by nuclear isoform of Casein Kinase 1 (CK1) promotes its cytoplasmic localization. On the other hand, dephosphorylation mediated by Ca^{++} -activated Calcineurin phosphatase facilitates its nuclear import. This specific requirement also suggests involvement of intracellular Ca^{++} level in eIF6 regulation and 60S ribosome biogenesis. This is a unique paradigm and inhibition of the process leads to defects in ribosome synthesis, ribosomal dysfunction and associated pathophysiology.

Keywords: eIF6, 60S biogenesis, CK1, Calcineurin, calcium

Introduction:

Initiation of eukaryotic protein synthesis (translation) requires a pool of free 40S and 60S ribosomal subunits from which 80S ribosomes gradually assemble in steps. Eukaryotic translation initiation factor 6 (eIF6; Tif6 in yeast), a monomeric protein of about 26.5 kDa, serves this purpose by binding specifically to the 60S ribosomal subunit preventing its association with the 40S (Russel and Spremulli, 1979; Valenzuela *et al.*, 1982; Miluzio *et al.*, 2009; Biswas *et al.*, 2012). Cryo-electron microscopic studies showed that eIF6 and the 40S subunit share a common binding site on the 60S subunit so that they cannot bind at the same time. Due to such ribosomal subunit anti-association role, the protein was labelled as an initiation factor (Biswas *et al.*, 2012). However, it should be noted that eIF6 cannot dissociate already preformed 80S complexes.

The protein was also shown to be highly conserved in nucleated species. Soon it was found that eIF6 plays a far more significant and essential role in 60S ribosomal subunit biogenesis. Specifically, it is required for pre-rRNA processing in the nucleolus. It is also needed to escort the pre-60S particles from the nucleus to the cytoplasm for their final maturation. eIF6

then has to recycle back to the nucleus for continued 60S biosynthesis. This nucleo-cytoplasmic shuttling of the protein has been found to be regulated by casein kinase 1 mediated phosphorylation at specific conserved serine residues and dephosphorylation by Ca^{++} -Calmodulin dependent calcineurin phosphatase, in mammalian cells. Calcineurin can associate with and promote nuclear transport of eIF6 only when it is Ca^{++} -activated. (Biswas *et al.*, 2012). This requirement indicates that intracellular Ca^{++} level might regulate eIF6 functioning and, consequently, nucleolar 60S ribosome biogenesis and assembly. This is a novel paradigm that implicates calcium signalling and a translation initiation factor in ribosome biogenesis. Moreover, inhibition of this conserved process has been shown to lead to defects in ribosome biogenesis and maturation and associated pathophysiology, including congenital ribosomopathy like the Shwachman-Bodian-Diamond syndrome (SBDS). eIF6 has also been implicated in tumorigenesis and cancer, making it a therapeutic target.

Study of eIF6 in yeast cells: Role in ribosome biogenesis

Cell growth and cell proliferation need coordinated ribosome biogenesis and translation. eIF6 is a highly conserved protein from yeast to mammals with no homologues reported till date in eubacteria. It can prevent premature binding between 40S and 60S ribosomal subunits, an essential feature of translation initiation. eIF6 was originally purified and studied from the post-ribosomal supernatant of wheat germ and mammalian cell extracts. Its isolation was based on an *in vitro* assay that measured its ability to bind specifically to the 60S ribosomal subunit inhibiting its association with the 40S. However, its role as a canonical translation initiation factor was not systematically characterized in details then. Later it was found that yeast and mammalian eIF6 both have 245 amino acids and are 72% identical in amino acid sequence. This made it possible to carry out in depth molecular genetic studies by examining the yeast eIF6 ortholog Tif6p in the yeast *Saccharomyces cerevisiae* to get an insight of the exact functional properties of this conserved protein (Sanvito *et al.*, 1999; Si and Maitra, 1999).

Genome sequencing studies have revealed that *eIF6* is a single gene in all species except in *Arabidopsis*. The yeast single copy essential gene was subjected to conditional mutation studies. Such mutant yeast strains showed that depletion of eIF6 adversely affects rate of *in vivo* protein synthesis and results in inhibition of yeast cell growth and viability. A detailed comparative analysis of the polysome profiles of wild-type yeast and eIF6-depleted cells revealed that eIF6 depletion causes a significant reduction in the number of polyribosomes. It was already known that cells depleted of an essential translation initiation factor (e.g., eIF5), show a reduction in the size of polyribosomes which is always accompanied by a conspicuous increase in the number of 80S ribosomes and both free 40S and 60S subunits. But, in striking contrast, the decrease in polyribosome content of eIF6-depleted cells was found to be accompanied by a decrease in the number of both 80S and 60S ribosomes and simultaneous accumulation of so called 'half-mer polyribosomes' that represented stalled 43S initiation

complexes at the 5'-UTR and at the AUG codon of mRNAs, presumably awaiting their association with 60S subunits. When eIF6-depleted cells were subjected to a direct estimation of their individual 40S and 60S subunit content, it was confirmed that there was a selective reduction of total 60S with respect to total 40S subunits, leading to a stoichiometric imbalance in the 60S/40S subunit ratio which, in turn, resulted in the formation of half-mer polysomes. Such selective reduction of 60S ribosomal subunits indicated severe inhibition in its biogenesis pathway and also suggested involvement of eIF6 in the process (Si and Maitra, 1999; Biswas *et al.*, 2012).

More detailed studies showed that in exponentially growing cells, yeast eIF6 ortholog Tif6p is localized primarily in the nucleolus where most of the steps of 60 S ribosome biogenesis occur, and that it is required in pre-ribosomal RNA processing. The lack of Tif6p specifically prevents the processing of the intermediate 27SB pre-rRNA to 7S and 25.5S pre-rRNAs that are the respective precursors of 5.8S and 25S mature rRNAs, the constituents of the 60S ribosomal particle. Tif6p has also been found to be a constituent of a multiprotein assembly complex associated with the pre-60S ribosomal particles in the nucleolus, in agreement with its functioning (Basu *et al.*, 2001; Biswas *et al.*, 2012).

It was also reported that Tif6p bound to the pre-60S ribosomal particles is needed to escort the pre-60S complex through the nuclear pore to the cytoplasm for its final maturation to make it translation competent (Johnson *et al.*, 2002; Biswas *et al.*, 2011; Biswas *et al.*, 2012). Once in the cytosol, two proteins namely, Efl1p and Sdo1p, facilitate the release of Tif6p from the pre-60 S particles in the cytoplasm via a structural rearrangement. It was proposed that the released cytosolic eIF6 is rapidly imported to the nucleus for continued 60 S ribosome biogenesis, which is essential to sustain rapid exponential growth of yeast cells (Senger *et al.*, 2001; Menne *et al.*, 2007; Biswas *et al.*, 2011; Biswas *et al.*, 2012). However, such nuclear import was not directly demonstrated in yeast.

Thus, it was established in yeast that this initiation factor is actually essential for 60S subunit biogenesis and maturation.

Additionally, lysates of yeast cells lacking eIF6 remained surprisingly active in translation of mRNA *in vitro*. This led to the conclusion that eIF6 may not function as a canonical translation initiation factor for global protein synthesis. Moreover, it was shown that the stability of mature 60S ribosomal particles (which had been synthesized in yeast cells in the presence of eIF6), was not significantly affected when eIF6 was removed from these cells (Biswas *et al.*, 2012).

It has not yet been directly shown whether eIF6 also plays a similar essential role in mammalian ribosome biogenesis. But such a function is strongly suggested since the protein is remarkably conserved from yeast to mammals and given that its total depletion results in lethality before embryo implantation and that human eIF6 can rescue yeast Tif6 mutations and

also that mammalian eIF6 is present in the nucleolus. Interestingly, mammalian eIF6 is predominantly cytosolic although a significant amount is nucleolar, suggesting some additional role for the cytoplasmic counterpart. However, in mammalian cells, if eIF6 be downregulated by using siRNA, then the nucleolar eIF6 pool is totally retained so that ribosomal biogenesis goes on as usual, whereas the cytoplasmic pool of eIF6 is reduced (Miluzio *et al.*, 2009).

Phosphorylation of eIF6:

In both mammalian and yeast cells, eIF6 (Tif6p) was found to undergo phosphorylation at Ser-174 and Ser-175 residues. Isolation and subsequent characterization of the protein kinase from rabbit reticulocyte lysates and from yeast identified the enzyme to be the nuclear isoform of casein kinase 1 (CK1) for mammalian eIF6 and yeast CK1 ortholog Hrr25p for yeast Tif6p. The serine residues involved were shown to be present in a highly conserved CK1 consensus sequence. eIF6 was also reported to possess a conserved docking motif for CK1 (FXXXXF, where X is any amino acid) at amino acid positions 34–38 (Basu *et al.*, 2003; Ray *et al.*, 2008).

Mutation of the two conserved serine residues to alanine or depletion of Hrr25p from yeast cells was shown to cause total abolition of eIF6 phosphorylation in yeast. Mutation of Ser-174 alone could result in major loss of yeast cell growth and viability. This suggested that CK1 mediated eIF6 phosphorylation is crucial and physiologically relevant.

Nucleo-cytoplasmic shuttling of eIF6:

As mentioned earlier, a small number of trans-acting protein factors, including eIF6 (Tif6p), remain bound to the nascent pre-60S particle and help it to exit the nucleus and enter the cytoplasm for final maturation. This is a vital step needed to obtain translation-competent ribosomes. In the cytoplasm, sequential and ordered release of these bound protein factors occur, by the action of specific cytoplasmic ATPases or GTPases. Tif6p and the nuclear export adapter Nmd3 are the last proteins to be released. Molecular genetic analysis studies in yeast cells revealed that two cytoplasmic proteins are involved in eIF6 release. One of them is the GTPase elongation factor-like 1 protein (Efl1p) and the other one is called Sdo1p. They have been shown to genetically interact with the pre-60S - bound eIF6 in the cytoplasm, and act cooperatively to facilitate the release of eIF6 from the pre-60S particles. Sdo1 happens to be the yeast ortholog of the highly conserved mammalian Shwachman–Bodian–Diamond Syndrome (SBDS) protein that is mutated in the inherited ribosomopathy SBDS, which is characterized by bone marrow failure and predisposition to leukemia (Senger *et al.*, 2001; Menne *et al.*, 2007; Biswas *et al.*, 2011; Biswas *et al.*, 2012).

Mutational studies showed that, unlike in wild type yeast, a large amount of Tif6p bound to the pre-60S particles accumulated in the cytoplasm of *efl1Δ* and *sdo1Δ* yeast cells which showed a slow growth phenotype. Multiple gain of function TIF6 alleles (carrying missense mutation that resulted in low affinity for pre 60S) could rescue the growth defect of *sdo1Δ* or

efl1Δ cells and could also restore the nucleolar localization of Tif6p. Further studies from independent laboratories confirmed that the mechanism of eIF6 release from the pre-60S particles in the cytoplasm is a highly conserved process between yeast and mammals. It involves cooperative interaction of SBDS and EFL1 in mediating GTP hydrolysis-dependent release of eIF6 from the pre-60S particles (Senger *et al.*, 2001; Menne *et al.*, 2007; Biswas *et al.*, 2011; Biswas *et al.*, 2012). After this release, eIF6 must be imported back to the nucleus to resume its role in pre-rRNA processing. Thus nucleo-cytoplasmic shuttling of eIF6 is mandatory for continued 60S ribosome biogenesis in the nucleus and its subsequent maturation in the cytoplasm.

Interaction between Ca⁺⁺-dependent calcineurin phosphatase and eIF6:

eIF6 does not have a nuclear localization signal (NLS) or a nuclear export signal (NES). This gave rise to the question how does the protein shuttle between nucleus and cytoplasm. A careful examination of the highly conserved amino acid residues surrounding the CK1 phosphorylation sites of eIF6 showed that in all nucleated species examined till date, the protein also possesses a sequence motif LQVP at amino acid positions 177–180, just adjacent to Ser-174 and Ser-175. It is well established that the Ca⁺⁺/calmodulin- dependent heterodimeric protein phosphatase calcineurin uses the moderately conserved consensus sequence, LXVP (X= any amino acid) as a docking motif on its substrates. Calcineurin is known to bind to this site at a hydrophobic pocket formed at the interface of its catalytic subunit A and regulatory subunit B. This hydrophobic pocket is targeted by the immunosuppressant-immunophilin complexes (e.g., cyclosporin A -cyclophilin complex or FK506-FK binding protein 12 complex). Accordingly, immunosuppressants cyclosporin A (CsA) and FK506 have been widely used in many studies as specific inhibitors of calcineurin-mediated dephosphorylation reactions *in vivo*. (Biswas *et al.*, 2011; Biswas *et al.*, 2012).

The presence of a putative calcineurin binding motif immediately adjacent to the CK1 phosphorylation sites on eIF6 suggested that it might be dephosphorylated by calcineurin similar to the mammalian NFAT family of transcription factors (essential for cytokine gene expression necessary for T cell activation) or like the stress-responsive transcription factor Crz1p in yeast. Co-immunoprecipitation studies showed that cytosolic eIF6 indeed binds to calcineurin, but only when the latter is activated by calcium or by calcium ionophore ionomycin. Calcineurin, however, did not interact with eIF6 under unstimulated condition, indicating a crucial role for changes in intracellular calcium level in eIF6 regulation (Biswas *et al.*, 2011; Biswas *et al.*, 2012). Yet unpublished data by Biswas *et al.*, suggest that mutation of the calcineurin docking motif LQVP to AQAA or presence of calcineurin inhibitors like CsA or FK506 can abolish interaction between eIF6 and activated calcineurin.

Phosphorylation-dephosphorylation mediated eIF6 recycling in mammalian cells:

Recent studies in mammalian cells have presented convincing data to establish that mammalian eIF6 recycles between the nucleus and the cytoplasm, although the distribution is predominantly cytosolic (unlike in yeast cells where it is mostly nuclear). eIF6 recycling can occur in the presence of a potent protein synthesis inhibitor cycloheximide which suggests that such shuttling is a reflection of dynamics of nuclear import and export of pre-existing eIF6 molecules instead of *de novo* new eIF6 synthesis (Biswas *et al.*, 2011; Biswas *et al.*, 2012).

Many proteins have been reported to shuttle continuously back and forth between the nucleus and cytoplasm of eukaryotic cells. The nucleo-cytoplasmic distribution of these proteins, is usually regulated by different mechanisms, including by their phosphorylation-dephosphorylation status. Like the mammalian NFAT transcription factors or the yeast Crz1 protein, eIF6 undergoes CK1 and calcineurin mediated phosphorylation-dephosphorylation. It was also shown that the nuclear export of eIF6, bound to the pre-60S particles, requires phosphorylation of Ser-174 and Ser-175 by CK1. Failure to phosphorylate at these sites either by mutation of the serine residues to alanine or by treatment of the cells with a CK1 inhibitor causes a significant fraction (>90%) of eIF6 to be retained in the nucleus. These observations strongly suggest that it is the phosphorylated form of eIF6 that is exported from the nucleus to the cytoplasm where it is subsequently released from the pre-60S particles by concerted actions of cytosolic SBDS/Sdo1 and EFL1/Efl1p, in a GTP hydrolysis coupled reaction. Calcineurin, following its activation by Ca^{++} , then associates with the released eIF6 that is still presumably in its phosphorylated form. Calcineurin appears to play an essential role in eIF6 nuclear import as the localization of eIF6 changes from cytosolic to nuclear subsequent to calcineurin activation *in vivo*. On the other hand, this event can be effectively blocked by the specific calcineurin inhibitor CsA, suggesting that the dephosphorylated form of eIF6 is imported to the nucleus. eIF6 being a rate limiting component, this nuclear re-entry is crucial for continued 60S biogenesis in the nucleus (Biswas *et al.*, 2011; Biswas *et al.*, 2012).

The requirement of Ca^{++} -activated calcineurin phosphatase in such nuclear import of eIF6 could provide a means by which changes in intracellular Ca^{++} levels could modulate nucleolar 60S ribosome biosynthesis. This is a unique paradigm in which calcium signalling has been implicated for the first time in ribosome biogenesis. A thorough molecular investigation of these steps is necessary to understand the entire mechanism to use it in therapeutics as this pathway provides an excellent platform where eIF6 phosphorylation-dephosphorylation regulators (including calcium signalling) can be assayed for their therapeutic potential in several ribosome-dependent pathophysiology.

eIF6 in SBDS ribosomopathy and cancer:

A number of congenital diseases result from defects in ribosome biogenesis and maturation. Shwachman-Bodian-Diamond syndrome (SBDS) is one such autosomal recessive

ribosomopathy, caused by deficiency of the highly conserved SBDS protein which is involved in 60S ribosome biogenesis. Characteristic clinical features include exocrine pancreatic insufficiency, neuro-cognitive dysfunction, bone marrow failure and leukemia predisposition (Menne *et al.*, 2007; Biswas *et al.*, 2012).

Mutations in the yeast ortholog of this SBDS protein (called Sdo1p) lead to defective 60S ribosome synthesis, which is rescued by gain-of-function of yeast eIF6 mutants, indicating that yeast eIF6 (called Tif6p) genetically interacts with yeast Sdo1p, as already mentioned before. In this context, release of cytosolic eIF6 from pre-60S particles followed by final maturation of the ribosomes and recycling of eIF6 back to the nucleus seem extremely crucial. Inhibition of this pathway leads to defects in both new 60S ribosome biogenesis and existing pre-60S maturation resulting in associated pathological symptoms in the SBDS patients ((Senger *et al.*, 2001; Menne *et al.*, 2007; Biswas *et al.*, 2012). SBDS, EFL1, eIF6 proteins and their regulatory factors (like phosphorylation-dephosphorylation mediators) can be implicated as critical, inter-dependent modulators of this essential and conserved pathway.

eIF6 has been reported to be consistently overexpressed in a variety of cancers including colon carcinomas and aggressive leukemias. Such eIF6 upregulation may be due to increased growth and proliferation rate and increased demand for ribosomes and translation in the transformed cells. As described above, eIF6 acts both as an essential ribosome biogenesis factor and a translation initiation factor. Recent data have indicated that a 50% reduction of eIF6 leads to a 90% reduction in oncogene-mediated transformation. Thus, eIF6 seems to take a part in tumorigenesis, although its exact role is poorly characterized and remains puzzling till date. Nevertheless, the observation that eIF6 activity affects transformation raises the hope that targeting its biochemical functioning and its regulators may be therapeutically beneficial (Miluzio *et al.*, 2009).

Conclusion:

eIF6, a highly conserved protein, plays an essential role in 60S ribosome biogenesis in the nucleus and its subsequent maturation in the cytoplasm. Opposing actions of casein kinase 1 and calcium-dependent calcineurin phosphatase regulate nucleo-cytoplasmic shuttling of eIF6 through phosphorylation and dephosphorylation events in specific serine residues. Such nucleo-cytoplasmic shuttling of eIF6 is analogous to that observed for the mammalian NFAT family of transcription factors and the yeast stress responsive transcription factor Crz1p. Two other crucial cytosolic proteins are also known to be involved in eIF6 recycling, namely SBDS/Sdo1 and EFL1/Efl1p. It is likely that other factor(s) may be involved in the process which are hitherto unknown. It is also not known whether calcineurin accompanies eIF6 into the nucleus or by what mechanism eIF6 phosphorylation in the nucleus is prevented till it has completed its function in pre-rRNA processing, since phosphorylation signals its nuclear export. Clearly, further studies

are needed to get a complete understanding of the pathway. Based on current data a schematic representation has been presented that depicts this process (Figure 1).

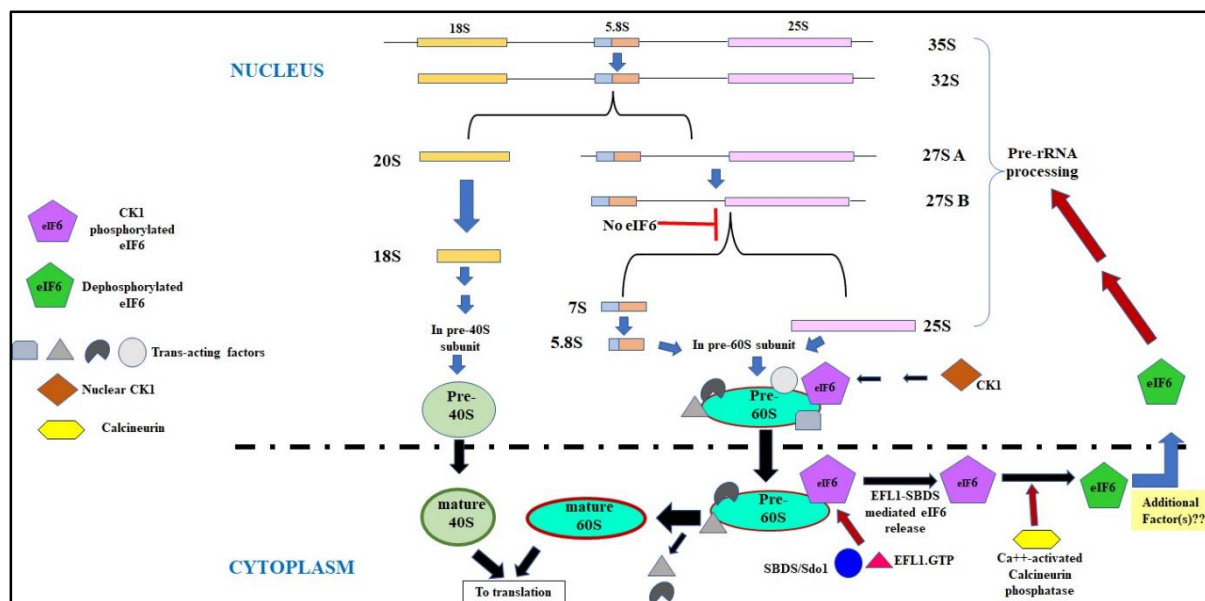


Figure 1: Schematic representation of the involvement of eIF6 in 60S ribosome biogenesis and maturation and its own nucleo-cytoplasmic shuttling. In the nucleus, eIF6 is essential for pre-rRNA processing to synthesize nascent pre-60S particles. The protein associates with the pre-60S particles along with many other trans-acting factors. eIF6 remains associated with the pre-60S particles during its nuclear export for final maturation in the cytoplasm. Nuclear export of eIF6 bound to the pre-60S particles requires phosphorylation of eIF6 at Ser-174 and Ser-175 by the nuclear isoform of CK1. In the cytoplasm, during the final maturation process of 60S, two cytoplasmic proteins SBDS/Sdo1 and EFL1/Efl1p interact with the pre-60S particles and catalyze the release of eIF6 in a GTP-coupled hydrolysis reaction by EFL1. The released eIF6 that is presumably in the phosphorylated form then interacts with Ca^{++} - activated protein phosphatase calcineurin for dephosphorylation. The dephosphorylated form of eIF6, either by itself or by interaction with additional factor(s)[unknown till date] is recycled back to the nucleus to participate in another round of 60S ribosome biogenesis

Acknowledgement:

The author is grateful to Albert Einstein College of Medicine, NY, USA and to CSIR, India for funding. The author would like to thank Prof. Umadas Maitra of Albert Einstein College of Medicine, NY, USA and Dr. Sasabindu Jana, Principal, Raidighi College, West Bengal, India for their support.

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MICROPLANT BIOTIZATION OF PLANT TISSUE CULTURES: POTENTIAL AND PROSPECTS

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Introduction:

Global growth in food demand, coupled with unequal distribution and imbalances in wealth distribution, is putting more and more pressure on food producers. At the same time, there is a growing consumer-led demand for reducing environmental damage and increasing sustainability in food production chains. Microplant biotization is a technique that uses PGPM (Plant Growth Promoting Microorganisms) to reduce the use of chemicals in crop production. Microorganisms that promote plant growth are involved in the decomposition, mineralization, solubilization of inorganic compounds, and the release of biologically active compounds such as chelating agents, plant hormones, and antibiotics. These are necessary for the overall health of the plant.

Unfortunately, PGPM has not been fully used in *in vitro* plant tissue culture, and few studies have reported inoculation of PGPM with micropropagation (Lopes *et al.*, 2019). This is because the presence of microorganisms in the *in vitro* environment has most often been perceived as negative in *in vitro* plant cultures (Orlikowska *et al.*, 2017). Elimination or prevention of contaminants is the key objectives in most of the research dealing with micropropagation and microbes.

However, many PGPMs can help root formation and shoot elongation, which can help with a successful acclimation period. In fact, they can protect against biological and abiological stresses that occur primarily during the hardening and acclimation stages of *in vitro* proliferation. Since PGPM is an important element of sustainable agriculture, it is difficult to promote the use of PGPM in micropropagation. This chapter aims to highlight the potential role of PGPMs in agriculture production, with special emphasis on micropropagation of plants under the following subheads.

Biotization in plant tissue culture

There are different stages in the *in vitro* propagation viz., Stage 0 (Plant stock immobilization and pre-treatments, selection of the explants), Stage I (Culture establishment), Stage II (Elongation and multiplication), Stage III (Rooting), Stage IV (Weaning, hardening, and

acclimatization) and Stage V (Transfer under natural conditions-to the field). The biotization can be done at all the above stages. In stages II and III of micropropagation by micro-cutting, PGPMs act generally as bio-stimulants by promoting elongation and increasing rooting, while in stage IV, they act as biocontrol agents and help to deal with biotic and abiotic stress factors (Diedhiou *et al.*, 2016). The stage of acclimatization that biotization of microplants seems to be most important (Orlikowska *et al.*, 2017). Numerous beneficial effects are found in plant growth promoting microorganisms (Fig.1)

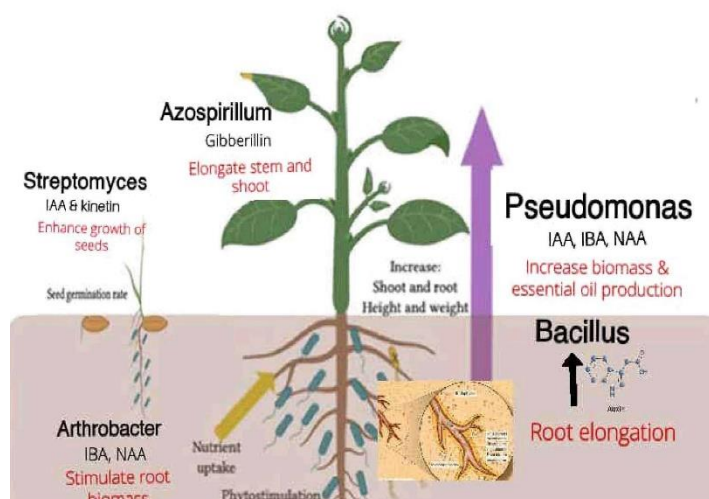


Figure 1: Beneficial effects of Plant growth promoting microbes

Beneficial effects of plant growth promoting fungi in *in vitro* plant culture

Fungi produce phytohormones such as auxins, cytokinins (CKs), abscisic acid (ABA), gibberellic acids (GAs), ethylene (ET), salicylic acid (SA), and jasmonic acid (JA). These hormones play a major role in biotic and abiotic stress by activating signaling pathways. The key issue to be addressed is how to create pure fungal inoculum free of impurities for micro propagation. At the moment, the spore disinfection and germination in agar Medium are challenging. Furthermore, fungi, particularly AMF, have a vital function in water intake and availability, enhancing the rate of photosynthesis and osmotic adjustment under environmental stress, increases the absorption of micro nutrients through their arbuscules and hyphae from soil to the plant. In case of *Hydrangea in vitro* plants that was inoculated with the AMF, *Glomus intraradices* (current name: *Rhizophagus intraradices*) at the acclimatization stage found with 100% survival rate and there was transplantation shock for the *in vitro* raised plants (Varma and Schuepp, 1994).

Role of Arbuscular Mycorrhizal Fungi (AMF) AMF in plants during water stress

Plantlets obtained through tissue culture are often adversely affected by water stress due to low water absorption capacity of their roots (Diedhiou *et al.*, 2016). This can be solved by inoculating Arbuscular Mycorrhizal Fungi (AMF) *in vitro* (Rai, 2011).

During the acclimatization phase, the contribution of Arbuscular Mycorrhizal Fungi is important. The *in vitro* plants which possess adventitious and weak root do not absorb water and nutrients at the early stage of development. This can be overcome through arbuscules and hyphae of AMF which helps to transfer nutrients from the soil to the plant (Chen *et al.*, 2018). Also it increases the photosynthetic rate and osmotic adjustment under stress condition. (Soumare *et al.*, 2017). Plants inoculated with this fungi also possess higher level of auxin when compared with non inoculated plants (Meixner *et al.*, 2005)

Certain endophytes are also phytopathogens and may limit the micro growth process. This is the case for *Fusarium equiseti*, which is suspected of causing bamboo rot and tail rot (Tyagi *et al.*, 2018). These problems can be solved by optimizing tissue culture protocol which ensure producing pure fungal culture without contaminants. The Murashige and Skoog medium (MS) most commonly used in micropropagation seems not to be favorable for the germination and growth of fungal spores (Rana *et al.*, 2019). Hence by adopting suitable media composition helps in producing pure fungal cultures.

Beneficial effects of plant growth promoting archaea in *in vitro* plant culture

Archaea are an important part of the Earth's planets and can play a role in C cycle and N cycle. There are few reports of archaeal-promoting plant growth, viz., phosphorus solubilization, nitrogen fixation, siderophore production, and indole acetic acid production. Plant growth promoting properties found in various archaea such as *Natrialba*, *Natrinema*, *Hallolamina*, *Halosarcina*, *Halostagnicola*, *Haloarcula*, *Natronoarchaeum*, *Halobacterium*, *Halococcus*, *Haloferax*, and *Haloterrigena*. Due to its unique adaptation to dramatically changing ecosystems, archaea are of particular interest in potential bioengineering applications in agriculture, medicine, and industry (Yadav *et al.*, 2017)

Beneficial effects of plant growth promoting bacteria in *in vitro* plant culture

Bacteria, fungus, actinomycetes, protozoa, and algae are among the tiny life forms found in soil. Among these microorganisms, bacteria are by far the most common (i.e., ~95%). They are generally not evenly distributed in soil. Bacterial concentrations around plant roots (i.e., in the rhizosphere) are often substantially higher than in the rest of the soil. The reason behind this is the presence of nutrients viz., amino acids, organic acids, and other small molecules from plant root exudates.

Production of phytohormones

The plants are instructed by the plant growth promoting rhizobacterias to produce growth hormones (PGPR). Gibberellins that is produced by bacteria interacts with other hormones and supports in shoot elongation (Bottini *et al.*, 2014).

Limited reports are found on other phytohormones such as abscisic acid and salicylic acid produced by PGPR. The growth hormones play a main role in senescence by modifying

ethylene levels and supports plant defense mechanism (Iqbal *et al.*, 2017). The plants resistance increase by decreasing the ethylene levels (Glick, 2015) Few examples of phytohormone producing bacteria and their role are listed in Table 1.

Table 1: List of phytohormone-producing bacteria and their ability to mitigate abiotic stresses

Microorganisms	Phytohormone	Host plant	Abiotic stresses
<i>Pseudomonas</i> sp., <i>Bacillus</i> sp.	IAA	<i>Sulla carnosa</i> (Desf.)	Salt stress
<i>Bacillus licheniformis</i>	IAA	<i>Triticum aestivum</i> L.	Salt stress
<i>Bacillus subtilis</i> , <i>Arthrobacter</i> sp.	IAA	<i>Triticum aestivum</i> L.	Salt stress
<i>Pseudomonas putida</i> , <i>Bacillus megaterium</i>	IAA	<i>Trifolium repens</i>	Drought stress
<i>Bacillus aryabhattai</i>	IAA, GA, ABA	<i>Glycine max</i> (L.) Merr.	Heat stress
<i>Bacillus licheniformis</i> , <i>Pseudomonas fluorescens</i>	ABA	<i>Vitis vinifera</i> L.	Water stress
<i>Bacillus amyloliquefaciens</i>	ABA	<i>Oryza sativa</i> L.	Salt stress
<i>Phoma glomerata</i> , <i>Penicillium</i> sp.	GA	<i>Cucumis sativus</i>	Drought stress
<i>Aspergillus fumigatus</i>	GA	<i>Glycine max</i> (L.) Merr.	Salt stress
<i>Bacillus subtilis</i>	CK	<i>Platycladus orientalis</i>	Drought stress
<i>Micrococcus luteus</i>	CK	<i>Zea mays</i>	Drought stress
<i>Serratia marcescens</i>	SA	<i>Zea mays</i>	Salt stress
<i>Burkholderia</i> sp.	IAA	<i>Solanum lycopersicum</i> L.	Cd stress
<i>Bacillus subtilis</i>	IAA	<i>Acacia gerrardii</i> Benth.	Salt stress
<i>Streptomyces coelicolor</i> , <i>Streptomyces geysiriensis</i>	IAA	<i>Triticum aestivum</i> L.	Salt stress
<i>Serratia</i> sp.	IAA	<i>Cicer arietinum</i> L	Nutrient stress
<i>Leifsonia</i> sp., <i>Bacillus</i> sp.	IAA	<i>Zea mays</i>	Cd stress
<i>Bacillus megaterium</i>	IAA	<i>Vinca rosea</i> L.	Ni stress
<i>Achromobacter xylosoxidans</i>	IAA	<i>Brassica juncea</i>	Cu stress
<i>Achromobacter xylosoxidans</i> , <i>Bacillus pumilus</i>	SA	<i>Helianthus annuus</i>	Drought stress

(Adapted from Egamberdievae *et al.*, 2017)

Production of volatile compounds

Volatile metabolites produced by bacteria play a role organogenesis (Gopinath *et al.*, 2015) and improve the efficiency of photosynthesis (Xie *et al.*, 2009). Plant growth promoting bacteria and fungi living in the rhizosphere play a major role against pathogens and insects by inducing systemic resistance (ISR). The immune system of the plants are activated during defense by Some PGPB (e.g., *Pseudomonas* and *Bacillus*) and some PGPF (e.g., *Trichoderma*) (Al-Ani and Mohammed, 2020).

Conclusion:

Application of PGPM in plant tissue Culture continues to be a viable option for improvement Plant growth and development in in vitro culture. They are considered as a potential biofactories since they instruct the plants for producing growth regulators. Development and use PGPM-based vaccines help reduce costs Production of in vitro plants in whole or in part. These beneficial microbes replace the commercial synthetic products by the way helps in reducing the cost of tissue culture plants. Hence studies should be focused in producing pure microbes in *in vitro* culture without contaminants and in effective utilization of PGPM for plant tissue culture.

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ENDOCRINE DISRUPTORS: AN ANTHROPOGENIC BUG – A REVIEW

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Abstract:

Hundreds of anthropogenic chemicals are released into the environment that can interfere with the normal endocrine system of living beings. These endocrine disrupting chemicals pose a global threat to the human beings and animals of present and future generations and are of great concern nowadays. This paper reviews in detail the mechanism of action of the endocrine disruptors and their adverse effects produced in the body. A review was conducted using published articles (Pubmed/ Medline) to study the different mechanisms of action of endocrine disrupting chemicals and the possible negative influences in the body on its exposure. Review article study shows that Endocrine disrupting chemicals target the endocrine system in the body, directly or indirectly interfering with the hormone receptor or interfering at the hormone concentration levels or by inducing epigenetic changes thereby altering normal neuroendocrine system. The adverse effects of Endocrine disrupting chemicals in the reproductive system, metabolic system, immune system, and thyroid system are vast and diverse and are discussed in detail. Therefore, necessary measures are to be taken with utmost care to reduce the critical windows of endocrine disruptor exposure. There also exists a great demand for data on the epigenetic aspects of endocrine disruptors.

Keywords: Endocrine Disruptors, Environment, Hormone, Mechanism, Plastics, Receptor.

Introduction:

We live in an environment where human beings and animals are exposed to a wide variety of chemicals; anthropogenic or plant origin. An escalation in industrial development has resulted in the release of vast majority of chemicals into the nature. Some of these chemicals act as endocrine disruptors, disturb the endogenous signaling pathways, and interfere with the normal developmental functions of living beings (Ying, 2012). US environmental protection agency defines endocrine disrupting chemicals (EDC) as, any exogenous agent that interferes with the synthesis, secretion, transport, metabolism, binding action, or elimination of natural blood-borne hormones that are responsible for maintaining homeostasis, reproduction, and developmental process of the body. EDC alters the endocrine system directly or indirectly through several mechanisms; disrupt hormone synthesis or breakdown, mimic hormones, act as hormone antagonists, and alters hormone binding or hormonal receptor expression (Kumar *et al.*,

2020). Among the environmental factors affecting global health deterioration, EDCs have gained much attention (Papalou *et al.*, 2019). Emerging epidemiological data highlights a close association between the increased exposure of endocrine disrupting chemicals to the development of modern industrialized world diseases such as obesity, hormone dependent cancer, and reproductive disorders (Swendenborg, 2009).

Historical perspective of endocrine disrupting chemicals

Though endocrine disruption has gained much attention recently, the phenomenon has been known for a long time. There were earlier reports on the endocrine disruption caused by feeding certain mouldy grain and clover in swine and sheep, respectively (Bennetts *et al.*, 1946; McNutt *et al.*, 1928). The publication of the book titled “Silent Spring” by Rachel Carson caught the widespread attention to the EDCs and later resulted in the ban on agricultural use of DDT (Darbre, 2019). Between 1940’s and 1971’s, Diethylstilbesterol (DES) was prescribed to several million women to prevent the miscarriage but later discovered of causing vaginal cancer in daughters born to these women who have taken diethylstilbesterol (Herbst *et al.*, 1971). The term Endocrine Disruptor was used for the first time at Wingspread conference in Wisconsin in 1991 (Markey *et al.*, 2002). In 2002, WHO issued the first global assessment of the state of the science of EDCs (Damstra *et al.*, 2002). Later in 2009, Endocrine society of the USA published its first scientific document on Endocrine disruptors, providing a wake-up call to the scientific community about the possible threats posed by these endocrine disruptors (Diamanti-kandarakis *et al.*, 2009), subsequently followed by the issuance of the second statement in 2015, detailing the possible mechanisms of EDC action (Gore *et al.*, 2015).

Multitudinous sources: Tip of the iceberg?

A myriad of chemicals has been identified as Endocrine Disruptors. The origin of these endocrine disruptors may be either natural or synthetic. Some of the chemical compounds naturally present in animal and plant food can alter the normal endocrine metabolism of the body (e.g., phytoestrogens like genistein and coumesterol) (Diamanti-kandarakis *et al.*, 2009). Increasing industrial and agricultural activities have resulted in the large-scale release of chemicals into the environment. Over 800 above chemicals are known for causing endocrine disruption, however, only a small fraction of these chemicals have been studied for their detrimental effect on the endocrine system (Bergman *et al.*, 2013). Since the endocrine disruptive action of a major proportion of synthetic chemicals used in our daily life has not been assessed, we have only looked at the “tip of the iceberg” (Ying, 2012).

The group of exogenous chemicals identified as endocrine disruptors is diverse namely; plastics [bisphenol A (BPA)], industrial solvents/lubricants and their by-products [polychlorinated biphenyls (PCBs), polybrominated biphenyls (PBBs), dioxins], plasticizers (phthalates), pesticides [dichlorodiphenyltrichloroethane (DDT), methoxychlor, chlorpyrifos,],

fungicides (vinclozolin), and pharmaceutical agents [diethylstilbesterol (DES)](Papalou *et al.*, 2019).

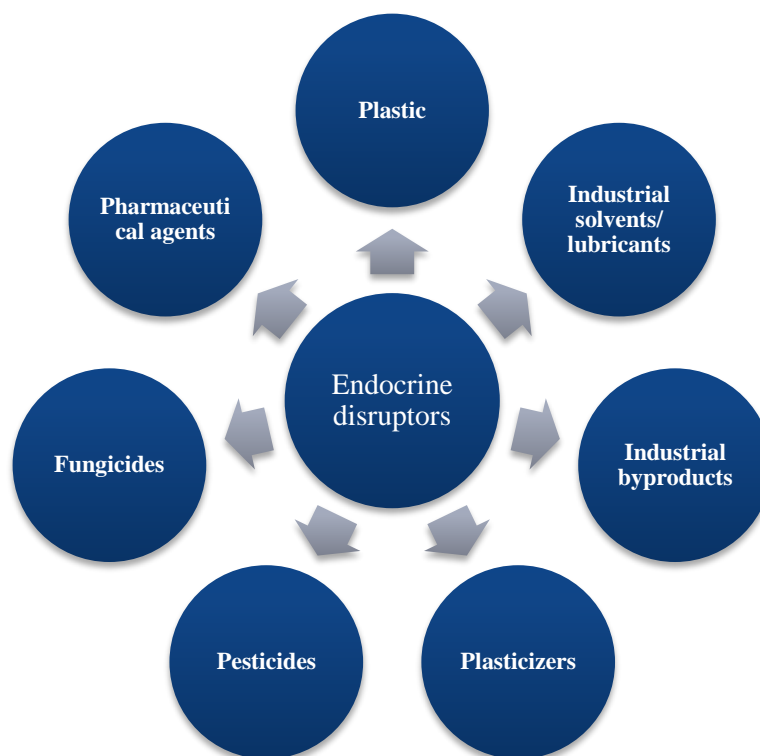


Figure 1: Sources of Endocrine disruptors

The constant release of chemicals from industries makes entry into the body by means of different mechanisms. Plastic, plasticizers, and industrial solvents/lubricants have become an inevitable part of the day-to-day activities, exposing to a major share of EDCs. Microplastic or nano plastic; the toxic components of plastic and drug delivery system (Betty *et al.*, 2010), make an entry into the environment by leaching or migration when exposed to high temperatures. Nanoparticles gain entry into the food via the packaging material (Hardy *et al.*, 2018). These microplastics/ nano polymers bioaccumulate in the food chain, thus directly or indirectly exposing humans and animals to the endocrine disruptors. Animals and human beings are at risk of exposure to EDC from contaminated drinking water through leaching of pesticides/fungicides, industrial waste discharge, and inadequate treatment of water supply for chemicals. Foetus is more vulnerable to EDC from EDC exposed pregnant mothers, transfer occurs through transplacental route and breast milk (Kumar *et al.*, 2020). A broad array of inevitable consumer products especially household consumables, cosmetic products, industrial solvents, etc increases the susceptibility of direct exposure to EDCs.

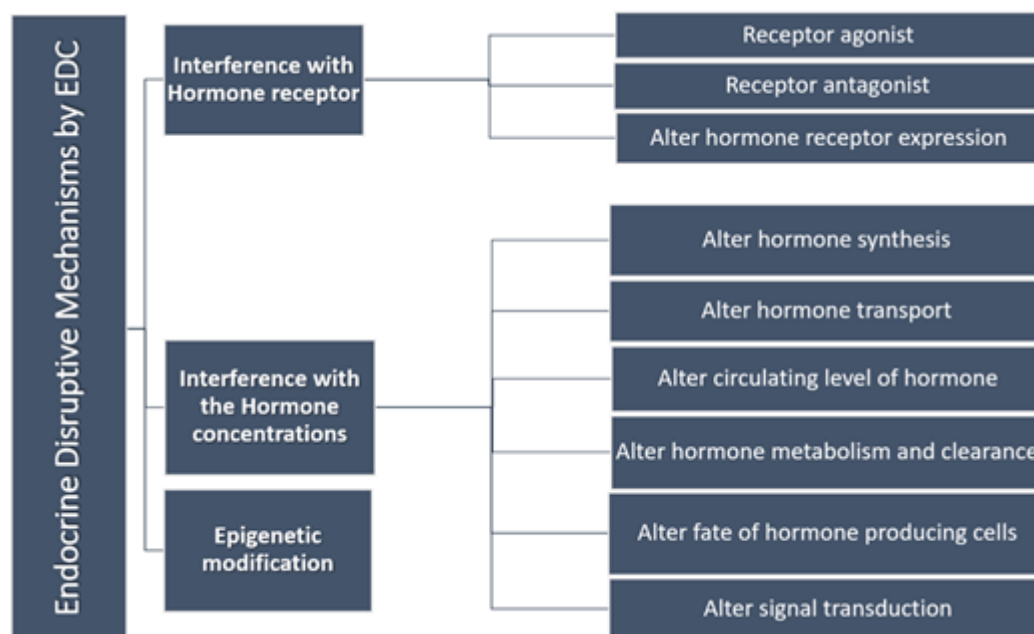
Mechanism of action of endocrine disruptors

The endocrine system comprises of the glands that secrete chemical messengers called as hormones, interacting with their receptors resulting in the regulation of body functions. Endocrine disrupting chemicals hinder the normal body metabolism by interfering with the complex endocrine mechanism in the body (La Merrill *et al.*, 2020). The mode of action of EDCs is dependent on the site of action, sex, stage of development/ age of exposure, Fig 2: Mechanism of Action of EDCs the concentration of the chemical, frequency of exposure, and many other factors (Zoeller *et al.*, 2012). The important mechanisms by which endocrine disruptors alter the endocrine metabolism are:

A. EDC interfering with hormone receptors

1. Receptor agonist or ligand

Hormones exert their effect by binding to its receptor (Jameson, 2016) located either in the cell membrane or in the cytoplasm, or inside the nucleus. Some of the known EDCs act as an agonist, inaptly binds and activate specific hormone receptors producing altered endocrinal metabolism (La Merrill *et al.*, 2020). The examples of the above said mechanism is; those EDCs that bind to oestrogen receptors (ER α and ER β) during developmental stages, thereby increasing the risk of infertility and reproductive tract cancer in both sexes(Lee *et al.*, 2013): Polychlorinated biphenyl (PCB) congener binding to thyroid hormone receptor and activating human thyroid hormone receptor- β -mediated transcription thus altering thyroid metabolism (You *et al.*, 2006). DDT exerts its endocrine disruptive action by binding to oestrogen hormone receptors ER α and ER β 33 (Legler *et al.*, 2002) and follicle- stimulating hormone receptors (Munier *et al.*, 2016).



2. Receptor antagonist

An antagonist is any chemical that blocks the responses elicited by the agonists. Endocrine disruptors by its antagonistic action, either inhibit or reduce the normal metabolic effect produced by the hormones. The hormonal receptor inhibition by the endocrine disruptors can be competitive or non-competitive depending on the extent of inactivation produced by the EDCs. Examples include herbicides such as linuron, vinclozolin, and their metabolites (Cook *et al.*, 1993). Antagonistic EDCs such as lindane and dieldrin, inhibits dihydrotestosterone binding to the AR (Androgen receptor)(Ostby *et al.*, 1999). DDE (dichlorodiphenyldichloroethylene), a DDT metabolite also exerts similar androgen receptor antagonistic action (Kelce *et al.*, 1995)

3. Alter hormone receptor expression

Hormonal actions are mediated through its receptor expression. EDCs induce changes in receptor expression, its internalization and degradation (La Merrill *et al.*, 2020) thereby altering hormone concentration in the body (Charlton, 2009). Examples include: DDT inducing a change in the internalisation of TSH receptor; decreased mineralocorticoid receptor expression in adult testis on exposure to di(2-ethylhexyl) phthalate (Martinez-Arguelles *et al.*, 2009) ; BPA inducing changes in receptor expression of oestrogen, oxytocin, and vasopressin in the brain nuclei (Adewale *et al.*, 2011; Patisaul *et al.*, 2012; Patisaul, 2017; Sullivan *et al.*, 2014; Wolstenholme *et al.*, 2012) and inhibiting androgen receptor expression (Qiu *et al.*, 2013)

B. EDC interfering with the hormone concentration

1. Alter hormone synthesis

Some of the EDCs are known to exert their endocrine disruptive action by interrupting the hormone synthesis mechanisms. EDCs such as Perchlorates block the uptake of iodine into the thyroid cells and decreases thyroid hormone production thereby affecting the thyroid metabolism (Wolff, 1998); Foetal rats exposed to Phthalates, reduce testosterone synthesis and hinder their reproductive efficiency (Mylchreest *et al.*, 2002). In contrast to the reduction of hormone production by EDCs, atrazine found in herbicides increases oestrogen synthesis (Hou *et al.*, 2012)

2. Alters hormone transport

Hormonal receptors being located in the cell membrane, cytoplasm, or inside the nucleus, hormones need to be transported to their respective receptor sites for the hormonal receptor complex formation. Steroid hormones, being lipophilic (including oestrogens, androgens, progestins and adrenal steroids) move through membranes passively and bind to its receptors. Amine, peptide, protein, and thyroid hormones need to be selectively transported across the membranes to bind to its receptors. EDCs targets these transport processes of the hormones. BPA exerts its endocrine disruptive action by reducing calcium entry into mouse pancreatic β - cells, thereby reducing insulin production (Villar-Pazos *et al.*, 2017).

3. Alter circulating levels of hormones

Hormones get transported to the target cells by the circulatory system; circulated as 'free', with or without conjugation or bound to various carrier proteins. The distribution mechanisms of the hormones such as carrier protein concentrations are marked by the EDCs. The bioavailability of the hormones at the target tissues can be decreased by a decrease in the concentration of the carrier proteins. EDC such as BPA and malathion decreases the circulating levels of testosterone by a decrease in the testosterone binding protein (La Merrill *et al.*, 2020). PBDE (Polybrominated diphenyl ether) downregulates the transport protein transthyretin (TTR) thereby decreasing the concentration of the circulating T4 in the blood (Boas *et al.*, 2012).

4. Alter hormone metabolism and clearance

The inactivation and clearance mechanism of hormones vary depending on their types. Protein hormones are broken down by the proteases in the blood whereas steroid and thyroid hormones are metabolized by enzymes, making them hormonally inactive and subsequently followed by its removal and excretion. EDCs targets the inactivation, clearance, and degradation processes of the hormone thereby altering the hormone concentrations in the body (La Merrill *et al.*, 2020). PCB, a major metabolite of the fungicide hexachlorobenzene reduces the rate of oestrogen clearance from the blood by the sulfation of oestrogen by oestrogen sulfotransferase (Journal *et al.*, 2002).

5. Alter the fate of hormone Producing/Responsive cells

EDCs can disrupt the normal differentiation, proliferation, and migration processes of the hormone producing/ responsive cell and may even cause death. For example, developmental exposure to PCBs can contribute to the abnormal morphology of thyroid gland cells due to its interference with the thyroid hormone signaling (Bansal and Zoeller, 2008). Developmental exposure to EDC can lead to abnormal mammary gland morphology (Newbold, 2012). Exposure to oxybenzone, UV filters found in personal care products increases mammary epithelial cell proliferation during pregnancy and lactation (LaPlante *et al.*, 2018). BPA is found to induce altered mammary epithelial cell differentiation and increased alveolar bud formation (Markey *et al.*, 2004).

6. Alter signal transduction mechanism of endocrine pathway

Hormones when binds to the receptor in hormone responsive cells, triggers specific intracellular responses thereby eliciting characteristic responses in the target cell. The signal transduction in the endocrine pathways is affected by the Endocrine disrupting chemicals. The extensively studied endocrine disruption by altering signal transduction is of cell membrane receptors and nuclear steroid hormone receptors (La Merrill *et al.*, 2020). Examples are BPA which interacts with Ras small G proteins and activates Ras signaling cascade (Schopel *et al.*, 2018). BPA also exerts its endocrine disruptive action by blocking low glucose- induced calcium

signaling from the glucagon- secreting α - cells in the pancreas (Alonso-Magdalena *et al.*, 2010). UV chemicals hinders calcium signalling in human sperm (Rehfeld *et al.*, 2017). Atrazine, the herbicide often used widely, causes cAMP accumulation by inhibiting cAMP-specific phosphodiesterase PDE4 (Kucka *et al.*, 2012). BPA increases the risk of breast cancer by activating signal transduction pathways involved in proliferation via epidermal growth factor receptor (EGFR) and G-protein coupled estrogen receptor (GPER) (Stillwater *et al.*, 2020)

C. Epigenetic modifications

1. Epigenetic modifications in hormone- producing or hormone- responsive cells

EDCs can exert permanent effects by making epigenetic changes via histone modifications and DNA methylation. Also, interfere with the hormones to induce epigenetic changes. EDCs also found to alter the expression pattern of noncoding RNAs. PCB alters the hypothalamic microRNA expression in a sexually dimorphic manner (Bergman *et al.*, 2013; Topper *et al.*, 2015). Early exposure to BPA results in DNA methylation of Fkbp5 gene in mice (Kitraki *et al.*, 2015). Prenatal exposure to TCDD in rats is known to induce hypermethylation of BRCA-1 promoter region and subsequently leading to the suppression of this gene expression in the rat offspring (Papoutsis *et al.*, 2015).

Effects of edc on endocrine system

Endocrine Disruptors have been reported to cause adverse health effects in the reproductive system, metabolic system, immune system, thyroid metabolism, and other major systems in the body. The adverse effects on each system are discussed in detail

1. Reproductive system

Adverse effects of EDCs in the reproductive system are the most extensively studied. Several chemicals are known to impair normal reproductive metabolism. EDCs can adversely affect the ovary, uterus, vagina, anterior pituitary, and/or steroid production (Gore *et al.*, 2015). The important reproductive problems associated with exposure to EDC are a)decreased sperm quality, b)decreased fertility, c)implantation failures, d)decreased steroidogenesis, e) decreased egg production, f)abnormal development of foetus and infants, g)reproductive tract cancer (vaginal cancer in females and prostate and testicular cancer in males), h) endometriosis and uterine lesions, i)oestrous cycle disruption, j) pregnancy dysfunction/ preterm birth, k)secondary sexual feminisation l) increased ovarian retraction m) abnormal puberty (You & Song, 2021). Diethyl stilbesterol (DES), BPA & Phthalates are the most commonly studied EDC in the reproductive system. DES is a major reproductive tract carcinogen causing vaginal tract cancer in female child of DES exposed pregnant mothers. Some of the pesticides such as DDT exerts its inhibitory action on enzymes required for ovarian steroidogenesis. For example, TCDD (2,3,7,8-tetrachlorodibenzo-p- dioxin, a Dioxin metabolite), is an endocrine disruptor found in plastics that affects the aromatase enzyme production. At low concentrations, BPA, which is prevalent in

plastic containers, cosmetics, and household items, can exert its effect directly on the mammary gland, increasing ductal development and breast cancer risk (the leading type of cancer in women)(Wang *et al.*, 2017). Gestational exposure to BPA has also been reported. According to previous research studies, BPA can be passed from mothers to their unborn children and even through lactation (Wolstenholme *et al.*, 2012). The major adverse effects reported in males from exposure to EDC are decreased sperm count, altered testosterone level leading to secondary sexual feminisation, cryptorchidism, and testicular abnormalities. Increased exposure to EDC have also been reported to cause lactational impairment and breast cancer in females (Gore *et al.*, 2015). Alterations in the various reproductive hormones on exposure to several EDCs such as phytoestrogens etc can lead to the development of hormone dependent cancers, especially in the reproductive tract of females.

2. Effects on thyroid metabolism

Hormones are carried to the target organ with the help of distributor proteins. Thyroid hormones are conveyed to their target organs mainly by means of three distributor proteins and often these distributor proteins are targets for multiple EDCs. The displacement of thyroid hormone from its distributor proteins will lead to a decrease in the circulating thyroid hormone levels due to changes in thyroid hormone metabolism by the liver. Adverse effects of disrupted thyroid metabolism are more pronounced during early pregnancy, as foetal brain is solely dependent on maternal thyroid hormone supply. Certain pesticides, flame-retardants, and per fluorinated compounds affect the circulatory thyroid level by displacing thyroid carrier proteins. Iodine deficiency and thyroid hormone deficiency are known to adversely affect brain development and increase the risk of neuro-developmental disease and IQ loss. A number of EDs such as perchlorate, nitrate, and thiocyanate, disrupt thyroid metabolism by decreasing iodine uptake by the thyroid gland. Perchlorate, another major EDC reported in amniotic fluid, is known for the modulation of maternal thyroid hormone during pregnancy. The adverse effects of Endocrine disruptors can be aggravated with the dietary Iodine deficiency, especially during the early gestation period (EU Petition, 2019).

3. Effects on the metabolic system

EDCs can cause adverse effects on the metabolic system of the body especially by disrupting glucose and lipid metabolism. A variety of chemicals such as BPA, phthalates, TBT (tributyltin), arsenic, PBDEs (polybrominated diphenyl ethers), PFOA (perfluorooctanoic acid), TCDD, PCBs, and DDTs are known to exert deleterious effects on the metabolic system. EDCs act as obesogens, diabetogens, and cardiovascular disruptors. DES, BPA, TBT, PCBs, and Phthalates are some of the common EDCs that act as obesogens, BPA, TBT, and POPS (persistent organic pollutants) act as diabetogens meanwhile TCDD and DDT act as cardiovascular disruptors. EDCs directly act upon the adipocytes and the brain to induce obesity,

generating insulin resistance, glucose intolerance, dyslipidaemia, and increases the risk of Type 2 Diabetes Mellitus (T2DM) and cardiovascular diseases (CVD). Changes induced by the EDCs in insulin signaling can result in metabolic syndrome. Exposure to EDCs also reported for producing fatty liver, dyslipidaemia in liver, and skeletal muscle generating T2DM and CVD conditions. Some of the EDCs such as BPA are known to exert its action directly on the heart, increasing the susceptibility to CVDs (Gore *et al.*, 2015).

4. Effects of EDC on the immune system

EDCs are known to produce adverse effects on the immune system by direct or indirect means, affecting the normal function and lifespan of the immune cells. The immune cells such as monocytes, neutrophils, dendritic cells, mast cells, lymphocytes, and natural killer cells are acted upon by the endocrine disruptors. EDCs exerts their disruptive action such as a decrease in the adhesion, chemotaxis, phagocytosis, and lifespan of monocytes and alter the TNF- α production. On exposure to EDC, the migration ability of the neutrophil is reduced, chemotaxis, adhesion and phagocytosis ability is reduced and apoptosis of the cell occurs at faster rate. Degranulation and IgE independent activation of the mast cells are altered by the endocrine disruptors. The release of chemicals from mast cells such as histamine, leukotrienes, and β hexosaminidase enzymes are also found to be disrupted by the EDCs. The decrease in the expression of the different types of CD receptors and altered cytokine production from the natural killer cells often make the immune system weak in EDC exposed animals (Nowak and Ratajczak-Wrona, 2019).

Untraversed cocktail effect

The mass industrialisation has resulted in the release of large amounts of chemicals into the environment, resulting in multiple exposure to the EDCs at the same time. Combined exposures may result from the multiple exposures from the multiple routes by the diverse use of chemicals. Combined exposure to EDCs can exert either an additive effect or synergistic effect on the body (EU petition, 2019). Very few studies are carried out on the interactive effects of EDCs. The interactive, synergistic, antagonistic, or cumulative effects of the EDCs needed to be studied in detail.

Future perspectives:

The research on EDCs so far known has not much focussed on the alternate pathways affected by these chemicals. The cocktail effect/ mixture effect on the combined exposure of the EDC is not studied in detail. Some of the individuals/ organisms are resilient or less vulnerable to the adverse effects of EDCs due to their enzyme polymorphism. Researches need to be conducted to identify their evading mechanisms from the endocrine disruption pathway. Early biomarkers for identifying the effects of EDC during neonatal and infancy period and early indicators of exposure to EDCs during fetal life are demanded due to the inevitable exposure of

EDCs starting from the foetal period to adulthood. Only a few EDCs and its deleterious effect on the health, have been detected so far, new methods have to be discovered to uncover new EDCs. One of the newer mechanisms of endocrine disruption by the EDC; epigenetic modulation is less studied. More scientific studies on the epigenetic effects of EDCs are awaited. The endocrine disruption caused by the EDCs are studied at a higher concentration of these chemicals in animal and cellular models, the low dose effects of these EDCs are uncertain. Nutritional deficiencies, metabolic disorders, stress, climatic conditions, etc. may exacerbate the effects of EDCs. Therefore, a systemic approach acting at multiple levels of biological organization is in at most importance. Since EDC exposure is inevitable, measures to reduce or reverse the endocrine disruption have to be studied in detail.

Conclusion:

We are constantly exposed to thousands of chemical residues through air, food, and water. Maternal exposure to the EDCs makes health of future generations at risk. EDCs possess a global threat as it interferes with tissue and organ development and function and alters the susceptibility to different diseases. Exposure to EDCs also accounts to the increased occurrences of modern lifestyle diseases such as obesity, cardiovascular diseases, etc. The newer chemicals discovered to replace the EDCs are also found to be a problematic/regrettable substitution. Therefore, need to improve research strategies, initiate preventive measures, and promote public knowledge to tackle the global deterioration of metabolic health caused by the EDCs.

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ANALYSIS OF FLORA AND FAUNA OF GONDWANA LAND FROM SIRONCHA AND ADJOINING AREAS (PRANHITA GODAVARI BESIN) OF GADCHIROLI DISTRICT MAHARASHTRA, INDIA

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Abstract:

The Kota Formation is primarily composed of sandstones, limestones, clays, and mudstones, and is considered to have been deposited in a lacustrine environment. The faunal assemblages of this region is reported to contain a rich assemblage of fossil fishes, sauropod dinosaurs, Pterosaur (Flying reptiles), Scutes of Crocodiles and Mammals, in addition to fossil Ostacords, Branchiopods and Insects. The floral assemblage constitutes mostly Pteridophytes Gymnospermous leaves and woods, dominating by *Dadoxylon*, *Araucarioxylon* and *Cupressinoxylon* woods. Most of them found in situ near Sironcha, at Waddham locality up to 40 - 60 feet long and 8 -10 feet in diameter. The overall faunal as well as floral assemblage are in favors of deposition under fresh-water conditions during Lower Jurassic period. However, presence of exclusively marine genera of fishes and Pterosaur, compared with thick limestone beds of large aerial extent, indicates existence of marine conditions towards the end of deposition of Kota formation. Floristic and other evidences suggest middle Jurassic or slightly younger age to the Kota Formation.

Keywords: Indian Gondwana Basins, Kota formation, Fauna, Flora, middle Jurassic age.

Introduction:

Gondwanaland is named after the Upper Palaeozoic and Mesozoic formation of the Gondwana district of central India. The Gondwana-land has been named after the Gond tribe of Madhya Pradesh ruled by the famous Rani Durgabati during the reign of Akbar. The name was coined by H. B. Medlicott in 1872 but actually published by O. Feistmantel in 1876 and later on Fox (1931). The Gondwana began to split in the Jurassic as shown by the intensive lava injections at that age. There was drifting apart of the continents in the Cretaceous, gradually bringing to their present positions. Another series of great mountain building waves built present dominant Mountains (Himalayas, Alps, etc.) on the location of the Sea of Tethys.

Gondwana period begins after the Carboniferous glaciations, from the Upper Carboniferous and extends right up to Upper Cretaceous, for about 205 million years. Generalised lithostratigraphic succession of the Gondwana sediments includes Talchir, Barakar,

Barren Measures, Kamthi (Lower Godwana Group), Maleri, Kota, Gangapur and Chikiala formations (Upper Gondwana Group).

The Upper Gondwana Group begins with 250 m thick Maleri Formation comprising reddish brown to greenish grey clay, siltstone, argillaceous sandstone and lime-pellet rock along with middle to late Triassic fauna. The succeeding Early-Middle Jurassic Kota Formation includes large-scale cross-bedded sandstone along with *Glossopteris* flora in the lower part, fish and dinosaur-bearing limestone in the middle and upper sandstone and clay with Gymnosperm conifer forest. Gondwana sediments occupy a large area between Pranhita and Godavari rivers trending in a NW-SE direction and covering Kota Sironcha-Ankisha area of Gadchiroli district

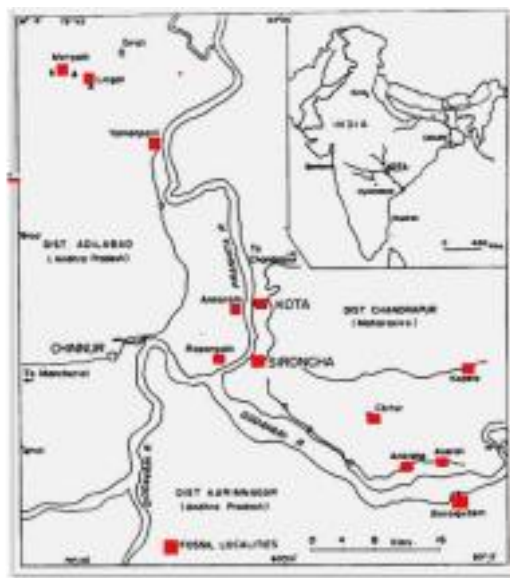


Fig. 1-Map showing fossil localities in the Kota formation.

in Maharashtra and Yemanapalli - Paikasigudem area of Adilabad District in Andhra Pradesh of upper Gondwana rocks (Kota formation). The

Kota Formation is named after the village Kota (Long 18°55' Lat. 79°59') situated on the east bank of the river Pranhita in the Gadchiroli District, Maharashtra.

Fossiliferous localities of Kota Formation (Sironcha and adjoining area, Fig.1) ■ Gadchiroli Dist. (Maharashtra)

- * Muldima (N 19°04.837' E 079°53.045')
- * Ramanpalli (N 19°25.895' E 080°03.844')
- * Papyapalli (N 19°044.979' E 079°53.593')
- * Tekada (N 18° 5.679' E 079° 59.256').
- * Gumalkonda (N-18° 43.387' E-080° 14.433')
- * Kota (N 18°46.275' E 080°06.984').
- * North of Pochampalli nala (N 18° 55.139' E 079° 58.874').
- * Rangayapalli (N 18° 54.250' E 079°59.658').
- * Amradi Road (N 18° 43.491' E 080° 08.617').
- * Ahmadali (N 18° 50'30'' E 080° 07'30').
- * Nandigaon bade line ka talab (N 18° 49.846' E 080°01.371').
- * Near Nandigaon villege site (N 18° 49.544' E 080° 02.151').
- * North-East of Sironcha (N 18°50.230' E 079°57.040').
- * North and west of Wadadham: (N -18° 44.473' E -080°04.375').
- * Wadadham, Towards River Bank (N 18°44.353' E 080°04.028')
- * North-East of Kottapalli-Pochmpalli (Dinosaur site) (N 18° 43.212' E 080° 05.885').
- * South-East of Chitur (N 18° 47.102' E 080° 06.660').
- * Boraigudem (Fossil Fish & woods); (N 18°46.275' E 080° 06.984').

* North-East Ankisa (N 18°45.066' E 080°08.738').

* Kopella nala (N 18°50.652' E 080° 14.215').

* Bodela nala (N 18°49.875' E 080° 11.826').

* Tekada (N 18° 59.679' E 079° 59.256').

* Amdeli (Ahmed Ali)

■ **Adilabad Dist. (Telaangna, old A.P.)**

* Kistaraopeta-padu (N 18°45' E 080° 03').

* West of Paikasigudem village

* Yemanapalli ((N 19°06.240' E 079°50.984)

* Mangnapalli ((N 19°06.823' E 079°49.705)

Methodology and Discussion

Biodiversity of Kota formation of Early-Middle Jurassic period of Pranhita-Godavari Valley.

Animal fossils (Fauna)

The Kota fauna includes an interesting assemblage of aquatic, semi-aquatic, terrestrial and aerial vertebrates. The Kota formation apparently contains two different kinds of sediments, viz., fluviatile sandstones and clays and marine limestones (Fig. 2). The lime stones have earlier been considered to be of fresh-water origin (Robinson, 1970; Tasch *et. al.* 1973, Govindan, 1975) in spite of the fact that the semionotids fishes recorded from Kota Formation resemble marine semionotids of Europe. Similar is the case with Pholidopholid and Coelacanth fishes. Detailed petrographic, mineralogic and geochemical analysis of limestones of Kota Formation, carried out by Bhattacharya (1981) have clearly shown that these were deposited “in marine intertidal flats where abundant terrigenous material was brought in by a river estuary”. Bhattacharya (1981) has classified these limestones as “a distinct marine unit in the pper Gondwana Formation of Peninsular India”. The deep-bodied and lanceolate fishes of Kota Formation inhabited the tidal-flat region of a sea on the shores of which lived the crocodiles etc. The Sauropod dinosaurs lived which close to the sea shore on the land which was also inhabited by a variety of mammals. The flying reptiles perhaps thrived on the fishes which were abundantly present in the tidal flat region.



Fig. 2- Fossiliferous section at Kota Formation

In India, Mesozoic mammals have been documented from the Upper Triassic Tikhi Formation (Datta and Das 1996), the Lower/Middle Jurassic Kota Formation (Datta 1981; Yadagiri 1984, 1985; Prasad and Manhas 1997), and from the Upper Cretaceous Deccan intertrappean beds (Prasad and Sahni 1988; Prasad *et al.* 1994; Prasad and Godinot 1994; Godinot and Prasad 1994; Das Sarma *et al.* 1995; Krause *et al.* 1997). Among these micromammal-yielding stratigraphic levels, the Kota Formation of the Upper Gondwana Group, Pranhita-Godavari valley (peninsular India), assumes great significance because of the time frame during which it was deposited. This formation has traditionally been considered as Lower Jurassic in age based on semionotid (Jain 1973), coelacanth (Jain 1974), and pholidophorid (Yadagiri and Prasad 1977), fishes, pterosaurs (Jain 1974) and palynofossils (Prabhakar 1986), despite an alternative Middle Jurassic age favoured by other works based on ostracodes (Govindan 1975; Misra and Satsangi 1979).

Sauropod remains are known from the basal beds of pebbly coarse sandstones as well as verlying strata. Prosauropod remains are known from the underlying clay beds. All other fossils are known from the limestone beds. Fish scales are amongst the common fossils of Kota formation.

The fauna so far known from the Kota formation is as under:

Invertebrate

Ostracords: *Candona kotaensis*, *Darwinula sarytirmensis*, *Timiriasevia digitalis*, *Limnocythera* sp., *Clinopyris*, *Cypridea*, *Stenocypris*, *Eucandona*.

Crustacea (Branchiopods) : *Cyzicus (Estheria) kotaensis*, *Palaeolimnadia* sp.

Insecta : *Blattoidae*, *Coleopton*, *Orthoptera*, *Hemiptera*.

Annelids : Undetermined worm tracks.

Vertebrate

The Kota Formation has been well known for its vertebrate fauna since the second half of 19th century. Despite the long history of vertebrate fossil collections including fishes, crocodiles, pterosaurs and dinosaurs (Egerton 1851, 1854; Owen 1852; King 1881; Jain *et al.* 1962; Jain 1973, 1974, 1983; Yadagiri and Prasad 1977; Yadagiri *et al.* 1979), mammals were discovered from this formation only at the beginning of the 1980s (Datta *et al.* 1978; Datta 1981, Bandyopadhyay 1999).

Fishes:

The Kota fauna is characterized by the presence of semiontid (*Paradapedium*, *Tetragonolepis* and *Lepidote*); coelacanth (*Indocoelacanthus*) and pholidophorid (*Pholidophorus*) fishes (Jain, 1959, 1973, 1974; Yadagiri and Prasad, 1977; Yadagiri *et al.*, 1980; Yadagiri 1982). In addition, sauropod dinosaurs (*Barapasaurus*) and a few other reptilian and mammalian remains are also known from these beds (Gain 1974; Jain *et al.*, 1975, 1979; Datta 1981; Yadgiri, 1982). The fishes were first recorded by Walker in 1841 which were described by Egerton (1851, 1854 and 1878). However, it was King (1881) who made a detailed study of the extent of Kota beds and found remains of fishes in the three limestone zones of what

he termed as Kota Group. Subsequently it was Jain (1959, 1974) who initiated detailed studies on fossil fishes during recent years. In G.S.I., pioneering work on Kota fauna was carried out by Rao and Shah (1969) who reported the first mammals from the Kota beds of Pranhita-Godavari Valley. The credit of discovering a rich Dinosaur bone bearing bed, just below the fish-bearing limestone beds, goes to Jain, Robinson and Roychoudhary (1962).

The fish specimens are generally fragmentary and would be described as below:

(I) Semionotid :- *Lepidote deccanensis*; *Paradapedeium egertoni*; *Tetragonolepies oldhami*.

Locality- Boraigudem, Ankisha. Nandigaon, Chitur

(i) *Lepidotes deccanensis* (Egerton, 1851).

Locality- Boraigudem, Ankisha.

In the Genus *Lepidotes*, Jain (1983) the trunk fusiform but moderately deep and only moderately compressed, marginal teeth styliform and slender or moderately robust; inner teeth may be styliform and slender, styliform and robust, always smooth. Vomers parried or coossifial, cheekplates variable

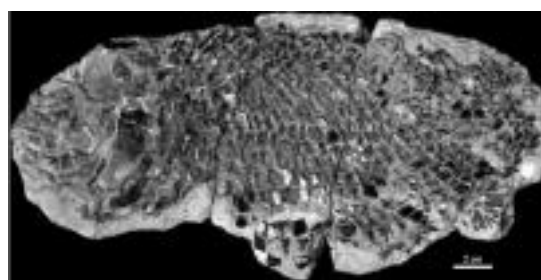


Fig. 3- *Lepidotes* fish

in number, never less than tub and only rarely less than four, and may be numerous in some species. Usually three antorbitals, never two one. Preoperculum shallowly curved and set almost vertically, Operculum deeper than wide, its width from about half to three quarters the length. Bronchiostegal rays, few Gular plate is absent. Ribs ossified, extending to the ventral border. Fin vulcra very large, present on all the fins, biserial. Paired fins relatively small, dorsal fin opposed to the space between pelvic and anal fins; caudal fins slightly forked, scales very robust, rhombic smooth or feebly ornamated or with reviating groovers; flank scales not much deeper than broad, with their wide overlapped margin produced forward at the superior and inferior angles; lateral line scales not deepened; scales of the ventral aspect nearly as deep as broad, dorsal and ventral ridge scales usually inconspicuous (fig.3). The transverse scales vary from 18-22. A few specimen collected from the Kota Limestone band north of Ankisa have been identified as *Lepidotes deccanensis*. The caudal fin appears to be shattered. There are thick rhombic scales on the lanceolate body form.

(ii) *Paradapedeium egertoni* (Jain 1973):-

Locality- Boraigudem, Ankisha, Nandigaon, Chitur.

In one specimen, which is 190 mm long and 130 mm deep, only a little anterior part of the head is missing but otherwise it is complete (Fig. 4). In another specimen, the body of the fish is completely preserved but the head part is a little shattered, although the fine teeth are also preserved.



Fig. 4- *Paradapedeium* fish

In another specimen, a good deal of posterior part is preserved. In one specimen, the head part is

completely preserved along with the anterior part of the body. Hence, it will be possible to determine various specific characteristic of this genus based on the different parts of the body preserved in different incomplete specimens. In all the *Paradapedium* specimens, the length /depth ratio is small because of deep body of fishes. Depth of body, below the lateral canal, is nearly two-third. The shape of the body is clearly hypsisomid and the entire body is covered with scales, which are generally elongated below the lateral line. It has been observed that the scales gradually become smaller and their rows become curved posteriorly. Orbit is well exhibited and is moderately large. Circum-orbital series is well observed. Median fans are well displayed. The dorsal fins extend from middle of the body to the end of the trunk portion. Anal, dorsal and caudal fins are well preserved.

Semionotids having abdominal region protuberant ventrally; head small in relation to the body. Postrostral absent. Mandible short, deep with coronoid elevation and anterior tooth enlarged. Teeth slender and pointed. Suborbitals, 9-10 extending beyond middle of orbit. Circum-orbital probably 15-16, Cleithrum arched; supra-scapular large, triangular, extra scapular, 3 bronchiostegial rays 6, small and inconspicuous. Hyomandibular slender, elongated-hyomandibular foramen at about mid length. No ossification around notochord. Neural spine fused the middle of the back and extending to about the tail, has about 35 lepidotrichia. Anal fin shorter, opposed to hinder end dorsal, has about 25 lepidotrichia. All lepidotrichia disply segmented and bifurcated and all fins with supporting fulcra rays. Complete squamation over trunk; flank and belly scales elongated dorso-ventrally. Dorsal ridge scales conspicuous, pectinat, or slightly denticulate. Ventral ridge scales slightly more conspicuous than dorsal, usually delicately pectinate. *Paradapedium egertoni* is a moderately large semionotid reaching 200-300 mm in length and 120-195 mm in depth (Fig. 4).

Specimens of *Paradapedium egertoni* were collected from north-east of Ankisha and south-west of Chitur and Kota river section and Nandigaon. Fishes was found in riverine sediment at Boraigudem limestone ridge, about 30 kilometers southeast of Sironcha. It is preserved in the museum of the Indian Statistical Institute- Kolkata.

(iii) *Tetragonolepis oldhami* Jain (1964, 1973).

Locality- Boraigudem, Ankisha, Chitur.

This species described originally by Egerton (1878) was restudied by Jain (1964). The body is hypsisomid and deep. The portion of the body below the central axis shows prominent lacramal arches and strong ribs. The ventral ridge scales are more prominent than dorsal and show fine pectinations at the ridges. The body of the fish is covered with scales, each of which has a sharply thickened ridge on its anterior border. *Tetragonolepis* attaining an estimated length upto 180 mm, with abdominal protumbeance about



Fig.5 - Tetragonolepis fish

twice that of the portion above the lateral line canal, Branchiostegal rays, three elongated, progressively larger starting from the anterior one. Preopercular angulate, slightly or markedly suborbitals four, symmetric. Caudal fin has 15- 16 haemal arc has supporting lower lobe (Fig. 5).

(II)- Coelacanth :- *Indocoelacanthus robustus*;

Pholidophoridae:

Division – Teleostei

Order – Pholidophoriformes

Family – Pholidophoridae

Genus- *Pholidophorous kingie* and *P. indicus* (Yadagiri & Prasad 1977).

Coelacanthidae:

Indocoelacanthus robustus (Jain 1974).

REPTILES :

Sauropod dinosaurs

Classification: Chordata, Reptilia, Dinosauria, Saurischia, Sauropoda

(i) *Barapasaurus tagorei* (Jain *et al.*, 1975)

Locality: near Pochampalli village, Gadchiroli district, Maharashtra, India.

Barapasaurus tagorei (Jain *et al.*, 1975) resembles with *Petagosaurus* of Patagonia & *Shunosaurus* from China. The best known Jurassic dinosaur from India is *Barapasaurus tagorei* (Jain *et al.*, 1975, 1979) from the Kota Formation (Early Jurassic) of the Pranhita- Godavari Basin. *Barapasaurus*, is a



Fig. 6- *Barapasaurus tagorei* Sauropod dinosaurs

large sauropod with rather slender limbs. Its spoon- shaped teeth bear coarse denticles on their sides. The cervical and anterior dorsal centra are opisthocoelous, whereas all others are nearly platycoelous (Jain *et al.*, 1975). The sacrum has four co-ossified vertebrae with “waisted” amphiplatyan centra. The girdles are typically saurischian in nature. Some interesting aspects of *Barapasaurus* have been highlighted by Jain *et al.* (1979). These include early attempts at gigantism, bone reduction and economy in the vertebrae and peculiar modifications in the region of the neural canal with the development of interlamellar hollows between the infradiapophysial laminae.

Barapasaurus is therefore one of the earliest known sauropods. *Barapasaurus* is known from approximately 300 bones from at least six individuals, so that the skeleton is almost completely known except for the anterior cervical vertebrae and the skull (Fig. 6). This makes *Barapasaurus* one of the most completely known sauropods from the early Jurassic.

Barapasaurus tagorei is housed at the Geology Museum of the Indian Statistical Institute in Kolkata. The skeleton is 47 feet long and has been dated to about 160 million years back. It is considered as one of the earliest known Sauropod.

(ii) ***Kotasaurus yamanapalliensis*** (Yadagiri 1988, 2001)

Locality- near the village of Yamanpalli, of Adilabad District, Andhra Pradesh.

Kotasaurus yamanapalliensis, first described by Yadagiri, 1988. The new species is compared with the amphidontid symmetrodonts from America (Wilson 2005) and Manchuria. It is tentatively included in the family Amphidontidae. *Kotasaurus* is the name given to a genus of dinosaur from the Early Jurassic period, about 208 million to 188 million years ago. It was an early sauropod, sharing some similarities with prosauropods.



Fig. 7 - *Kotasaurus yamanapalliensis*

Extensive and well preserved sauropod materials belonging to more than twelve individuals were recovered from the Early Jurassic Kota Formation of India. The sauropod *Kotasaurus yamanapalliensis* is characterized by simple dorsal vertebrae and a low iliac blade. It is believed that those species are the inhabitants of current India. Partial skeletal remains of at least 12 individuals, though the skull is unknown, were discovered in the Kota Formation of Andhra Pradesh near the village of Yamanpalli.

In late 1970s, total of 840 skeletal parts have been found and later it was described by Yadagiri categorizing it under a new genus and species of sauropod, *Kotasaurus yamanapalliensis* (Yadagiri 1988). The researches revealed that the creature was a large, quadrupedal herbivore with long neck and tail. The body length is estimated approximately of 9 meters long with straight femur and columnar limbs. The overall structure of the creature was predicted approximately 30 feet long and 10 feet tall (Fig. 7). Like all sauropods, the species also had the spoon shaped teeth with other basal features of relatively short and slightly twisted humerus. The skeletal structure of *Kotasaurus* were built massive that has been in contrast with the other genus of *Barapasaurus* group.

Kotasaurus is the oldest known sauropod, a group of giants who were among the largest animals to ever walk the earth. These plant-eaters first appeared during the Jurassic. Sauropods evolved from prosauropods, also herbivores that first appeared during the last part of the Triassic, as far back as 225 million years ago.

Pterosaur (Flying reptiles)

Classification: Order – Pterosauria

Sub-order – Rhamphorhyncoidea

Family – Campylognathidae

Genus-*Campylognathus indicus*

Ramphorhynchus sp.

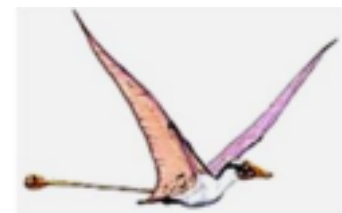


Fig. 8 – *Campylognathus* flying reptile

Campylognathus is a medium-sized flying reptile. The diapsids of the upper unit a pterosaur *Campylognathus* a Telosaurus like mesosuchid crocodylomorph. A nearly complete skull and

other fragmentary material of the crocodylomorph shows that it is a short snouted form and may be a telosaurid (Fig. 8).

Armoured dinosaur: (Nath *et al.* 2002)

Ornithischia : *Ankylosauria*.

For the first time, skeletal remains of an armoured dinosaur (Ornithischia: Ankylosauria) were found in the red clay bed of the Kota Formation, Pranhita-Godavari Valley, Andhra Pradesh. The bed occurs 2 m below the marker limestone unit of the Kota Formation. The collection includes parts of skull, 30 specimens of body armour, vertebrae and parts of girdle bones. The characteristics of armour plates, skull and teeth indicate that these fossils belong to *Ankylosauria*. The ankylosaurs are less known from the Lower Jurassic period. The detailed studies of the present material are likely to throw light on the evolutionary history of these dinosaurs.

Archosauria (Chrocodylomorph) :- Scutes of Crocodiles.

Cryptodiran turtle

Family- Indochelyidae: *Indochelys spatulata* (Datta et.al, 2000)

A primitive cryptodiran turtle, *Indochelys spatulata* is described from the Early Jurassic Kota Formation, a member of the Upper Gondwana Group in the Pranhita-Godavari Valley. The shell morphology of *Indochelys* differs substantially from that of the Triassic *Proganochelys* of Germany but is significantly similar to the oldest known Early Jurassic cryptodire, *Kayentachelys*, from the Kayenta Formation of Arizona. *Indochelys* also shares many shell characters with the Late Jurassic North American turtles, in particular *Dinorchelys*. The new family Indochelyidae is proposed, which probably has the same phyletic status as that of Kayentachelyidae, with both evolving simultaneously in different regions.

Fossil lizard

Bharatagama rebbanensis gen. et sp. nov (Evans *et al* 2002)

Locality: west of Paikasigudem village

The Mesozoic lizard fauna of Gondwana is virtually unknown. Report of fossil lizards from the Jurassic Kota Formation of peninsular India (Evans *et al*, 2002). It is usually considered to be of Early-Middle Jurassic age. The dominant form, *Bharatagama rebbanensis* gen. et sp. nov., has a predominantly acrodont dentition. Comparison with living and extinct taxa suggests that this new genus is a primitive acrodont iguanian distinct from the Cretaceous priscagamids. It predates known records of iguanian lizards by some 80 Ma, and provides evidence that iguanians had begun to diversify before the break-up of Pangea.

The microvertebrate assemblage recovered from the Paikasigudem site of Adilabad District, also includes two new taxa of Sphenodontid reptiles (Evans et al. 2001) and one new taxon of Agamid lizard as well as theropod and ornithischian dinosaur teeth.

MAMMALS:-

Triconodont mammals (Mammalian teeth):

Kuehneotheriidae: *Kotatherium Haldanei*, *Kotatherium yadagirii* (Datta 1981). **Locality-** west of Paikasigudem village

The first mammalian tooth recovered from the Kota Formation was referred to a then new genus and new species, *Kotatherium haldanei* Datta, 1981, of uncertain familiar affinities within Symmetrodonta (Datta 1981). Yadagiri (1984) described two other supposed lower molars of symmetrodonts from this formation and placed each of them in a new Mudstones (Prasad *et al.*, 2002) associated with the limestone bands of Kota Formation, west of Paikasigudem village, 10 km east of the nearest town Rebbana in Adilabad District, Andhra Pradesh (state), India.

Incertae sedis:

- *Trishulotherium kotaensis* (Yadagiri 1984).
- *Indotherium pranhitai* (Yadagiri 1984).

Amphidontidae: *Nakunodon paikasiensis* (Yadagiri 1985).

Locality- west of Paikasigudem village, Adilabad District, Andhra Pradesh State

Trishulotherium kotaensis and *Indotherium pranhitai* (family- incertae sedis). Subsequent to this, Yadagiri (1985) reported as new an amphidontid symmetrodont, *Nakunodon paikasiensis* Yadagiri, 1985, from this formation. Prasad and Manhas (1997) described an upper molar under a new species of *Kotatherium*, *K. yadagirii*, and included the two species of *Kotatherium* and *Trishulotherium kotaensis* in the symmetrodont family Tinodontidae. The type of *Trishulotherium kotaensis*, Yadagiri, 1984, originally described as a lower molar, has recently been considered as an upper (Prasad and Manhas 1997) discussed the taxonomic position of *Indotherium pranhitai* and tentatively referred it to the order Triconodonta, as closely allied to morganucodontids. Continued search for early mammals from the Kota Formation has led to the recovery of a few additional teeth referable to docodonts and triconodonts from a stream section exposed west of Paikasigudem village, Adilabad District, Andhra Pradesh State.

Plant fossils (flora)

In addition to the fossil fauna, the Kota formation has also yielded excellent specimens of silicified fossil wood (Shah, 1931). Fossil tree trunks ranging in length up to 10 m and girth up to 5 meter, with well developed annular rings and nodes etc., are seen embedded in conglomerate and calcareous sandstones in the Sironcha Reserved Forest area, around Ahmed Ali, in between Indravati and Godavari rivers (Anon). King (1881) considers fossil wood as a constituent of Kota formation but Yadagiri (1979) considers fossil wood as a constituent of Kota formation since he found fossil embedded in calcareous sandstone of Kota formation in Yerrapalli area, A.P.

Fossil flora and stratigraphy:

The term “Kota flora” is generally applied to the plant fossils reported from the plant beds of the Kota group as defined by King. This flora having an intermediate status stratigraphically between Early Jurassic “Rajmahal flora” and late Jurassic to early Cretaceous “Jabalpur flora” is involved in two controversies. The first controversy related to the Triassic fish

fauna found in Kota limestone overlaying the plant beds in the Godavari valley area; the second related to early Cretaceous fauna found associated with this Middle to late Jurassic flora. The paper presents a stratigraphic appraisal of this controversial flora in the light of recent mapping, palynological and radiometric data available from related Upper Gondwana formations. These point to the possibility that Rajmahal Trap and Deccan Trap flows were initiated probably during Aptian times (Early Cretaceous).

The floral assemblage is dominated by gymnosperms (conifers, cycadophytes and ginkgophytes respectively). Pteridophytes have not been recorded. Few Pteridophyte foliage also reported.

The assemblage comprises dominated

Leaf genera: *Cladophlebis*, *Sphenopteris*, *Pagiophyllum*.

Wood genera- *Araucarioxylon*, *Podocarpoxyton*, *Taxaceoxylon*, *Cupressinoxylon* and *Ginkgoxylon*. Floristic and other evidences suggest Middle Jurassic or slightly younger age to the Kota formation (Rajanikanth and Sukhdev, 1989).

Pteridophyta

Foliage (Leaves)

Filicopsida-**Osmundaceae-** *Cladophlebis indica*, *C. whitbyensis*, *C. reversa*.

Protocypatheaceae- *Protocypathe trichinopoliensis*.

Dicksoniaceae- *Dicksonia* sp.

Uncertain- *Rhizomopteris ballii*.

Unclassified Ferns- *Alethopteris medlicottii*.

-Sphenopteris sp.

Genus:- *Cladophlebis* sp.

Frond fragmentary, measuring 9 mm X 7 mm, imparipinnate. Rachis distinct. slender, Pinnules small, alternate, connected at base and making an angle of about 70°- 85° to rachis. Margin entire. Apex obtuse or rounded. Midrib present, lateral veins arising at an angle of about 30°- 40°, forked once or twice (Fig. 9).

Genus:- *Sphenopteris* sp.

Fragmentary pinna 5 mm X 2 mm in size. imparipinnate.

Rachis distinct. slender. Pinnules alternate, short, lanceolate, dissected into 2-3 forwardly directed lobes. Pinnules of the same side connected at the base by narrow lamina. Margin entire. Apices of lobes acute or subacute. Each lobe having a vein which further gives rise to a few small veins (Fig. 9).

Gymnosperms

(i) Foliage (Leaves)

Cycadofilicales- *Pachypteris ellorensis*.

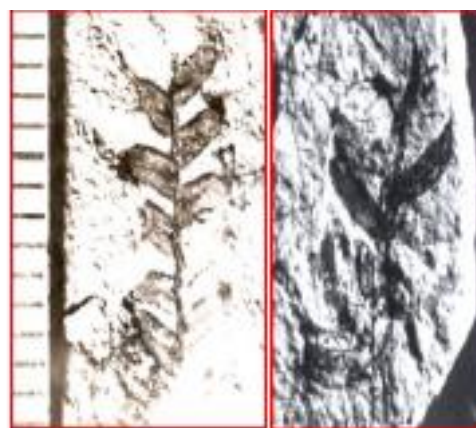


Fig. 9 - *Cladophlebis* & *Sphenopteris* leaf

Cycadopsida- *Taeniopteris ensis*, *T. maclelandi*, *Williamsonia* sp., *Otozamites abbreviatus*, *O. bunburyanus*, *Zamites proximus*, *Ptilophyllum acutifolium*.

Coniferales- *Alethopteris medlicottii*, *Palissya (Elatocladus) jabalpurensis*, *P. indica*, *Brachyphyllum rhombica*.

Araucariaceae- *Pagiophyllum*, *Araucarites cutchensis*, *A. latifolia*.

Taxales- *Taxites (Elatocladus) tenerrima*, *Elatocladus plana*, *Torreyites constrictus*.

(ii) Petrified woods

Coniferales- *Mesembrioxylon*, *Cupressinoxylon kotaensis*.

Araucariaceae- *Dadoxylon*, *Araucarioxylon santalense*, *A. pranhitaensis*. **Podocarpaceae-** *Podocarpoxyylon krauselli*,
P. chandrapurensis.

Ginkgoales- *Ginkgoxylon dixitii*.

Taxales- *Taxoxylon*, *Taxaceoxylon sahnii*.

Coniferales

Leaf

Araucariaceae

Genus- *Pagiophyllum* Heer, 1881

Leafy twigs, measuring 3 cm X 1.8 cm, branched. Branches slender, 2-5 mm wide, making an angle of about 20° - 35°, Leaves spirally borne, straight or slightly falcate, lanceolate, directed forward at an angle of about 10° - 30°, typically 2 mm long and less than 1 mm broad. Leaf-base decurrent, concealed by lower leaves. Margin entire. Apex acute or obtuse (Fig. 10).



Fig. 10 - *Pagiophyllum* Leaf

Woods

Family- Araucariaceae

Genus- *Araucarioxylon* Kraus, 1870

The fossil wood showing pinoxylic secondary woods with araucaroid pitting are generally described either under *Dadoxylon* Endlicher (1847) or *Araucarioxylon* Kraus (1870, in Schimper 1870-72). Seward in 1963, 1991 mentioned that fossil woods with araucaroid pitting from the Palaeozoic be assigned to *Dadoxylon* and those from

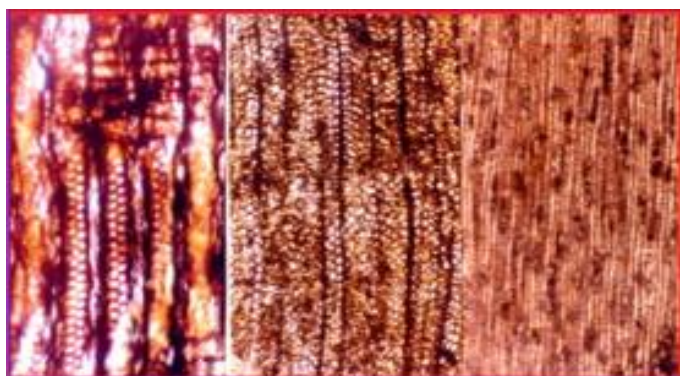


Fig. 11- Anatomy of *Araucarioxylon* wood

the younger strata to *Araucarioxylon* because *Dadoxylon* type of woods were thought to belong to Cordaitales and *Araucarioxylon* to Araucariaceae. Potonie (1902) preferred to retain the name *Dadoxylon* irrespective of age of the wood. Maheshwari (1972) suggested that the name

Araucarioxylon be used form the secondary woods with arau caroid radial pitting and cupressoid crossfield pits having uniseriate or rarely partly biseriate rays. Accordingly Bose and Maheshwari (1974) transferred some Indian Mesozoic *Dadoxylon* species to *Araucarioxylon*. But, it has been found that there is considerable variation in the height and width of xylem rays in the woods (Bailey and Paul, 1934; Dayal, 1972).

***Araucarioxylon pranhitensis* n. sp.**

Growth rings present; resinous cells scattered; wood parenchyma absent; radial wall pits uni-to tri -seriate, mostly uni-or biseriate, alternate. sometimes sub-opposite or opposite, hexagonal, bordered, contiguous; cross-field pits 3.6, arranged in groups, circular or elliptical, 6-8 μm in size, xylem rays mostly uniseriate, sometimes bi-triseriate, long, 1-52 cells in height, cells longer than broad, walls smooth and thin (Fig. 11).

***Araucarioxylon* sp.**

Wood light brown in colour; Growth rings present, transition from early to late wood gradual. Early wood zone 24-42 cells wide. Late wood zone narrow, 3-5 cells wide. Resin cells scattered. Early wood tracheids squarish to polygonal, 32-58 X 36-78 μm in size, wall 16-24 μm thick. Late wood tracheids nearly squarish, 14- 24 μm X 16-40 μm in size. Radial wall pits mostly uniseriate, contiguous, 13-16 μm in size, more or less hexagonal, 5-6 μm in diameter. Cross-field pits 2-5, bordered, arranged in groups. 4-5 μm in size, oval, sometimes circular. Xylem rays mostly uniseriate rarely partly biseriate, 1-18 cells (18-430 μm) in height. Cells longer than broad, walls smooth and thin (Fig. 11).

Family - Podocarpaceae

Genus-- *Podocarpoxylon* Gothan, 1905.

***Podocarpoxylon krauselii* n, sp.**

Growth rings distinct, transition from early to late wood abrupt, Xylem parenchyma scattered; Early wood tracheids polygonal, 20-38 μm X 25-55 μm in

size; late wood tracheids round or polygonal, 16-25 μm X 25-32 μm in size; radial wall pits uniseriate,

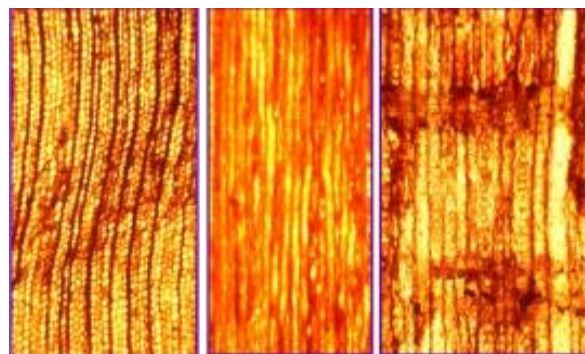


Fig. 12- Anatomy of *Podocarpoxylon* wood

circular, bordered, contiguous or separate, aperture oval to round; cross-field pits 4-5, elliptical, arranged in groups, bordered; xylem rays mostly uniseriate, 2-28 cells in height, cells oval or beed-like, cell wall smooth and thin.

***Podocarpoxylon chandrapurensis* n, sp.**

Growth rings distinct, transition from early to late wood abrupt, resinous parenchyma scattered; early wood tracheids 6-54 cells wide, cells squarish, 18-32 μm x 19-30 μm in size; radial walls pitted, pits mostly uniseriate, rarely biseriate, contiguous, separate, simple bordered, circular, 10-12 μm in size, aperture oval; cross-field pits 1-2, bordered, round; 6-7 μm , pore round; xylem rays mostly uniseriate, simple 1-18 cells high, ray cell longer than broad (Fig.12).

Family - **Taxaceae**

Genus-*Taxaceoxylon* (Krausel & Jain, 1964)

Taxaceoxylon sahnii n. sp.

Growth rings present, transition from early to late wood gradual, resin cells and parenchyma absent; tracheids squarish to polygonal, 26-54 X 34-66 μm in diameter. Radial wall pits mostly uniseriate, sometimes biseriate, opposite, circular, bordered, contiguous, 14-16 μm in size, pore oblique, sometime round, crassulae absent. Spiral thickenings mostly double 5-6 μm thick, occur at a distance of 12-20 μm at an angle of 30°-45°; cross-field pits 2-5 cupressoid, bordered, pore oblique; xylem rays uniseriate, 1-9 cells in height, cells oval to elliptical, spirals on tangential walls mostly double, arranged at a distance of 8-20 μm at an angle of 30°- 42°.

Taxaceoxylon sp.

Growth rings faintly marked variable in size, transition from early to late wood gradual. Resinous parenchyma and resin canals absent. Early wood tracheid squarish to nearly round, thin-walled, cells 32-52 μm x 28-58 μm in size, radial wall thickness 8.5-10 μm , tangential wall thickness 10-12.5 μm . Late wood zone 8-10 cells wide, tracheids angular, 17-28 μm in size, wall thickness 2.5-3 μm . Pits on radial walls of tracheids uniseriate, circular, bordered, contiguous or separate, 12.5-15 μm in size, pore round, 4.5-5 μm in diameter. Spiral thickenings on radial walls of tracheids making an angle of 40°- 80°, 4-5 μm thick, single and double, sometimes thickenings horizontal, 2-5 μm thick, band like cross-field pits 2-4, cupressoid, 3.5-5 μm in size, bordered, pore round or oval. Xylem rays uniseriate, rarely partly biseriate, simple, 1-22 cells (25-575 μm) in height, mostly 3-12 cells, cells barrel or dumb-bell shaped, 30-55 μm broad, walls smooth and thick,

Family- **Cupressaceae**

Genus- *Cupressinoxylon* (Goeppert 1850)

Cupressinoxylon sp.

Growth rings faintly marked; xylem parenchyma scanty, scattered; resin cells present; tracheids mostly round, 24-52 X 28-54 μm in size; radial wall pits uni-biseriate, in the latter condition opposite, bordered, contiguous or separate, 14-18 μm in size, pore oblique,



Fig. 13:- Anatomy of *Cupressinoxylon* stem

crassulae present, 6-8 μm thick; cross-field pits 2-4, cupressoid; xylem rays uniseriate, 1-13 cells, mostly 2-6 cells in height, cells oval, cells wall smooth and thin (Fig. 13). *Araucarioxylon santaleme* (Sah & Jain) Bose and Maheshwari (1974), described from Rajmahal Hills is commonly found in the Kota Formation. *Taxaceoxylon rajmahalense* (Bhardwaj) Krausel and Jain is another species in the Kota Formation which closely resembles *T. rajmahalense* from the Rajmahal Formation. This study is suggestive of close floral similarity between Kota and Rajmahal formations. The flora of Rajmahal is considered to be Early Cretaceous in age

(Sukhdev, 1987, 1988). Broad leaved cycadophytes and other characteristic fossils of the Rajmahal flora are not recorded in the present assemblage.

Planoxylon indicum (Vagyani and Mahabale, 1974).

A coniferous wood collected at Adhari, District Chandrapur shows combination of Characters found in the families Abietineae and Araucarineae. Its characters agree with those of the genus *Planoxylon* but the species is new and named as *Planoxylon indicum*. It is from the lower Triassic horizon (Kamthi stage). The wood shows distinct 7-8 growth rings, secondary xylem differentiated in spring and autumn wood; pith absent. Medullary rays uniseriate and biseriate, mostly uniseriate 2-28 cells high, average height 11 cells. Abietineous pits present on medullary ray-cells in T. S. and T.L.S., radial pits uniseriate, circular, separate, and circular pits are in clusters of 3-4; also multiseriate hexagonal pits present. Cross-field pits 4-8, circular to oval.

Taxopitys indica (Prasad and Chandra, 1979).

A new species of *Taxopitys indica* is described from the Kamthi Beds Kanhargaoon Village, Chandrapur District. The wood shows homogenous pith, mesarch primary xylem, distinct annular rings in the secondary xylem. 1-30 cells high uni or partly Bi-seriate xylem rays. Spiral thickening on tracheidal walls. Bordered hexagonal pits on tangential and radial walls of the Tracheids.

Ginkgoxylon dixitii (Biradar and Mahabale 1978)

A species of Ginkgo like wood *Ginkgoxylon dixitii* sp. nov. from Kota (80°2'18"55") a village 12 km north of Sironcha. It is characterized by growth rings; disorderly appearance of tracheids in T.S., uni- biseriate slightly radially flattened bordered pits, 2-4-6 cross-field pits and 1-2 or 1-3 xylem ray cells. It belongs to Kota stage of Upper Gondwana.

Sclerospiroxylon marguerierae (Prasad, 1982).

An annotated synopsis of Indian Palaeozoic gymnospermous wood; pith small with vertical columns of sclerotic cells, heterogenous with sclerotic cells dispersed in the parenchymatous ground tissue and these sclerotic cells arranged in respective conditions; primary xylem incipient, appear to be endarch; secondary xylem shows Araucaroid-type pitting with spiral thickenings; tracheid pitting and cross-field pits in the secondary xylems.

DISCUSSION

The present floral finds together with earlier records from the Kota Formation include 17 genera and 33 species. This assemblage is dominated by conifers and pteridophytes. Cycadophytes and Ginkgophytes are poorly represented

These are chiefly represented by the genera *Equisetites*, *Hausmania*, *Cladophlebis*, *Sphenopteris*, *Otazamites*, *Elatodadus*, *Ptyllophyllum*, and *Elatocladus*, *Pagiophyllum*, *Araucarites* which constitute the flora of the Jurassic-Lower Cretaceous periods. The other constituents of this assemblage are conifer woods belonging to *Araucarioxylon*,

Podocarpoxylon, *Taxaceoxylon* and *Cupressinoxylon*, common to the Rajmahal flora. Moreover, among these conifer woods, *Araucarioxylon santaleme* (Sah and Jain) Bose and Maheshwari (1974), described from Rajmahal Hills is commonly found in the Kota Formation. *Taxaceoxylon rajmahalense* (Bhardwaj) Kruusel and Jain (1964) is another species in the Kota formation which closely resembles *T. rajmahalense* from the Rajmahal Formation. This study is suggestive of close floral similarity between Kota and Rajmahal formations. The flora of Rajmahal is considered to be Early Cretaceous in age (Sukh-dev, 1987). Broad leaved cycadophytes and other characteristic fossils of the Rajmahal flora are not recorded in the present assemblage.

Stratigraphically the Kota Formation conformably overlies Dharmaram Formation. The flora from the Dharmaram Formation is not definitely known except *Palissya conferius* and *Chirolepis munsteri* recorded from the Annaram beds (King, 1881; Bandopadhyay and Rudra, 1985). Faunal evidences of Dharmaram Beds supported an Upper Triassic age (Kutty, 1969). The Kota sediments are unconformably overlain by the Gangapur beds which show a fairly well represented flora of Early Cretaceous age (Rao and Shah 1960, Sukh-Dev & Rajanikanth, 1988).

Age

The age of the Kota Formation was considered Lower Jurassic on fish evidences (Jain, 1973, 1983; Satsangi and Shah, 1973; Robinson, 1970; Yadagiri *et al.*, 1980). On the basis of ostracod Middle Jurassic age has been suggested to these beds, (Govindan 1975; Misra and Satsangi, 1979; Tripathi 1975). Overall palaeontological, geological and palaeobotanical evidences favour middle Jurassic or slightly younger age for the Kota formation. The view expressed by Rajeshwar Rao *et al.* (1983) that the Middle to Upper Jurassic strata are missing in the Upper Gondwana sequence of Pranhita Godavari Valley is not in concurrence with the present Study. It was probable that during the time of deposition of Kota sediments there was abundant vegetation comprising of mostly conifers with probably subtropical environment with moderately good rainfall. Presence of growth rings in the woods points out alternating seasonal variations. The fish and ostracod evidences indicate the presence of freshwater expanding and very shallow lake (Govindan, 1975, Jain, 1983). The presence of some fish and pterosaur which are elsewhere known from marine deposits indicates the deposition of Kota sediments was not far away from the sea (Shah and Pant, 1979), and also the occurrence of coccoliths in limestones of the Kota Formation indicates marine transgression as well (Bhattacharyya, 1981).

Conclusions:

- Flora and Fauna of Sironcha and its vicinity constitute Upper Gondwana land, in the Pranhita-Godavari basin belongs to Upper Carboniferous to lower Jurassic period.
- A narrow triangular and layered concretionary as well as massive in nature.
- Recent field work carried out in this region has brought a rich assemblages of fossil Pteridophytes and Gymnospermous leaves and woods, dominating by *Dadoxylon*, *Araucarioxylon* and *Cupressinoxylon* woods. Most of them found in situ at Waddham locality up to 40 feet long and 8 feet in diameter.

- The faunal assemblages of Gondwana land of this region is reported to contain a rich assemblage of fossil fishes, sauropod dinosaurs, Pterosaur (Flying reptiles), Scutes of Crocodiles and Mammals, in addition to fossil Ostacords, Branchiopods and Insects.
- Well preserved fossil fishes collected from the limestone beds occurring in the neighbourhood of Nandigaon, Chitur and Ankisha. A few insitu at Boraigudum. Fragmentary remains of dinosaurs bones in patch of upper Gondwana rocks occurs in the Godavari valley near Sironcha. This patch is well known by Kota stage the name after the Kota village 5 km away from Sironcha, Gadchiroli district.
- The Kota formation (Mesozoic Gondwana) comprises limestones, sandstones, siltstones, mudstones and red clays with pebbly sandstone at the base. The limestone band is grey colored are collected from clay beds underlying the limestone horizon near Sironcha, Kottapali and bank of Godavari near Wadadham.
- The collected fossil fishes indicate marine environment for the deposition of Kota limestone. The Kota formation as a whole appears to have been deposited in a mixture estuarine and marine environment.
- The Kota-Sironcha-Ankisha area of the Pranhita- Godavari Valley appears to be one of the most promising areas for the collection of fossil fishes, dinosaurs and Gymnospem woods.
- The overall faunal as well as floral assemblage are in favor of deposition under fresh-water conditions during Lower Jurassic period. However, presence of exclusively marine genera of fishes and Pterosaur, compared with thick limestone beds of large aerial extent, indicates existence of marine conditions towards the end of deposition of Kota formation.

Fish-

Lepidotes deccanensis,
Paradapedium egerteri.
Tetragonolepis oldhami.
Pholidophorous kingie
Indocoelacanthus robustus .

Pterosaur (Flying reptiles) -
Campylognathoides indicus.

Sauropod dinosaurs -

Barapasaurus tagorei
Kotasaurus yamanapalliensis
Teleosaurid Crocodiles

•The floral assemblage:-

leaf genera:

Cladophlebis,
Sphenopteris,
Pagiophyllum

wood genera-

Araucarioxylon,
Podocarpoxylon,
Taxaceoxylon,
Cupressinoxylon
Ginkgoxylon.

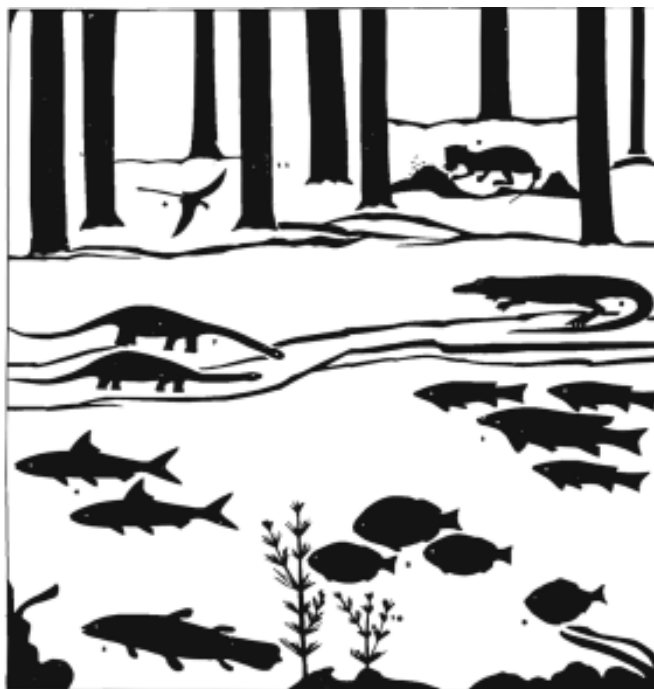


Fig.14 - Restoration of Kota fossil assemblage in silhoutte to indicate suggested ecological roles :

Figure 14: An Attempted restoration of the main Kota fossil assemblage in silhoutte to indicate suggested ecological roles (Jain, 1980)

Suggestions/Recommendations:



Fig. 15 - Wadadham exposure before park



Fig. 16 - Wadadham Fossil park Work in Progress

In view of the above observations, it is desired that that Kota Type area may be studied in details as it shows interesting faunal and floral elements of great palaeoenvironmental and stratigraphical values. This area has already yielded excellent specimens of dinosaurs, including sauropods, Fishes and Gymnosperm forest. Government of Maharashtra or Geological Survey of India should preserve these fossil localities of this region and established a unique **“Gondwana Jurassic Fossil park”** at Wadadham which shows in situ excellent preserved Gymnosperms wood forest. ". These are probably the thickest and largest tree fossils not found anywhere in India."

Acknowledgements:

Special thanks to Chief Conservator of Forest, Gadchiroli circle, for providing facilities for this project work. Miss. Nusrat Shaikh, Botany Dept. Shivaji College- Sironcha, for her cooperation in exploration work & Donation of her collection of fossil specimens & Dinosaur Bones to the Forest Department's fossil Museum. All the staff members of Sironcha Forest Division. Thankful to, Assit. Conservator of Forest- Shri L. M. Belekar, Sironcha Division; Range forest officer- Shri A. D. Karpe, Sironcha; Range forest officer- Shri M. B. Kusnaik, Gimalgattha Range; Range forest officer- Shri S. N. Shende, Asarali Range; Range officer- Shri S. T. Navghare, Wadadham range, Shri Mallikarjun, President of J.F.M.C. Wadadham and Forest guard- Shri Dangi for their help in exploration work.

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HUMANS, ENVIRONMENT AND COVID-19

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Abstract:

Human life depends heavily on the environment and cannot sustain without it. World Environment Day is celebrated as 5th June of every year to emphasize it. To curb the overexploitation of natural resources and protect the environment, public should be made aware of these facts. During the Corona period, a new dimension of nature has become evident which clearly shows that protecting the environment and keeping the world beautiful naturally is not so tough. During this short span of time, human activities, especially which are harmful for the nature and biodiversity was reduced to a great extent. This resulted in the building up of natural environment, cleaning of rivers and the chirping of unseen bird species was heard by all. Protection of nature and environment is one of the most challenging tasks of 21st century. Appropriate steps have to be taken by not only the governments, but by authorities as well as by the public in general to keep our environment clean and inhabitable. The present study deals with the effects of human activities on environment and is an effort to suggest some solutions for this problem.

Keywords: Environment, Covid-19, Biodiversity, Epidemic.

Introduction:

Coronavirus is a new hazardous disease. In history, the world faces many dangerous diseases like malaria, AIDS, measles, others, etc. These are all terrorist attacks and now COVID-19. The first outbreak of SARS was registered in 2003. After some years zero cases were reported around the world. But in 2012 in Saudi Arabia the second outbreak of coronavirus was reported. World health organization called Covid-19 a pandemic on 31 December 2019. All these diseases attack the respiratory system. The symptoms of the coroner are the same as some other diseases but the coronavirus reason is different from others.

COVID-19 stands for coronavirus disease. CO stands for corona, VI stands for the virus and D stands for disease. The name of the coronavirus was registered on 11th Feb 2020 in the World Health Organization. This virus has pin like structures on its outer surface. That's why we call it coronavirus. The size of Corona virus is between 50nm and 140nm. This virus spreads through respiratory droplets. The distance maintains by the person who is affected due to the

corona. By touching those people who were ill due to cornering the chances of illness increased. Indications of corona infection are dry cough, fever, and issues in the inhaling system. The chances of infection in old people and those people who take medicine regularly are more than others. The people who suffer other diseases like BP and cancer the chances of corona are more than healthy. No antibiotics work on a person who is affected by this virus. No medicines are available for corona infection. In some cases, home remedies are found to be more effective than antibiotics as they decrease the chance of this virus.

After one month of lockdown, it has been noticed that pollution of water and air has decreased. This virus has also affected the animals. Lockdown due to the spread of corona virus has also affected industries. This lockdown has affected every field of life. In negative effects health issues, joblessness and others many difficulties people faces. This lockdown impacts the economic growth of every country. At this time whole world faced this issue. However, there are some positive effects as well. Some of them are decreased air and water pollution along with increased quality of environment and biodiversity.

Positive impacts of lockdown on air quality

Lockdown has given us blue sky and natural air back. After the lockdown, 60% NO was reduced by average. In lockdown, transport, industries and power plants were closed because they are all the main reasons for air pollution. Similarly, 50-70% reduction was recorded in the emission of SO₂. Significant reduction in the emission of greenhouse gases such as CO and CO₂ was also recorded.

Effect of lockdown on global environment

Lockdown left a massive impact on the environment. During the lockdown, the air pollution decreased, and the blue sky made the nature beautiful. Maybe the improvement in air and water quality is unstable, but it is exemplary for the whole world.

Impact of lockdown on the ozone layer

The blue surface in the upper atmosphere is called the ozone layer. Before the lockdown, air pollution increased day by day and some gases like SO and NO caused ozone depletion. But after the lockdown, ozone depletion decreased. Lockdown proved to be a blessing for the ozone layer. The main reason for ozone depletion is air pollution.

Effect of lockdown on water pollution

Lockdown has also impacted the water pollution. Some times ago, industries and power plants threw their wastes in rivers. Such waste consisted of many dangerous chemicals that mix with water and makes the water very hazardous. The result of that reaction of deadly chemicals and water is harmful and dangerous for humans and animals. But after lockdown and intermesh of industries, the levels of water pollution have decreased significantly. As the industries were shut due to lockdown, the industrial effluents came to zero discharge and this

lead to huge reduction in water pollution levels. Several Indian rivers such as Ganga, Yamuna, Gomti etc. that were full of polluted water were also cleaned significantly and their water quality was also improved very much.

Energy consumption

Before lockdown, a huge amount of energy was being used in power plants and industries. But after lockdown, all industries were closed and the need for energy reduced by almost 30%. In different countries like Italy, France, USA, etc. consumed a lot of energy in their works. But in the era of coronavirus, the consumption has decreased a lot.

Negative impacts of lockdown on environment

The negative effects of lockdown on environment are as under:

Increase in Biomedical Waste

During the corona period, a lot of biomedical waste has been generated. Doctors and other hospital staff used gloves, face mask, and virus-resistant clothes. Different substances were used to make all these things, and this created a huge pile of disposed medical waste. Because of Corona, the use of mask was raised by thousand times. Most of the people showed the habit of throwing the used mask, gloves and other waste anywhere, which caused huge increase in the quantity of biomedical waste.

Use of safety types of equipment

During the whole lockdown, medical staff required large quantities and varieties of safety instruments which were necessary to decrease the chances of coronavirus infection. During the lockdown, the need for these instruments increased.

Effects on economic growth

During the lockdown, the economy of every country was decreased. The shut down of industries, education, and power plants are the main reasons for the reduction in economic growth.

Another vital negative effect was faced by the people living in developing countries like India, Bangladesh, Pakistan, Sri Lanka and others. They used the water of the river, and the river water also was contaminated by the virus. Hence, the water supply from the river also became dangerous for the people. China has established clean water projects for its people. But this facility is not for Indians and Bangladeshi people. That's why lockdown was dangerous for the people of these countries.

Reduction of recycling

In the whole lockdown period, people didn't go to the hotel. Home delivery was available at every hotel and restaurant. That's why a massive quantity of waste increased because waste recycling factories were closed due to restrictions of the lockdown.

Influences of lockdown on biodiversity

Lockdown has left massive effects on biodiversity. Some effects are positive and some are negative for biodiversity. Biodiversity is the proficiency of whole species.

Positive effects of lockdown on biodiversity

After the restriction of human's activities in the forests, animals are in calm environment. People did not go to parks and jungles. The animals are not being disturbed. The natural air is finest for animals.

Deforestation and unlawful trades of admirable animals have also decreased. Animals love to live in forests. Due to human fear, they do not feel safe there. Because of lockdown, the incidents of forest destruction have decreased a lot.

Negative impacts of lockdown on biodiversity

Tourism

Lockdown has also decreased tourism ratio. Every country collected a massive amount of money from tourism. But during the lockdown, tourism revenues have decreased. Almost 30% of its income for a country is earned from tourism. A huge amount of money has lost due to lockdown. The animals living in parks and houses face issues for food. Due to lockdown, food industry is facing problems. During lockdown, the wildlife officers and staff were not allowed to touch any animals. That's why many issues were faced during their study. Many animals and other species were decreased yearly because they need good environment for a good life.

Decline in pet shops and jobs related to pet animals

Sudden countrywide lockdown in India caused a number of very unprecedented problems to all. There are tens of thousands of pet shops across India and lakhs of people engaged in businesses related to delivery of pets and items related to them. Millions of pets kept in those shops were locked continuously for several months and died inside the shops. The people related to pet shops and other businesses became jobless and the pets were died. This was a position which nobody could think of.

Conclusion:

Coronavirus is a dangerous virus. World never faced such type of crisis ever before. There is only one method to prevent Corona virus infection and it is lockdown and keeping distance. In every field of life, the world is facing serious crisis and failures. In my opinion, lockdown will end by the last of this year completely. If we follow the restrictions, we can control coronavirus and get freedom from lockdown very soon. If whole world gets vaccinated, all the world's activities can start again. Otherwise, it will be impossible. Lockdown is a threat for business. If lockdown remains, the economy of every country dropped. Joblessness has increased due to Lockdown. In some cases, lockdown is good but in some cases it is bad. According to my mind, lockdown is a difficult time for everyone.

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STORAGE BEHAVIOUR OF PRIMED SEEDS

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Abstract:

Seed vigour successively declines after physiological maturity, depending upon the storage condition and chemical compositions of the seed. The importance of seed storage has been known ever since domestication of plants. Seed priming is a pre-sowing seed treatment in which the seeds are hydrated and dehydrated, altering the physiological and biochemical properties of the seed. Priming is used to enhance seed performance after storage. But priming before storage may be harmful for seed performance and it can lower the seed longevity. Hydration-dehydration treatments increase the enzymatic activities in germinating seeds while decreasing membrane damage that elongates seed storability.

Keywords: storage behaviour, priming, longevity, seed enhancement.

Introduction:

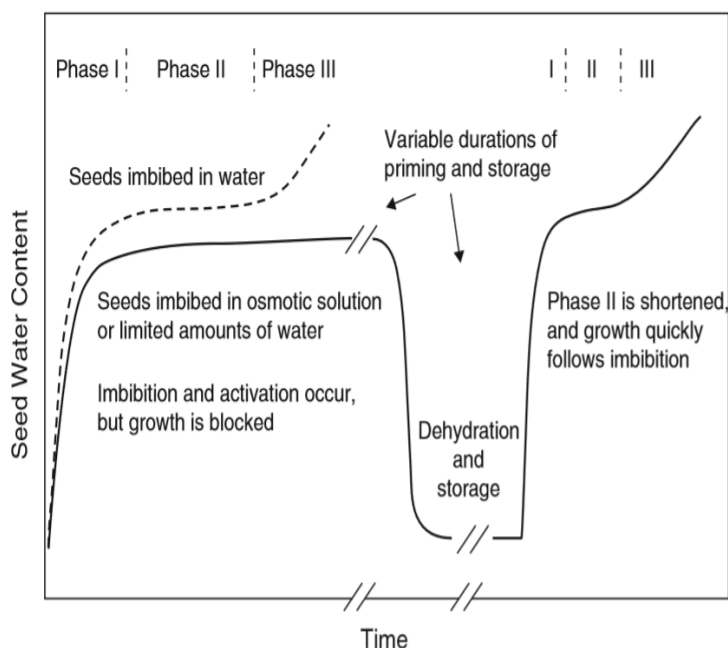
Seed attains highest vigour during the physiological maturity and then successively declines depending upon the storage condition and chemical compositions of the seed. The importance of seed storage has been known ever since man began to domesticate plants. The duration of storage depends upon the objectives of the particular storage effort. Farmers need to maintain viable seeds from one growing season to the next while seed producers may wish to maintain carry-over seeds for a few years whereas for several years in case of seed conservation activities. Seed priming is a technique by which seeds are partially hydrated to a point where germination-related metabolic processes begin, but radicle emergence does not occur (Heydecker and Coolbear, 1977). Seed priming is used to enhance seed performance when sown in fields. Priming enhances germination percentage, seed vigour, uniformity in germination, speed of germination and seedling establishment. But priming before storage may be harmful for seed performance and it can lower the seed longevity. Once primed, seed attains the maximum beneficial effects of priming and there is decrease in the effects if seed is stored for longer durations. There are some kind of particular priming treatments i.e. hydration dehydration, that are given in middle of seed storage and these treatments help in increased storability of seeds. Reports says that some particular priming chemicals i.e. KNO₃, KH₂PO₄ etc., enhance seed

longevity even after storage. But basic and simple priming treatments i.e. hydropriming, osmo-priming, solid matrix priming; are not to be given before seed storage but before sowing only. As these treatments do not enhance seed longevity and all the priming beneficial effects are lost during storage and seed deterioration take place even faster resulting into seed death. After seed priming retains some water and not fully dehydrated. Seed desiccation tolerance is lost after priming and seed is more liable to deterioration. Seed priming is utilised to some extent to reduce seed degradation during storage. Priming before to storage can control seed deterioration during storage (Georghiou *et al.*, 1987), since priming activates antioxidant enzymes in seed that scavenge reactive oxygen species and lower lipid peroxidation in seed (Braccini *et al.*, 2000). Antioxidants are molecules that protect the cell membrane from oxidative damage caused by oxidants (Temple, 2000). Seed priming is a pre-sowing seed treatment in which the seeds are hydrated and dehydrated, altering the physiological and biochemical properties of the seed (Basu, 1976). According to Gu *et al.* (1993), hydration-dehydration treatments increased the activity of catalase, superoxide dismutase, and peroxidase in germinating tomato seeds while decreasing melondialadehyde concentration and membrane damage. This chapter discusses about storage behaviour of seed after priming and methods to be adopted for higher seed longevity after priming.

Priming

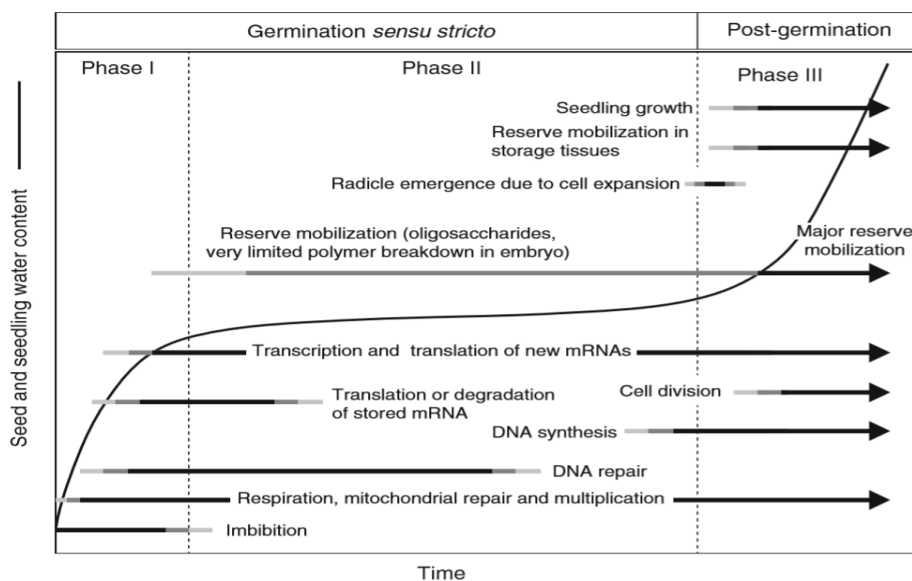
Seed germinates when a dry seed imbibes water and the water potential of the seed reaches a critical physiological level (varies between 0 to -2MPa). Seeds generally go through three reorganizable stages of germination, as well as three stages of water uptake. (Bewley, 1997). Seed priming is a technique by which seeds are partially hydrated to a point where germination-related metabolic processes begin, but radicle emergence does not occur (Heydecker and

Coolbear, 1977). Priming actually results in an extension of Phase II of seed imbibition, essentially restricting the seed within the lag phase. It prevents the seeds from taking in enough water to enter Phase III of hydration. Primed seeds usually display increased germination rate, greater germination uniformity and higher stand establishment.



Metabolic changes during triphasic uptake of water

Seeds imbibe water when placed in water and it is a physical process. Seeds generally go through three reorganizable stages of germination, as well as three stages of water uptake. (Bewley, 1997). First change after imbibition is increased rate of respiration, mitochondrial repair and DNA repair. Translation of stored mRNA and synthesis of new mRNA take place. Rapid initial uptake of water and repairment of metabolic activities are basic characteristics of phase I of imbibition. During phase II there is a very small water intake and new synthesis of cell metabolites i.e. mRNA, proteins, DNA, mitochondria etc.

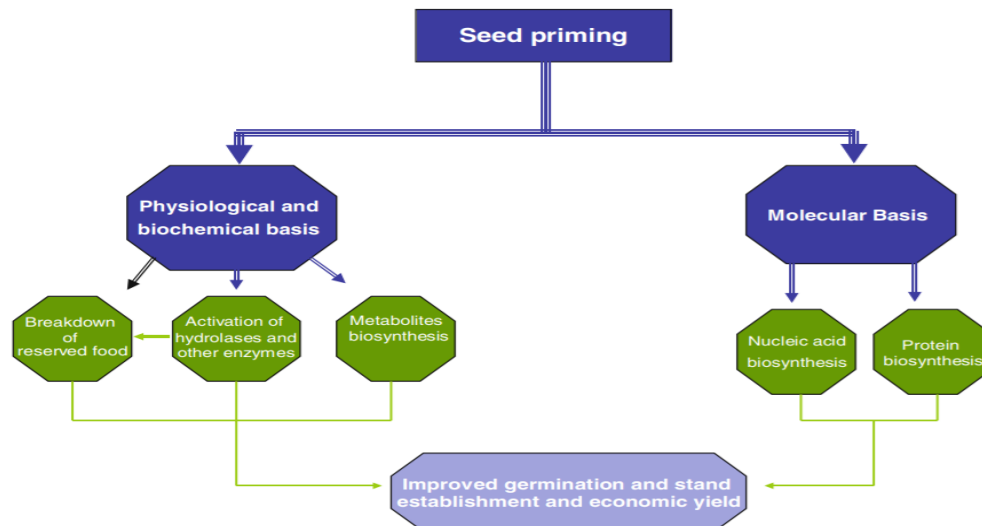


Activation of hydrolases and antioxidants take place before germination. These antioxidants counter excess reactive oxygen species (ROS) produced during respiratory activities in mitochondria. New mitochondria synthesis and DNA synthesis during phase II of imbibition. Cell elongation starts in phase II but cell division take place in phase III i.e. after germination and radicle emergence. Reserve mobilisation mainly take place in phase III after seed germination and a very limited reserve mobilisation take place before germination. Phase III is started where seed starts germination and radicle comes out of the seed. A large amount of water intake occurs after seed germination starts i.e. in phase III. High increase in growth of embryonal axis due to major reserve mobilisation and higher water intake by seed in phase III.

Changes in seed during priming

Phases I and II represent the germination processes (Bewley, 1997), and they are the cornerstone of good seed priming, which involves bringing seed to a seed moisture content just short of radicle protrusion. At phases I and II of germination, the seeds are desiccation tolerant, which allowed the primed seeds to be dried back to their original moisture content. The germination process is launched during priming, and as a result, changes such as the leakage of inhibitory chemicals, the breaking down of reserves, and the accumulation of enzymes required

for endosperm breakdown occur within the seed. Seed priming is linked to an increase in total protein content, as well as the amounts of enzymes involved in respiration, breakdown, and storage reserve mobilisation. (McDonald, 2000). During priming, the levels of aldolase, isocitrate lyase, G-6-phosphate dehydrogenase, and α - and β - amylases increased while the levels of alcohol dehydrogenase declined. During priming, the enzymes involved in the antioxidation of damaging free radicals created by lipid peroxidation are also enhanced. Priming increases the respiratory activity of seeds by increasing ATP, energy charge (EC), and the ATP/ADP ratio. The improvement in germination that comes with priming is followed by an increase in 4C DNA, indicating that the cell cycle has progressed from G1 to G2. Osmo-priming also increases RNA content, with ribosomal RNA accounting for the majority of the increase, while mRNA concentration remains unchanged (McDonald, 2000). During priming, changes like as metabolic repair during imbibition, the accumulation of germination-promoting chemicals, and osmotic adjustments occur.



Why Primed seeds are poor storers...

The storage potential of low vigour seeds is improved by hydration treatments, but the longevity of high vigour seeds is reduced (Powell *et al.*, 2000). Increased K(initial seed viability) following priming and a slower rate of deterioration are linked to longer seed lifetime in low vigour seeds. The scenario was the opposite in high vigour seeds, presumably due to an over-advancement of metabolic processes during the aerated hydration treatment, making the seeds more sensitive to drying and deterioration after storage. Following aerated hydration, the fraction of 4C DNA and β -tubulin increased in high vigour seeds, indicating that germination advancement occurs. Cells with 4C DNA in the G2 phase of the cell cycle are more susceptible to drying and degradation. The ability of low vigour seeds to perform metabolic repair during the early stages of hydration may explain their low 4C DNA, showing that most cells are in the G1 phase.

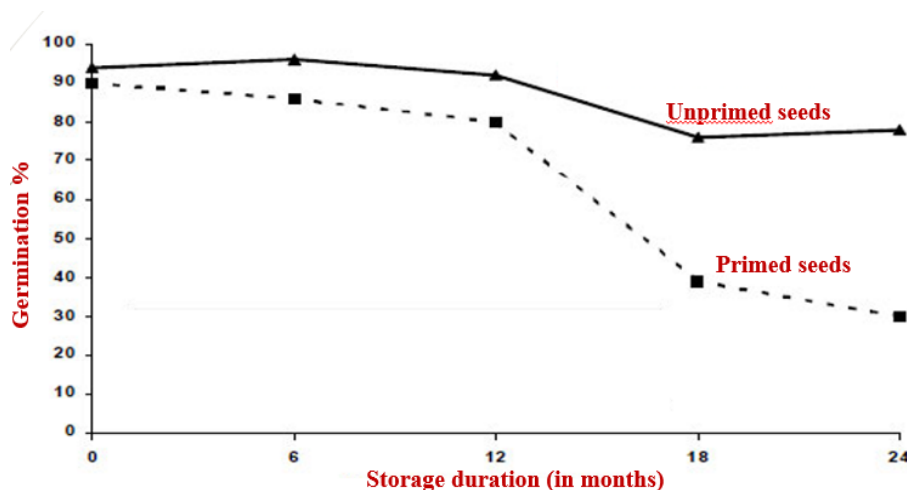


Fig.-Germination of primed seeds of melon stored upto 24 months at 25°C

(W.M. Nascimento, 2002)

Under artificial ageing, hydropriming and ascorbic acid priming of cotton seeds preserved germination and antioxidant enzyme activity such as POX, CAT, glutathione reductase, ascorbate peroxidase, and SOD. PEG osmopriming lowered hexanal levels, which linked with decreased loss in viability of soybean seeds during storage (Braccini *et al.*, 2000). Solid matrix priming in moistened vermiculite decreased lipid peroxidation, increased antioxidative activities and increased seed vigour of solid matrix primed sh-2 sweet corn seeds maintained at sub-zero temperatures (Chiu *et al.*, 2002). Sugar and sugar derivatives, particularly sucrose and raffinose family oligosaccharides (RFOs), are thought to play a role in seed life. Sucrose and raffinose, in addition to maintaining membranes and proteins during dehydration, are linked to the ability of tissues to acquire a glassy state (Leprince *et al.*, 1993). The raffinose concentration of entire lettuce seeds dropped throughout hydration and priming and was associated with a decrease in median potential viability (Gurusinghe and Bradford, 2001). Short-term drying in a vented oven at 29°C followed by incubation at 32, 37, or 40°C before quick drying did not result in consistent changes in RFO or planteose content. These findings do not rule out the possibility that oligosaccharides have a role in seed lifespan, but they suggest that changes in oligosaccharide content are unlikely to be responsible for the decrease in longevity during priming. During seed maturity, several heat shock proteins (hsps) have been linked in the acquisition of desiccation tolerance. BiP, or immunoglobulin binding protein, is an ER-resident homolog of cytoplasmic hsp-70. BiP is involved in protein repair activities in plants in response to heat shock and other stimuli, in addition to being expressed constitutively throughout normal growth. Tomato seed longevity was restored after post-priming treatments, which was associated by greater levels of BiP accumulation (Gurusinghe *et al.*, 2002). In the absence of a post-priming heat treatment, treatment of primed seeds with the calcium ionophore calcimycin increased BiP protein

accumulation and extended prospective seed longevity. As a result, increased BiP expression could help primed seeds last longer after postpriming treatments.

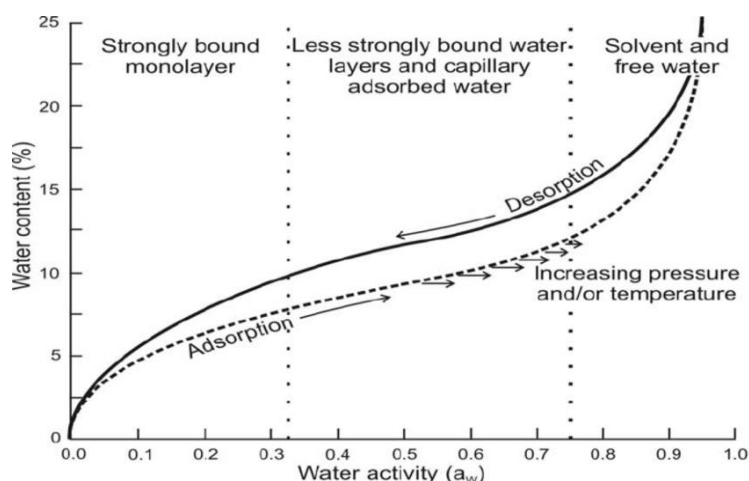
On dehydration of seed during priming, free water remains inside the seed. During dehydration water is replaced with small air pockets but some amount of water is retained inside seed that cause in faster seed deterioration. Repair of damaged RNA/DNA to ensure the availability of error free template for replication and transcription.

Storability of primed seeds depend on Initial seed vigour

Generally priming improves the life i.e. storability, of low vigour seeds, but reduces that of high vigour seeds. The high vigour seed is at a more advanced physiological stage after priming nearly at phase III and thus more prone to deterioration. When a low vigour seed is primed, it requires more time to repair the metabolic injuries thus preventing further deterioration. Low vigour seeds are at edge of seed death and when they are primed, their energy stored and enzymatic activity is enhanced, increasing their life under storage. But on the other side, seeds having high vigour are already full of energy and enzymatic activity ia also at its peak. When these seeds are primed, there is not much enhancement effect but seed is deteriorated due to some amount of moisture retained after dehydration after priming and seed is deteriorated even faster. Powell *et al.* 2000, observed that hydration treatments improve storage potential of low vigour seeds and decrease the longevity of high vigour seeds.

Chemical composition of seed

Seed longevity also depend on major storage material in seeds. Oilseeds have poor storability because they release the imbibed water slowly and some amount of water is retained by the seeds after dehydration after priming treatments. Thus, the seeds having higher oil content are more prone to deterioration. Oilseeds have larger hysteresis loop, more water is retained by oilseeds followed by proteinaceous seeds, followed by starchy seeds. Thus, starchy seeds can be stored for longer durations after priming treatment as compared to oilseeds and proteinaceous seeds.



Priming treatment

In a study, Seeds were primed with GA₃ (100 ppm), KNO₃ (2%), KH₂PO₄ (5000 ppm) and CaCl₂ (1%) and recorded for germination and field emergence after 8 months of storage. The seeds treated with GA₃ (100 ppm) showed rapid decline in the germination and field emergence from 3rd month onwards upto eight month of storage. Seeds primed with KNO₃ (2%) recorded higher germination per from third month onwards upto eight months of storage followed by KH₂PO₄ and CaCl₂. Nitrogen in KNO₃ provide additional substrate for ageing and membrane repair mechanism (Khan *et al.*, 1978). Phosphorus in KH₂PO₄ activates the respiratory enzymes involved in the biosynthesis of seed and extends the seed storability. Results shows that quality of the seed, priming treatments and period of storage greatly influence the storability of the seeds (Pushpalatha (2008). The seed treated with plant growth regulators has promotory effect during initial few months of storage followed by rapid decline in seed germination. Pretreatments with growth regulators stimulated the rate of germination but they are not stored for longer period. Thus, there are treatments that may enhance storability but there is a time limit for which seed can be stored after a priming treatment.

Storage duration

There is a limited duration for which the beneficial effects of priming are retained in seeds. With time the beneficial effects are lost and seed starts deteriorating and that too even faster than unprimed seeds. This is because of the energy and enzyme activity that was enhanced during priming is all deteriorated with time if seed is not sown.

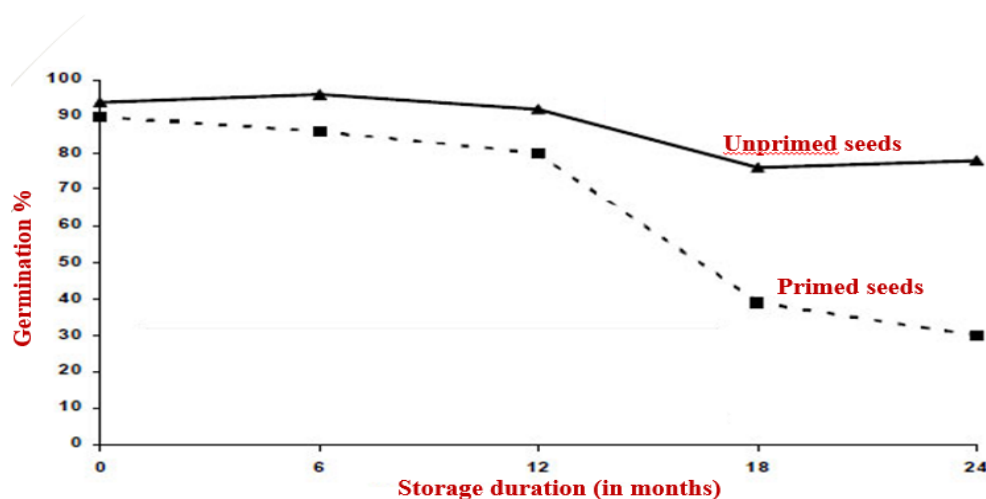


Fig.-Germination of primed seeds of melon stored upto 24 months a (W.M. Nascimento, 2002)

Nascimento W.M. (2002) conditioned melon seeds for nine days at 25° C in aerated KNO₃ solution (0.35M). The conditioned or non-conditioned seeds were packed in aluminized envelopes and stored for 0, 6, 12, 18 and 24 months under laboratory conditions. Upto 12 months of storage there was a small decline in germination % of primed seeds as compared with

unprimed seeds. After 24 months only 30% of primed seeds germinated, compared with 78% of unprimed seeds. Primed seeds showed a greater deterioration with storage duration.

Enhancing storability of primed seeds

When a primed seed is stored under conducive conditions (low temperature and low moisture) most of the beneficial effects of priming are retained. Low temperature and moisture decrease the rate of respiration and thus increases longevity. But seeds used in large quantity for sowing are difficult to store under these favorable conditions as they need more space and cost is increased. Thus, for maximum enhancement results generally priming is used just before sowing. Slow drying after priming of seed, is another method to retain the beneficial effects of seed priming for longer durations. Slow drying of osmo primed *B. oleracea* improved the performance of the osmo primed seed and increased storability. The enhanced expression of two stress tolerant genes (Em6 and RAB 1/8) during slow drying improved longevity. Enhanced BiP expression via seed treatment may contribute to the improved longevity of primed seeds following postpriming treatments.

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AN OVERVIEW ON THE RECENT TRENDS IN CIRRHOSIS AND ALCOHOLIC LIVER DISEASE

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Abstract:

The alcoholic liver disease covers a spectrum of disorders beginning from the fatty liver, progressing at times to alcoholic hepatitis and culminating in alcoholic cirrhosis, which is the most advanced and irreversible form of liver injury related to the consumption of alcohol. Different factors, such as metabolic, genetic, environmental, and immunological, collectively play a role in alcoholic liver disease. There are 3 types of liver disease related to alcohol consumption: fatty liver, alcoholic hepatitis, or cirrhosis. Management of alcohol liver disease depends on the extent of the disease. Medical Treatment includes alcohol abstinence, enrollment to detoxification programs, nutritional support and therapeutics managements.

Keywords: Alcoholic liver disease, cirrhosis, fatty liver, ascites and alcoholic hepatitis.

Introduction:

The liver weighs up to 1500g in adults and as such is one of the largest organs in the body. The main functions of the liver include protein synthesis, storage and metabolism of fats and carbohydrates, detoxification of drugs and other toxins, excretion of bilirubin and metabolism of hormones. It is noted for its capacity to regenerate rapidly. However, once it has been critically damaged multiple complications develop involving many body systems. The distinction between acute and chronic liver disease is conventionally based on whether the history is less or greater than 6 months.

Alcoholic liver disease also called as alcohol related liver disease (ARLD) that encompasses the liver manifestation of over consumption

Definition:

(ARLD) is caused by damage to the liver from years of excessive drinking. Years of alcohol abuse can cause the liver to become inflamed and swollen. This damage can also cause scarring known as cirrhosis. Cirrhosis is the final stage of liver disease. Alcoholic liver disease is common, but can be prevented.

Types of ALD:

There are 3 types. Many heavy drinkers progress through these 3 types over time-

Alcoholic fatty liver disease:

Drinking a large amount of alcohol, even for just a few days, can lead to a build-up of fats in the liver. This is called alcoholic fatty liver disease, and is the first stage of ARLD.

Alcoholic hepatitis:

Alcoholic hepatitis, which is unrelated to infectious hepatitis, is a potentially serious condition that can be caused by alcohol misuse over a longer period. The liver damage associated with mild alcoholic hepatitis is usually reversible if you stop drinking permanently.

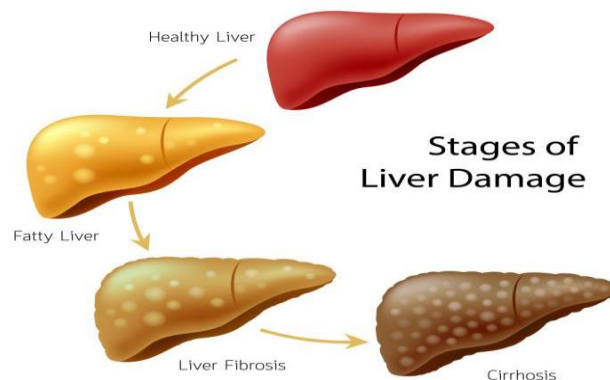
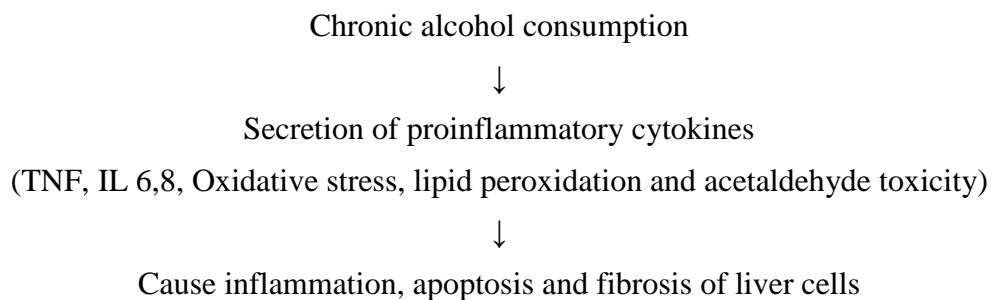
Alcoholic cirrhosis:

Alcoholic cirrhosis is the destruction of normal liver tissue. It leaves scar tissue in place of the working liver tissue. It's generally not reversible, but stopping drinking alcohol immediately can prevent further damage and significantly increase your life expectancy.

Pathophysiology:

Alcohol metabolism by the liver is primarily via two enzymes:

1. Alcohol dehydrogenase
2. Aldehyde dehydrogenase



These may lead to any one type of ALD:

a) Fatty Changes (steatosis) –

- Accumulation of fatty acids in liver cells.
- Alcoholism leads to large fatty globule throughout liver.

- Mechanism: Alcohol in presence of ADH converted to acetaldehyde. Acetaldehyde in presence of aldehyde dehydrogenase produce acetic acid, further acetic acid oxidized to H₂O and CO₂. This process generates NADH, increases the ratio of NADH/NAD⁺. It increases fatty acid synthesis and decreases fatty acid oxidation, leads to accumulation of fatty acid in liver cells causes fatty liver.

b) Alcoholic hepatitis:

- Inflammation of hepatocytes. This condition is also called as alcoholic-steato-necrosis and the inflammation appears that predispose to liver fibrosis.
- Inflammatory cytokines involve in initiation of liver injury. Kupffer cells of liver then phagocytose endotoxin, stimulate release of TNF this will trigger apoptotic pathway leads to cell death.

c) Cirrhosis:

- This is late stage of serious liver disease marked by inflammation (swelling), fibrosis (cellular hardening) followed by scarring and necrosis.

Clinical presentation:

Fatigue, pruritis, malaise, anorexia, weight loss, encephalopathy, hepatomegaly, splenomegaly, jaundice, ascites, ankle edema, pleural effusion and respiratory difficulties.

Risk factor:

- Quality of alcohol taken – consumption of 60-80g/day
- Pattern of drinking- before meals increases the risk
- Sex- 2 times more susceptible to ALD
- Hepatitis C infection- concomitant infection, increase liver injury.
- Genetic factors- polymorphism of alcohol metabolism enzyme ADH, CYP4502E1
- Iron overload- hemochromatosis
- Diet: Vitamin A and E deficiencies, decreases regeneration of hepatocytes.

Diagnosis:

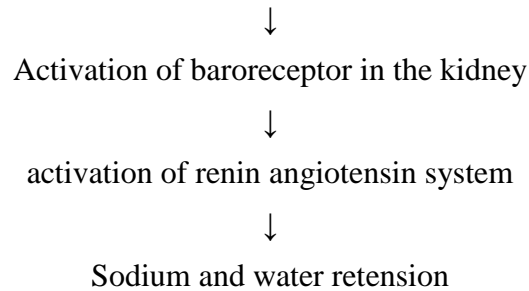
- LFT
- Radiological test
- Liver biopsy

Based on the Child- Pugh Score we can evaluate the severity of liver disease-

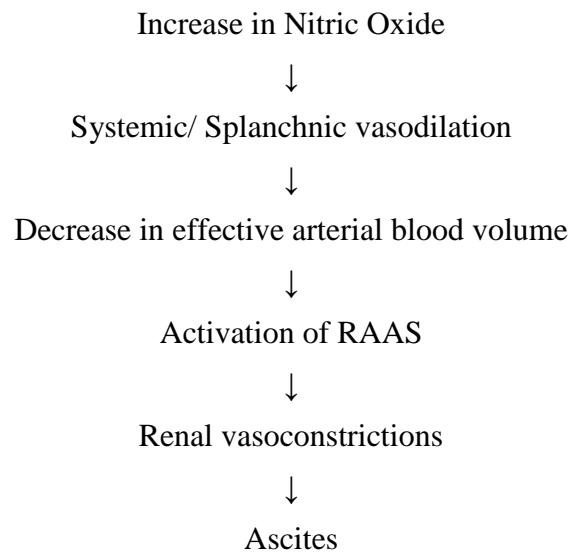
Child-Pugh score is determined by scoring **five clinical measures of liver disease and the possibility of eventual liver failure**. A score of 1, 2, or 3 is given to each measure, with 3 being the most severe.

Anatomic and physiologic effects of cirrhosis (complications)

- 1) **Ascites:** development of portal hypertension in conjugation with systemic arterial vasodilation

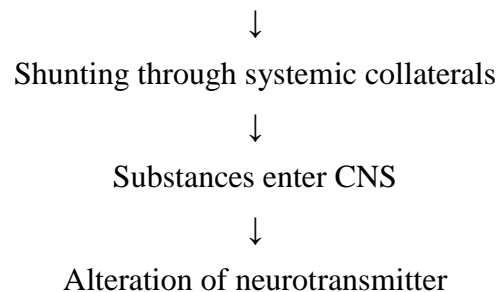


- 2) **Portal hypertension:**



- 3) **Hepatic encephalopathy** (neuropsychiatric syndrome)

- Results from an accumulation of gut derived nitrogenous substances in the systemic circulation



- Elevated arterial ammonia concentration leads to liver insufficiency.

Hepatic encephalopathy (HE) is divided into 3 types:

- a) Acute HE: Altered sensorium last for less than 4 weeks, followed by complete recovery.
- b) Chronic HE: Cognitive or neuropsychiatric abnormalities that persist for at least 4 weeks.
- c) Subclinical HE: Alteration in neuropsychiatric functions.

4) Coagulation defects:

Coagulation disarrangements can occur in cirrhosis, leads to reduction in the synthesis of coagulation factors. More than 40% of cirrhotic patient have abnormal prolongation of bleeding times to more than 10 minutes and platelet count less than 1,00,000 lakhs.

Management

Non-pharmacological treatment:

1. Limit or stop the consumption of alcohol.
2. Include low-sodium diet.
3. Include healthy balanced diet every day.
4. Take adequate sleep.
5. Perform mild to moderate physical exercise or walking.

Pharmacological treatment

Goal of treatment:

1. Identify and eliminate the cause of cirrhosis (e.g., alcohol abuse)
2. Evaluate the patients for clinical signs of ascites and manage with pharmacological therapy.
3. Hepatic encephalopathy can be life threatening, focus on therapy to lower ammonia levels.
4. Frequent monitoring for signs of hepatorenal symptoms, pulmonary insufficiency and endocrine dysfunction is necessary.

Treatment:

1) Portal hypertension

- The treatment recommendation for portal hypertension is beta adrenergic blockers like propranolol and nadolol. It works by decreased blood flow to vascular system and decreased portal vein pressure.
- Initiate therapy with oral propranolol 10mg TID or nadolol 20mg OD
- Nitrates may be considered for the patient younger than 50 years of age and well compensated cirrhosis.
- Initiate the therapy with isosorbide mononitrate 20mg BD and increase upto 20mg TID after 1 week if tolerated well.

2) Variceal bleeding:

- The main goal is to coagulopathy, thrombocytopenia and control of bleeding.

- Vasoactive drugs (somatostatin, octreotide and terlipressin) and beta blockers can also be used to manage the condition.
- Initiate octreotide with IV bolus 50-100mcg, followed by continuous infusion 25mcg.
- Monitor: blood glucose level with diabetic patient and assess for cardiac conduction abnormalities.
- Interventional and surgical treatment includes;
- Balloon tamponade, Trans jugular intrahepatic portosystemic shunt (TIPS), Surgical shunting and sclerotherapy.

3) Ascites and spontaneous bacterial peritonitis

- Patient with cirrhosis fail to maintain normal extra cellular fluid. Patient have complaints of edema, ascites, abdominal swelling, respiratory difficulties, malaise, anorexia and weight loss.
- Based on American Association for the study of Liver Disease (AASLD), recommended therapy for;

Ascites: Diuretic therapy -furosemide 40mg maximum dose is 160mg and Spironolactone 100mg maximum dose is 400mg.

Spontaneous bacterial peritonitis- cefotaxime 2gm every 8th hourly, fluroquinolones and amoxiclav are the choice of drugs.

4) Hepatic encephalopathy

- Lactulose is the first drug of choice.
- Inhibit urease producing bacteria by using drugs like neomycin, metronidazole or vancomycin.
- Zinc is also recommended as it is required for ammonia metabolism, thereby it increase urea formation and lower ammonia level.

5) Systemic complication:

- It includes hepatorenal, hepatopulmonary, coagulation disorders and endocrine dysfunction.
- In case of hepatorenal exclude nephrotoxin (NSAIDS/ Aminoglycoside)
- Hepatopulmonary requires supportive therapy with supplemental oxygen therapy.
- Coagulation disorder recommended option is fresh frozen plasma.

6) Miscellaneous treatment:

- Liver supplements – LIV 52, Silymarin
- Oxygen therapy
- Vitamin supplements
- Liver transplant

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GC-MS ANALYSIS OF ETHANOLIC LEAF EXTRACT OF *LANTANA CAMARA* IN SEARCH OF POTENT PHARMACOLOGICALLY BIOACTIVE COMPONENTS

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Abstract:

Lantana camara is well recognized plant with active secondary metabolites. The present investigation was carried out to identify these bioactive components from ethanol extract of leaves of *Lantana camara*. This identification was done through Gas chromatography and Mass spectrometry (GC-MS). The mass spectra of these bioactive components were matched with the database available at National Institute of Standard and Technology (NIST) library. GC-MS analysis leads to identification of 32 compounds out of which there are 7 major components. 13-(Z) Decosenamide is present in highest amount and is known bioactive agent to enhance neovascularization in regenerating skeletal muscle and also modulates water balance in the visceral organs and in the cerebrospinal fluid. This is pioneering work to identify the above compound present in the leaves of *Lantana camara*. This research reveals that ethanol extract of *Lantana camara* leaves is a good source of bioactive components and its active principles can be utilized to discover novel drug molecules.

Keywords: Anticancer, Bioactive components, *Lantana camara*.

Introduction:

Lantana camara is an angiosperm belonging to family Verbenaceae that grows near the forest edges, agricultural fields and grasslands. It was introduced in many parts of the world as a perennial ornamental shrub of garden courtyard though it is a common weed found in central and South America. In India, it was brought in around 19th century in areas with moderate temperature and well drained soils. But the plant is tough enough and can survive even on sandstone derived soils if it gets sufficient moisture throughout its growth period. Different Indian languages got different names for the plant namely, Raimuniya (Hindi), Chaturangi (Sanskrit) and Ghaneri (Marathi) [1, 2].

Lantana camara has high medicinal value as recognized by Indian traditional folkhealers. Scientific explorations further support the therapeutic importance of the plant with array of medicinal uses. Each and every part of the plant has one or other pharmacologically bioactive secondary metabolic components of medicinal importance [3]. The present study investigates the presence of these bioactive components in ethanol extract of *Lantana camara* leaves which

attracted modern researchers to clinically examine these compounds as a potent source of many dreadful diseases such as anticancer as novel leads for drug development.

Material and Methods:

Collection and taxonomical identification of plant

Plant was collected from outskirts of Gondia district of Vidharbha, Maharashtra; India. The plant parts needed for study were authenticated by Dr. (Mrs.) Rani V. Choubey, taxonomist and visiting faculty Department of Environmental Sciences, Institute of Science; Nagpur-40001. The specimen was submitted to the herbarium of department with authentication code, Research/Env./112.

Sample preparation drying

Lantana camara leaves were shed dried for a week and crushed in a home based grinder. The fine powder was used for extraction of crude drug using ethanol as a solvent by cold extraction technique [4, 5].

Cold extraction

100 g. of the plant material (fresh/dried) was put to extraction using ethanol as a solvent. This weighed plant materials, fresh/dried, was put into a well stoppered brown glass containers with 250 ml. solvent and allowed to stand at room temperature for a period of 3 days with frequent agitation until the soluble matter get dissolved. The extract thus obtained was decanted and filtered. The clear extract was concentrated using rotary vacuum evaporator.

The advantage of cold extraction is that temperatures do not rise high and therefore oxidation and free radical damage to the phytochemicals are kept at a minimum. The collected extract was then used for further phytochemical screening.

Investigative GC-MS studies

GC-MS is an important analytical technique used in knowing the unknown components of plant origin. GC-MS ionizes the individual compounds in the mixture according to mass numbers. Ionization though carried using method of Chemical Ionization (C.I.) and Electron Ionization (E.I.) but E.I. gives better results as far as quantitative screening is concerned with more selectivity avoiding interfering components. Mostly separation of volatile components in the test sample is possible using suitable column coated with non-polar, semi-polar and polar chemicals [6-9].

GC-MS instrumentation

The ethanolic extract was tested for active bioactive components by using GC-MS analytical technique. The study was done at SAIF Chandigarh and the set-up details are as under:

GC program

Column: TG 5MS (30m X 0.25mm, 0.25 μ m) Equipment: Trace 1300 GC
Carrier gas: Helium

Carrier flow: 1 ml /minute

Detector: Mass detector: MS TSQ 8000

Sample injected: 1.0 µl

Oven temperature program

60°C - 2.0 minutes hold

Up to 280°C at the rate of 10°C/minute - 10.0 minute hold

Injector temperature: 250°C

Total GC time: 31minute

MS program

Library used: NIST

Inlet line temperature: 280°C

Ion source temperature: 230°C

Electron energy: 70 eV

Mass scan: (m/z) 50 – 700

MS Time: 34 minute

Analysis protocol

The test sample with mixture of components was evaporated in the injection port of the GC equipment and separated in the column by adsorption whose temperature was controlled by temperature programme software. The components were eluted from the column according to boiling point of each of the segregated component [10-13].

The GC column was heated between 60- 280°C placed in an oven. The time for elution of individual components is termed as Retention time (RT) [14, 19].

Observation:

Table 1: Major components recorded in the ethanol extract of *Lantana camara*

SN	Library search compound	Molecular formula	Molecular weight	Retention time	% Area
1	2',6'-Dihydroxyacetophenone, bis(trimethylsilyl)ether	C ₁₄ H ₂₄ OSi ₂	281.1	5.99	6.74
2	Phthalic acid, butyl hept4ylester	C ₁₉ H ₂₈ O ₄	341.0	18.25	3.26
3	Astaxanthin	C ₄₀ H ₅₂ O ₄	477.1	24.54	3.64
4	13-Decosenamide	C₂₂H₄₃NO	320.3	25.16	21.85
5	1-Monolinoleoylglycerol trimethylsilyl ether	C ₂₇ H ₅₄ O ₄ SiO ₂	479.1	29.69	5.87
6	Glycine,N[(3à,5á,7à,12à)24oxo3,7,12-tris[(trimethylsilyl)oxy]cholan24yl],methyl ester	C ₃₆ H ₆₉ NOSi ₃	405.1	29.92	8.74
7	Betamethasone acetate	C ₂₄ H ₃₁ FO ₆	411.2	29.97	13.02

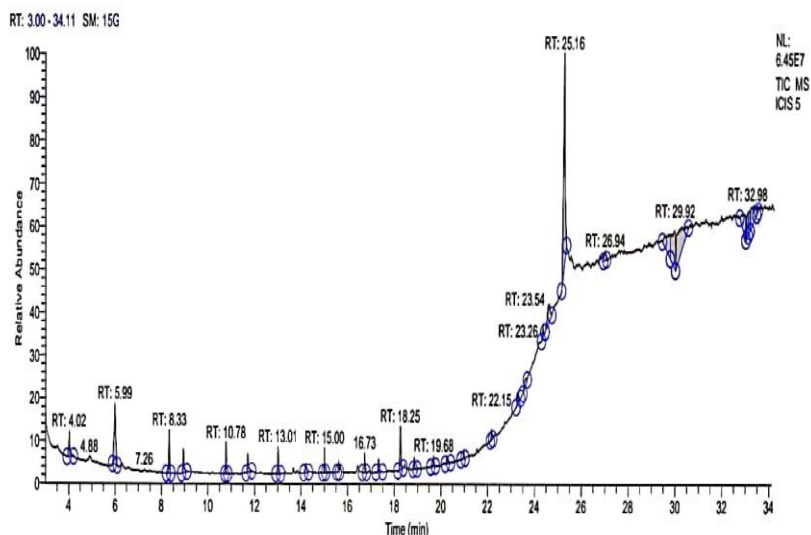


Figure 1: Chromatogram for ethanol extract of *Lantana camara*

The extracts subjected to GC-MS analysis were found to contain components as identified using standard data of NIST library by comparing the unknown compound data with the standard data of library of molecules. Retention time, molecular weight, molecular formula and percentage composition helps in identification and quantitative specification of components [14, 19]. The presence of various components in ethanol extract of *Lantana camara* as identified in GC-MS analysis with different retention times as illustrated in Table 1 and Figure 1.

Result and Discussion:

The ethanol extract of *Lantana camara* shows the presence of 7 major components viz. 2',6'-Dihydroxyacetophenone (**6.74%**), bis(trimethylsilyl)ether (**3.26%**), Phthalic acid, butylhept-4-yl ester (**3.26%**), Astaxanthin (**3.64%**), 13-(Z) Decosenamide (**21.85%**), 1-Monolinoleoylglycerol trimethylsilyl ether (**5.87%**), Glycine, N[(3 α ,5 α ,7 α ,12 α)24-oxo-3,7,12-tris[(trimethylsilyl) oxy]cholan-24-yl] (**8.74%**), Betamethasone acetate methyl ester (**13.02%**).

It was understood from the present study that the extract of *Lantana camara* contained many bioactive components as revealed by GC-MS analysis.

This study was only a preliminary one as the mere presence of any compound is not a sufficient for discovery of potent new drugs. . These findings of were good enough to reflect its importance as further studies with separation of individual components. This emphasizes on mechanism of action of extract/compound as carried out in reference of a particular diseases.

The serrated leaves of *Lantana camara* are aromatic and result has shown that its ethanol extract is composed of mainly monoterpenes, sesquiterpenes and triterpenes. Thirty-two probable compounds, representing 100% composition of extract were identified by GC-MS. Out of which seven components had shown more than 3% in constitution as represented in results.

Astaxanthin is a red pigment that belongs to the carotenoid family like β -carotene has been reported to have antioxidant activity [20].

13-(Z) Decosenamide is a fatty acid amide known to enhance neovascularization in regenerating skeletal muscle and modulate water balance in the visceral organs and in the cerebrospinal fluid [21]. This component has highest % area in the chromatogram which shows it forms the major part of the ethanolic extract. GC & MS of this component is shown in the Figure 2. and Figure 3. respectively.

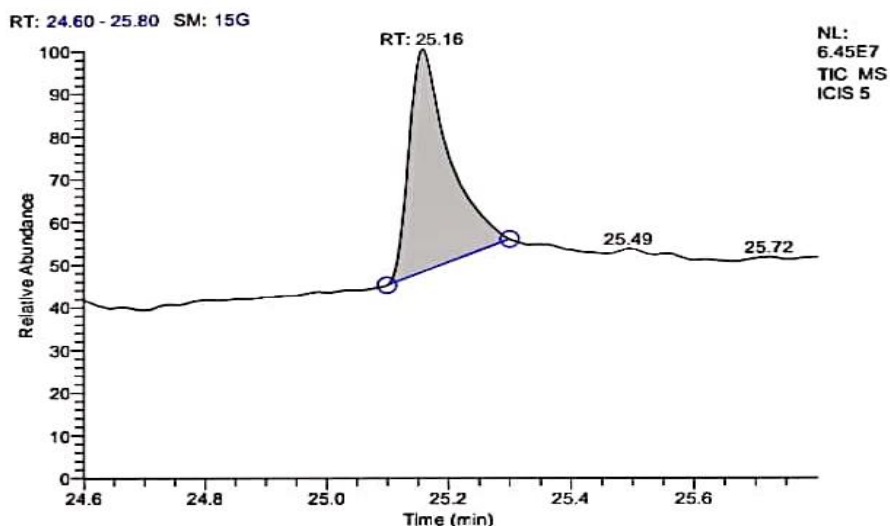


Figure 2: GC for 13-Decosenamide in ethanol extract of *Lantana camara*

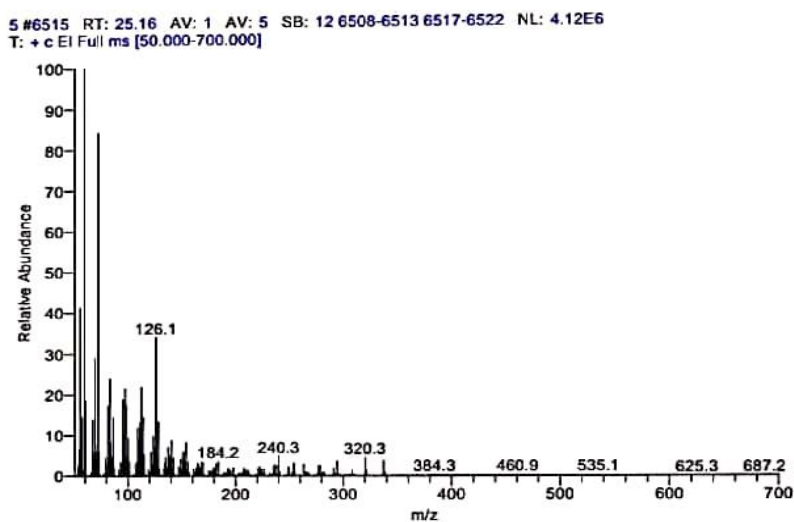


Figure 3: MS for 13-Decosenamide in ethanol extract of *Lantana camara*

Results from this study have shown that the essential oil of *Lantana camara* leaves contains compounds with proven pharmacological effects.

The continued demand for preparation of synthetic flavorings and fragrances for use in pharmaceutical, food, drink and cosmetic industries makes the essential oil of *Lantana camara* worth exploiting for use by relevant industries [3].

Conclusion:

GC-MS explores and promulgates the active principles in plants at the preliminary point of study to find active biomolecules such as flavonoids, terpenes which are thought to possess good anticancer properties. This may help to isolate individual bio-components which may proceed to hit upon a novel drug by evaluating pharmacological activity of that molecule. This study is only an initiating step in search of certain specific properties of ethanol extract and in-depth study will provide a good concrete base for all the biochemical and phytochemical functions mentioned above. New scientific strategies for the evaluation of natural products with specific biological activities require the implementation of large screening process.

Acknowledgement:

The author acknowledge Principal, Government Science College, Pardi for necessary laboratory facilitates and Mr. Pankaj Samual, Application Scientist, Saif Chandigarh for GC-MS analysis. I am also thankful to Dr. (Mrs.) Rani V. Choubey, taxonomist and visiting faculty Department of Environmental Sciences, Institute of Science; Nagpur for identifying the plant taxonomy.

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PIGMENTS IN NATURE: USES AND ITS HEALTH BENEFITS

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Biological pigments or Biochromes, are nothing but true pigments or colouring substances produced and expressed by the living organisms resulting from particular wavelength of colour absorption and reflection. Biological pigments include both plant and animal pigments. These are natural colouring matters found in plant or animal cells or tissues. Plant pigments present in plant parts like leaf, fruits, root and flowers. In animals many biological structures such as eyes feathers, fur, skin and hair contain pigments. In some species pigments develop for a certain period till the organ development, but in some it is expressed longer periods throughout an individual's life duration. Pigment colour is same at all sides, differs structural colour coming from organ thickness and selective reflection.

Plant pigments:

A plant pigment is any type of colored material synthesized by a plant. Plant pigments are unique complex chemical compounds which absorb light energy at visible ranges and convert it into chemical form of energy. These pigments give various colour to different organs of the plant body like leaves, flowers, fruits etc. and maintain important physiological mechanisms. The assimilation process, photosynthesis requires green coloured pigment called chlorophyll, absorbs radiant energy and converted into carbohydrate from carbon dioxide and water. This energy trapping process needs chiefly chlorophyll pigment along with other accessory pigments as carotenoids, phycobilins. Chlorophyll and carotenoids are organic solvent soluble pigments and phycobilins are water soluble pigments. Other essential pigments are flavonoids, phytochrome betalains, rhodospins and flavins, performs other important plant functions (Delgado-Vargas, 2000). All organisms on earth depend upon photosynthesis for food, either directly or indirectly. Various plant pigments are played a major role to control photoassimilation, growth and developmental processes in plants. Pigments produce bright visible colourations to attract agents such as insects, birds and other animals for cross pollination and promote seed dispersal when consumed their fruits. Pigments in plants protect them from harse effects of UV and Visible light (Tanaka *et al.*, 2008).

Chlorophylls:

Chlorophylls are green coloured pigments found in all photosynthetic organisms. These are important plant pigments present in the plastids, carry on photosynthesis in plants. Chlorophyll occurs in all groups of green plants, algae, cyanobacteria (BGA) and photosynthetic

bacteria. A molecule of chlorophyll consists of a porphyrin head and a phytol tail. Head is made up of tetrapyrrole rings with Mg present at center. This porphyrin head bears an alcohol component with 20 carbon atoms-the phytol tail, is hydrophobic in nature.

Chlorophylls are Chl-a, Chl-b, Chl-c, Chl-d and Chl-e. Chlorophyll a and b, both are predominantly present in angiosperms, exhibit different absorption spectra. These pigments are also the characteristics of algae and photosynthetic bacteria. Chlorophyll-a is common in higher plants, green algae, BGA and green bacteria-chloroxybacteria. Higher plants and green algae also have chlorophyll-b. Where chloroxybacteria also performs photosynthesis with chl-a, chl-b and carotenoids. Other marine brown algae and red algae have Chl-c or Chl-d. Chl-e mostly found in Xanthophyceae members (yellow-green algae). Among all these five types of chlorophyll pigments, chlorophyll a and b are considered as the primary photosynthetic pigments. In higher plants and algae, chlorophylls are located in the grana region of chloroplasts, the membrane bound cell organelles present in the cytoplasm of plant cells.

Carotenoids:

These are located in variously colour plastids called Chromoplasts, which may be yellow, orange and red, synthesized by algae, fungi, bacteria and plants. In plants, carotenoids occurs in all parts as in leaves, stems, roots, seeds, flowers, and fruits. Carotenoids play a measure role during light harvesting process of photosynthesis. Carotenoids are carotenes and xanthophylls. Carotene is orange-red in colour and xanthophylls are yellow in colour. Carrots (orange) and Tomatoes (red) owe their colour due to β - carotene and Lycopene respectively, are the best known carotenoids. All carotenoids are tetraterpene pigments, consist of 40 carbon atoms.

Carotenes are unsaturated hydrocarbons made up of carbon and hydrogen only. The most abundant carotenes are α and β carotene, β -carotene is the most common carotene. Carotenes are unsaturated hydro carbons made up of carbon and hydrogen. Xanthophylls are oxygen containing derivatives of carotene, have one or more oxygen atoms. The xanthophylls are lutein, zeaxanthin, neoxanthin, violaxanthin, where lutein is the most common. Xanthophylls are distributed more, than carotenes in living organisms.

Carotenoids shield the chlorophyll molecules against photo-oxidation, protect plants when over-exposed to sunlight, as well as trap solar energy of short wavelengths of light and transfer the same to chlorophyll-a, reaction centre during light phase of photosynthesis. Along with this carotenoids are antioxidants and plant hormone precursors, also involved in photomorphogenesis and development (Nisar *et al.*, 2015).

Phycobilins:

Water soluble photosynthetic pigments are Phycobilins. Phycobilins are the chromatophore moiety of bili protein. They are heat sensitive, chemically chlorophyll like but absence of phytol tail and Mg., not present in higher plants, but occur in red algae and the

cyanobacteria (blue green algae). The common phycobilins are phycocyanin (blue in colour) and phycoerythrin (red in colour).

Flavonoids:

Flavonoids are widely distributed plant pigments. These are water soluble, commonly occur in cytoplasm and vacuoles of plant cells. Flavonoids have 15-carbon skeleton with one heterocyclic and two phenyl rings. The best known flavonoids are the anthocyanins, flavonols, and flavones. Flavonoids are yellow coloured pigments, abundantly found in fruits like lemons, grapes, oranges, apples, cherries, tea leaves, soybean and in yellow flowers also.

Anthocyanins are flavonoid type of pigments, naturally occurs in all type of tissues of higher plants. This pigment provides colour to various parts as leaves, stem, roots, flowers and fruits, which gives a colour range of red, blue to purple basing on their pH. In acidic condition, some anthocyanins appear red, purple at neutral pH and the color changes to blue at alkaline pH gives its colour stability. Blueberries, cranberries, black berries, cherries, black rice, grapes, red cabbage and violet petals are rich in anthocyanins. Flavonoids in flowers and fruit provide visual cues for animal pollinators and seed dispersers to locate their targets.

Many flavones and flavonols strongly absorb UV radiation and form significant UV colour patterns on flowers, are the reason for insect pollination. Flavones and flavonols are in the leaves of many species, protect plants from harmful ultraviolet radiation of the sun.

Phytochrome:

Phytochrome is a blue light receptor plant pigment regulates plant vegetative growth and development, seed germination, pigment synthesis and initiation of flowering. They occur in two forms in plants as red absorbing Pr (660nm) and far-red absorbing Pfr (730nm) form. Phytochrome occurs in very low concentrations in plants, but not visible unless chemically purified.

Betalains:

The betalains are red-violet betacyanins and yellow-orange betaxanthins pigments. They are water-soluble and never co-occur in plants with anthocyanins, found abundantly in the class of Caryophyllales and leaves of some *Amaranthus* spp. Betalains are responsible for the deep red color of beets. Bougainvillea, certain cacti, and amaranth are also other examples of occurrence of betalains. Betalains in the flowers and fruits attract animals, so helps in pollination and dispersal of seeds.

Rhodopsin:

Rhodopsin pigment in many species of algae controls light-regulated movements as phototaxis and photokinesis. Rhodopsin is a purple, pigment-containing sensory protein that converts light into an electrical signal, found in organisms like some algae, fungi and bacteria.

Purple light-sensitive receptor proteins also involved in signalling and photo-response in a variety of organisms.

Flavins:

Flavins are orange-yellow pigments often associated with proteins, found in flavin mononucleotide (FMN) and flavin dinucleotide (FAD) forms. Some flavins control phototropism and various developmental responses in plants. Animals are dependent on plant sources for them. Flavin mononucleotide, a form of vitamin B2 quite useful for animals. Flavins are synthesized only by bacteria, yeasts, and green plants. Similar to phytochrome, flavins also occur in low concentrations and visible only when purified. Flavoproteins in plants are involved with a wide range of biological processes, including oxidation-reduction reactions during oxidative stress, photosynthesis and essential for cellular respiration etc.

Health benefits

Plant pigments have lots of health benefits so as used in human diets. According to Health line, "Chlorophyll plays an important role in making plants green and healthy. Human get benefit as these are the source of vitamins, antioxidants, and have some therapeutic properties to boost our immunity.. Chlorophyll is present in green vegetables, and some people take it as a health supplement. The potential benefits of chlorophyll include improving health, aids in weight loss, oxygenates our body, detoxifying the blood, reduces risk of cancer, helps in skin healing, boosting energy, and fighting illnesses.

When human take carrots or foods containing carotenoids, during digestion process liver splits the carotenoid into molecules of vitamin-A. Lycopene, anthocyanin, and betacyanins are natural red pigments of carotenoid group found in fruits, leaves and vegetables, possess antioxidant properties beneficial in sun protection, prevention of cancer and cardiovascular health. Many of these effects are shown to be related to the free-radical scavenging and antioxidant properties of anthocyanins, or to their ability to modulate the intracellular antioxidant systems.

Foods like strawberries, blueberries, walnuts, grapes, cabbage and cinnamon contain flavonoids, which lower the cholesterol levels and also possess some antioxidant properties. Phytochromes are the sole dichromic red/far-red photoreceptors actively regulate plant growth, development and flowering induction mechanism to maintain its nutritious value, indirectly pay the benefit to human health.

Betalains are phytonutrients found in plants, characteristics of Caryophyllales which produce yellow pigment used as food colorants and red color pigment to provide health benefits as antioxidant, anti-inflammatory and anticancer properties. (Leong *et al.*, 2018).

Interestingly, humans and many other animals use rhodopsin for their vision. Rhodopsin is the specialized photo receptor proteins present in the rods of retina provides vision even in low light.

Flavins are synthesized only in plants, for this reason, animals are dependent on plant sources for them, including riboflavin (vitamin B2) the most prevalent member of the group. FAD and FMN flavoproteins are the coenzymes involve in many energy producing metabolisms and synthesis of various biomolecules in the body. FAD protects hemoglobins from oxidative damage. Flavin mononucleotide protects from cataracts, sores in mouth and throat, reduce blood pressure, anemia, hair loss etc.

Animal pigments:

It is a natural colouring substance found in cells or tissues. The most common animal pigments are the heme of hemoglobin (red), carotenoids (yellow-orange), melanins (black or brown), and guanine (white and iridescent). The last three mostly resulted the surface coloration in most animals. Hemoglobin in blood cells is red because of iron in heme pigment needed to carry oxygen. Melanin is one of the only pigments that can be synthesized in the body of mammals. Other animals can't make many other pigments, whereas getting their colors through the food chain. Colour plays a multitude of roles in the natural world, used to entice, to camouflage, or to warn other creatures. Various colourations in animals may be with any combinations of structural coloration, chromatophores or bioluminescence. Animals directly produced pigments in visual coloured cells containing coloured materials and indirectly produced chromatophores, the special pigment containing and light-reflecting cells, may respond to hormonal or neuronal control mechanisms, mostly found in animal group like amphibians, fish, reptiles, crustaceans, and cephalopods. Chromatophores may change their sizes and allow redistribution of colours within the cells, also stimulated by visible light, UV radiation, pH, temperature, chemicals, etc., thus varying the colour and pattern in the animals. Chromatophores are classified into subclasses depend on their colours under white light as xanthophores (yellow), erythrophores (red), iridophores (iridescent), leucophores (white), melanophores (black/brown) and cyanophores (blue). Amphibians such as frog skin and the pattern of alternating golden and blue stripes on zebra fish is due to three major types of pigment cells, are xanthophores, melanophores, and iridophores.

Pigment coloration based on its chemical properties which viewed same at all angles. Structural colors produced interference effect with visible light from nano structures, sometimes in combination with pigments, producing different bright colors at different views. Most of structural colors showing range of pigment colors especially in birds and insects. Peacock tail feathers are colored through a combination of pigments, when light reflected off the feathers to create iridescence, occurred usually because of multilayer structures. Examples of colors arising

from iridescent and diffractive structures can be found in feathers, the blue/green gloss on the plumage of ducks, many butterflies and beetles colors are created by structural coloration.

Another coloration also because of bioluminescence occurs due to emission of light by living in organisms, results a chemical reaction within their body. Luciferins are a class of light emitting molecule found in these organisms, when oxidized by luciferase produce light. Bioluminescence occurs widely in marine vertebrates, invertebrates, algae, microorganisms including some fungi and bioluminescent bacteria, is mostly blue-green or green. In land animals such as fireflies and other beetles, the color is most commonly green or yellow, and sometimes red.

Hemoglobin:

Haemoglobin is the red pigment, an iron-containing protein that carries oxygen in the blood of many animals (vertebrates) and transports it to all the parts of the body. Each hemoglobin chemically with made up molecules of four heme groups and a protein globin, . Heme contains iron and gives a red color to the molecule. Red blood cells contain haemoglobin in it. Hemoglobin forms an unstable reversible bond with oxygen. In the oxygenated state, it gives a bright red color, called oxyhemoglobin and in the reduced state (oxygen released), it is deoxyhemoglobin, gives purplish blue color. So in cold-blooded animals, blood appears blue because of central copper atoms and then binds to oxygen.

Melanin:

Animals manufacture their own melanin pigment by the body and cannot make many other pigments. Melanin pigments are colored chemicals in animal tissues. It is the main pigment found in mammals; produce a huge range of colors. Melanin is responsible for most of the color (or lack of color) in their skin, hair, eye and fur. Naturally occurs red, brown, gray or black in color. Basically five types of melanins found in organisms, are eumelanin, pheomelanin, neuromelanin, allomelanin and pyomelanin. The most common type is eumelanin, of which may be brown or black. Melanin in their skin, eyes and fur is mostly to protect themselves against sunburn caused by ultraviolet light (Proctor and McGinness, 1986).

Carotenoids:

Carotenoids exhibit yellow, orange to red, are the most abundantly found pigments in nature, present in photosynthetic bacteria, archaea and fungi, algae, higher plants, and animals. Animals are incapable of synthesizing carotenoids de novo. Thus for animals, plant and plant products are the only source of carotenoids, either directly accumulated from food or modified them through metabolic reactions. These are the most important source of dietary vitamin A, also play major roles such as excess light radiation-protection(sunburn),immunity enhancers, beneficial antioxidants and signaling during reproduction (Maoka, 2020). Many animals use carotenoids in sexual signaling, social signaling, and parenting social signaling, and other

important specific roles as recognition, camouflage, mimicry, distraction, sexual selection, advertising, warning and crypsis. The deep red of a North American bird Cardinal and the bright pink feathers of a Flamingo, are all produced by color carotenoids from plant sources as food. Flamingo eats pink shrimps which do not synthesize carotenoids but derive their body colour from the feed microscopic red algae as diatoms and dinoflagellates (planktons). The carotenoid pigments of the tasty berries that the red Cardinal enjoys in the summer are laid down in the feather follicles. The wide variety of summer fruits and seeds that form the bird's diet provides the pigments in its vibrant plumage. Even same types of seed eaten by House Finch appear yellow, while Cardinal appear pink, as converts the pigment metabolically to a red pigment. However, carotenoids have not yet been detected in butterflies' wings.

Flavonoids:

Flavonoids are plant pigments that cannot be synthesized by Lepidoptera, an order of insects that includes butterflies and moths, but are consumed in the diet of the caterpillar. In almost all families of butterflies (Papilionoidea) white and yellow flavonoids have been detected.

Green and Blue colors:

Though it is believed that green coloration of many insects is due to chlorophyll taken from plant food. Caterpillars are often green in body color. A green coloration has also been observed in the hemolymph and integument of various insects. But the green color forms in combination of blue pigment with yellow pigments, thus the green is therefore resulted not due to chlorophyll pigments. While most animals are unable to make green and blue pigments, their green and blue colors are created through structural effects. Thus green and blue colors of bird feathers and insect carapaces are produced by structural effects not by pigments at all. Where most green colors in fishes, reptiles, amphibians, and birds are created by a reflection of blue light through an overlay of yellow pigment.

White/iridescent (Guanine) color:

White animals are often found in nature and sometimes thought as the effect of albinism. Albinism is a hereditary condition, occurs in mammals (including humans), fish, birds, reptiles, and amphibians. White peacocks and white lions that appear white, has been attributed to leucism, but do not have albinism effect. Leucism is a condition characterized by less pigmentation in animals causing white, pale or patchy coloration of the skin, hair, feathers, scales, or cuticles, but not the eyes. It affects all pigments, even the primary pigment melanin. The animals with leucism identified with having normal eye color, while animals with albinism have red eyes.

Iridescent colors generated by structural colorations, are the interaction of light with biological tissues that are microstructured surfaces to produce thin films interfere with visible light. Iridescence caused when an animal's color appears to change due to view or the angle of

illumination changes(Singh, 2014). Certain structural pigments in the animal's skin interfere with reflected light; causing different colors of light to scatter in every direction give a silvery luster view. The silvery luster is due to crystals of guanine, produced in the body as a waste product.

Guanine is one of the four bases common to DNA and RNA. Like melanin, guanine crystals are also formed from the metabolism of proteins. The crystals are deposited in the skin just closest to the adjacent muscle. Guanine crystals are rhombic platelets and stack of multiple transparent layers, but they have a high refractive index that partially reflects and transmits light from layer to layer, thus producing a pearly luster, provides a pearly iridescent effect, results spectacular structural colors.

Structural colors are iridescent colors created when light transmitted through multilayered tissues, depends on structure, shape, arrangement of the scales or tissues, or the chromatophores themselves. The silver sheen or silver-iridescence in the scales and eyes of fishes comes from guanine crystals. Iridescent coloration found in animals, including the sparkling elytra of beetles, the shimmering scales of butterfly wings and the flashing gorgets of hummingbirds.

Conclusion:

Pigmentation occurs in nearly all living organisms. Almost all plants synthesize their own pigments for their metabolisms, protective against climate change and stresses, which having various human health benefits, where heterotrophic animals getting these pigments from plants directly as food or few they synthesize themselves, require for maintaining their metabolism, maintenance and life events.

Plant pigment phytochemicals or bioactive compounds play a vital role in restoring human health. Generally the colour of a food can influence our attention towards health and corresponding nutrition. Studies has reported that people who eat plenty of fruits and green vegetables, particularly deeply pigmented varieties, have less heart ailments, cancer, diabetes, arthritis, osteoporosis, and neurological disorders. Phytochemicals, which are naturally occurring compounds present in plant foods, have various health benefits. Particularly, plant originated pigments like carotenoids and flavonoids, are the most valuable phytochemicals for their antioxidant functions as well as potential preventive medicinal benefits.

Color also plays most important and effective roles in the natural animal world, used to entice, to camouflage, or to warn other creatures by performing physical protection, distraction, temperature regulation, mimicry, sexual selection and signaling (Mallet, 2001). The function and adaptive usefulness of pigment patterns in animals are generally considered as a means of avoiding predators, maintaining body metabolism and signaling to select mates, though the reasoning behind the colorations is basically unique to each species. Hence, pigment formation and pattern development will therefore be an exciting subject in the field of biology. In spite of

their precious occurrence in plant and animal life showing adaptation, adjustment and diversity in organisms that inhabit our planet earth.

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PHYTOPHARMACOLOGICAL REVIEW ON *GARDENIA GUMMIFERA*

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Abstract:

Gardenia gummifera L.f. is a deciduous tree belongs to the Rubiaceae family. It is commonly known as gummy gardenia. This plant is claimed to have numerous medicinal properties possessing astringent and carminative properties that are used in the management of dyspepsia and hemorrhoid. It is also useful in the treatment of flatulence for cleaning foul ulcers and wounds, anticonvulsants. The present review focuses on the phytochemical constituents and pharmacological properties of *Gardenia gummifera*.

Keywords: *Gardenia gummifera*, phytochemical constituents, pharmacological properties

Introduction:

The usage of plants as medicines throughout the past 60,000 years has been thoroughly documented in the scientific literature. For their well-being, millions of people still rely on medicinal plants today. In the tropics, medicinal plants are frequently utilised on a regular basis in rural areas where access to medicines is difficult or non-existent. In contrast, in westernised countries, medicinal herbs are often employed as a complement or alternative to conventional medicine. Medicinal plants are significant for people, not only as a primary source of medicines but also as phytochemical building blocks for development of new drugs.

Taxonomy:

Kingdom	:	Plantae
Subkingdom	:	Viridiplantae
Infrakingdom	:	Streptophyta
Superdivision	:	Embryophyta
Division	:	Tracheophyta
Subdivision	:	Spermatophytina
Order	:	Gentianales
Family	:	Rubiaceae
Genus	:	Gardenia
Species	:	<i>Gardenia gummifera</i>

Vernacular names:

- Common name: Gummy Gardenia, Cambi gum tree
- Gujarati: Dikamalli

- Hindi: Dekamali
- Kannada: Bizhke, Bilke, Bikke, Kadu Bikke
- Malayalam: Kambimaram
- Marathi: Dikemali
- Sanskrit: Nadihingu
- Tamil: Sirukkambil
- Telugu: Chittamali

Distribution and Pharmacognosy:

The plant is a small tree or shrub and grows in rocky areas in slightly undulating terrain along with other species. It occurs in specific habitats having well drained shallow soil with exposed rocks at places usually on plateaus, which are not too dry. A large shrub with white bark, sub-sessile shining simple leaves which are apposite and decussately arranged. Leaf size is 3 to 8 cm long, and 12 to 16 pairs of secondary nerves. Flowers are large (2 to 3 cm) with white fragrant. Fruit is ovoid (2 to 4 cm) with fleshy mesocarp, and is edible. Flowering occurs during June to July. The plant flowers every year during onset of monsoon rains. However sporadic flowering takes place during other months of the year. Fruiting is during August to October. The fruiting medium has 20 to 40 fruits per bush. Seeding is medium in extent. Some fruits dry prematurely owing to very hot and dry weather conditions. The regeneration is usually by seeds, which are spread by birds that feed on the fruits. Sometimes mature fruits fall on the ground releasing seeds, which germinate under favorable conditions. Growth is slow. In the study area most of the plants were in bushy form.



**Figure 1: *Gardenia gummifera*
Linn. plant**



**Figure 1.2. *Gardenia gummifera* Linn. f.
plant with flower and fruit**

Phytochemistry:

A number of flavanoids such as Gardenin A, B, C, D & E were isolated from Dikamali in the past (Krishnamurthi *et al.*, 1971; Chhabra *et al.*, 1977). The gum yielded flavones, including gardenin, de-Me-tangeretin and nevadensin; wogonins, isoscutellarein, apigenin and de-MeO-sudachitin (Khare, 2007; Warriar, 1995; Mir *et al.*, 2010). Recently, a number of new cycloartanes Dikamaliartane - A, B, C, D, E & F and Gardenin E were reported from this source, of which Dikamaliartane-A is the main cycloartane (Kunert *et al.*, 2009). Oleanoic aldehyde, sitosterol, D-mannitol, erythrodiol and 19-hydroxyerythrodiol were isolated and characterized from *G. gummifera* stem bark (Reddy, 1977). Plant also contains Acerosin, Apigenin, 3',4'-Dihydroxywogonin, 3',4'-Dimethoxywogonin, Gardenin A, B, E, 4'-Hydroxywogonin, 3-O-Methylkaempferol, Nevadensin, 5,7,3',4'-Tetrahydroxy-6,8- dimethoxyflavone, 5,7,3',5'-Tetrahydroxy-8,4'- dimethoxyflavone, 5,7,4'-Trihydroxy-6,8- dimethoxyflavone, 3',4',5'-Trihydroxywogonin, 3',4',5'-Trimethoxywogonin, Hexacosyl-p- coumarate, mixture of long chain (C22-C26) esters, Dikamaliartane-A

Pharmacological properties:

Hepatoprotective effects:

The study showed that fruit methanolic extract of *Gardenia gummifera* L.f. exhibited potential protective action against the hepatotoxicity induced by CCL4. The extract showed significant antioxidant activity in DPPH and Nitric oxide radical scavenging. Quantitative phytochemical assay determines the presence of alkaloids and phenolics. GC-MS analysis of aromatic extract resulted in 36 compounds. Among them, compounds 2, 3-Dihydro-3,5-dihydroxy-6-methyl-4 h-pyran-4-one, 2-furancarboxaldehyde 5-(hydroxymethyl) and Quinic acid are the major ones. The fruit methanol extract showed significant in vivo hepatoprotective activity by altering the levels of liver function biochemical parameters. Histology of the liver section also confirms the hepatoprotective activity of *Gardenia gummifera* L.f. fruit methanol extract . Molecular docking of GC-MS profiled phytochemicals with the target protein TGF- β 1 and PPAR α also confirmed the therapeutic effect with good hydrogen bonding and hydrophobic interactions. The hepatoprotective role of *Gardenia gummifera* L.f. fruit methanol extract might be due to the presence of chemical constituents like phenolic compounds and alkaloids. These phytochemicals may offer antioxidant activity and thus preventing the oxidative stress-induced in the liver. Hence, GFME may act as a prophylactic as well as a curative drug in treating hepatotoxic conditions.

Anti-ulcer effects:

The effect of anti-ulcer and anti oxidant activity of methanolic extract of *Gardenia gummifera* (MEGG) whole plant in ulcer induced rats and in-vitro anti-oxidants method respectively was evaluated. The efficacy of methanolic extract of *Gardenia gummifera* was

evaluated against aspirin-induced ulcers in rats. *G. gummifera* at doses of 150 mg/kg and 300 mg/kg was administered orally once daily for 6 days. Results showed that extract treatments prevented ulcer area and gastric secretion in a dose-dependent manner. Administration of 2000 mg/kg extract did not show any toxicity in albino mice. In preliminary phytochemical studies identified the presence of flavonoids in the methanolic extract of *Gardenia gummifera*. According to the results, it was concluded that the whole plant of *G. gummifera* has significant antiulcer activity due to the presence of potent antioxidants like flavonoids.

Antibacterial activity:

The antibacterial efficiency of the extracts were screened by agar well diffusion method against human pathogens viz, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Vibrio cholera*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Klebsiella pneumoniae*. Ethanol stem bark extract showed the pronounced antibacterial effect with 12.67 ± 0.33 mm and 12.33 ± 0.33 mm inhibition zone against *S. typhi* and *S. aureus* respectively and leaf extract showed 12.33 ± 0.33 mm and 12.83 ± 0.60 mm inhibition zone against *S. typhi* and *S. aureus* respectively. Leaves ethanol extract exhibited highest inhibitory activity against *S. aureus* with MIC value of 8.33 μ g. The obtained results have supported the traditional claim of *Gardenia gummifera* stem bark and leaves to control microbial infection.

Diuretic effect:

The diuretic activity of ethanolic extract of aerial parts of *Gardenia gummifera*. Lipschitz test in Wistar rats was used to evaluate diuretic effect of ethanolic extract of *Gardenia gummifera* with furosemide as a standard and normal saline as control. The urine volume (in mL) measured at 5 h. The urine volume and urinary electrolyte excretion (Na^+ and K^+) were found to be significantly higher in rats treated with *Gardenia gummifera* as compared to normal rats. This present study indicates that Ethanolic extract of aerial parts of *Gardenia gummifera* has potential diuretic and naturetic property.

Antiradical and insecticidal potential:

The antimicrobial, antiradical and insecticidal potential of leaf and fruit of *Gardenia gummifera* L. f. (Rubiaceae) was investigated. The leaf and fruits were shade dried, powdered and extracted by maceration process using methanol. Antibacterial activity was evaluated against Gram positive and Gram negative bacteria by Agar well diffusion assay. Antifungal activity was determined against six seed-borne fungi by Poisoned food technique. Antiradical activity of leaf and fruit extracts was evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2-azinobis 3-ethylbenzothiazoline 6-sulfonate (ABTS) radical scavenging assays. Insecticidal activity of leaf and fruit extracts, in terms of larvicidal and pupicidal activity, was assessed against larvae and pupae of *Aedes aegypti*. Both the extracts inhibited all test bacteria. Marked antibacterial activity was displayed by fruit extract when compared to leaf extract. *S. epidermidis* and *E. coli* were

inhibited to highest and least extent by both extracts respectively. Fruit extract was found to exhibit higher antifungal effect when compared to leaf extract. Leaf extract and fruit extract exhibited highest inhibitory activity against *A. niger* and *A. flavus* respectively. Leaf and fruit extracts scavenged DPPH radical's dose dependently with an IC₅₀ value of 49.01 µg/ml and 2.53 µg/ml respectively. The scavenging of ABTS by leaf and fruit extracts was dose dependent and the IC₅₀ value for leaf and fruit extract was 2.58 µg/ml and 2.31 µg/ml respectively. Fruit extract was shown to exhibit marked antiradical activity when compared to leaf extract. Leaf and fruit extracts exhibited dose dependent insecticidal activity in terms of larvicidal and pupicidal activity and the susceptibility of larvae and pupae to extracts was in the order II instar larvae>IV instar larvae>pupae. Fruit extract displayed marked insecticidal potential when compared to leaf extract. Overall, fruit extract of *G. gummifera* exhibited marked antimicrobial, antiradical and insecticidal activity when compared to leaf extract. The plant can be used for developing agents/formulations effective against infectious microorganisms, oxidative stress and insect vectors that transmit dreadful diseases. The observed bioactivities could be ascribed to the presence of active principles which are to be isolated and characterized.

Cytotoxic activities:

Both the plant leaves were extracted by ethanol, extracts were subjected to cytotoxic activity study against MCF-7 cell lines using the MTT assay. MTT assay was the technique utilized for cell survival determination. Measurements were performed and the concentration required for a 50% inhibition of viability (CTC₅₀) was determined graphically. The concentration of ethanolic extract yields the value of CTC₅₀ as 170.00±2.0 µg/ml and 200.00±1.6 µg/ml respectively for *Gardenia latifolia* Ait. and *Gardenia gummifera* Linn. The results showed that the *Gardenia latifolia* Ait. had potential cytotoxicity than the *Gardenia gummifera* Linn. against MCF-7 cell lines.

Insecticide:

The study was carried out to investigate antimicrobial, antiradical and insecticidal potential of leaf and fruit of *Gardenia gummifera* L. f. (Rubiaceae). The leaf and fruits were shade dried, powdered and extracted by maceration process using methanol. Antibacterial activity was evaluated against Gram positive and Gram-negative bacteria by Agar well diffusion assay. Antifungal activity was determined against six seed-borne fungi by Poisoned food technique. Antiradical activity of leaf and fruit extracts was evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2-azino-bis 3-ethylbenzothiazoline 6-sulfonate (ABTS) radical scavenging assays. Insecticidal activity of leaf and fruit extracts, in terms of larvicidal and pupicidal activity, was assessed against larvae and pupae of *Aedes aegypti*. Both the extracts inhibited all test bacteria. Marked antibacterial activity was displayed by fruit extract when compared to leaf extract. *S. epidermidis* and *E. coli* were inhibited to highest and least extent by

both extracts respectively. Fruit extract was found to exhibit higher antifungal effect when compared to leaf extract. Leaf extract and fruit extract exhibited highest inhibitory activity against *A. niger* and *A. flavus* respectively. Leaf and fruit extracts scavenged DPPH radical's dose dependently with an IC₅₀ value of 49.01 µg/ml and 2.53 µg/ml respectively. The scavenging of ABTS by leaf and fruit extracts was dose dependent and the IC₅₀ value for leaf and fruit extract was 2.58 µg/ml and 2.31 µg/ml respectively. Fruit extract was shown to exhibit marked antiradical activity when compared to leaf extract. Leaf and fruit extracts exhibited dose dependent insecticidal activity in terms of larvicidal and pupicidal activity and the susceptibility of larvae and pupae to extracts was in the order II instar larvae>IV instar larvae>pupae. Fruit extract displayed marked insecticidal potential when compared to leaf extract. Overall, fruit extract of *G. gummifera* exhibited marked antimicrobial, antiradical and insecticidal activity when compared to leaf extract. The plant can be used for developing agents/formulations effective against infectious microorganisms, oxidative stress and insect vectors that transmit dreadful diseases. The observed bioactivities could be ascribed to the presence of active principles which are to be isolated and characterized.

Antihyperlipidemic activity:

Ethanollic extract of twigs and gums of *Gardenia gummifera* (EEGG) was investigated for antihyperlipidemic activity. It was evaluated via in vivo model i.e. Poloxamer-407 induced hyperlipidemia and suspension of Cholesterol + cholic acid induced hyperlipidemia. Poloxamer-407 is an acute model used for determination of preventive antihyperlipidemic properties of EEGG and determination of most efficacious dose i.e. EEGG (250mg/kg), which further used as treatment dose for chronic model (cholesterol +cholic suspension induced hyperlipidemia model). In Poloxamer-407 induced hyperlipidemia models, blood samples were withdrawn at 48th hrs and serum lipid levels and atherogenic index were analyzed. EEGG exhibited significant inhibition of serum TC and LDL levels while as atherogenic coefficient decreased significantly. Effective dose obtained from Poloxamer-407 induced hyperlipidemia model also demonstrated significant inhibition in serum lipid levels (TC and LDL) and atherogenic index (AI,CRR and CR). EEGG also significantly increased serum HDL levels when blood samples were analyzed at 28th day. Liver enzyme assays and Histopathological showed enough evidence to prove its hypolipidemic properties.

Antioxidant activity:

Preventive and curative effect of methanolic extract of *Gardenia gummifera* Linn. f. on thioacetamide induced oxidative stress in rats. To evaluate the antioxidant and antihepatotoxic effect of methanolic extract of *Gardenia gummifera* Linn. f. root (MEGG) on thioacetamide (TAA) induced oxidative stress in male Wistar rats. Methods: In the preventive study, rats were administered with 125 and 250 mg/ kg of MEGG for 9 days prior to TAA administration (100

mg/kg s.c.). In post-treatment groups, rats were treated with MEGG at doses of 125 and 250 mg/kg, 2, 24 and 48 h after TAA intoxication. Silymarin was used as a standard drug control (100 mg/kg). Hepatotoxicity was assessed by quantifying the serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH). The antioxidant potential of MEGG was evaluated by the estimation of catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GST), reduced glutathione (GSH) and lipid peroxidation thiobarbituric acid reactive substances (TBARS)] in hepatic and renal tissues. Histopathological changes were also evaluated. MEGG significantly prevented the elevation of serum AST, ALT, ALP, LDH and tissue malondialdehyde levels in both experimental groups, when compared to the TAA alone treated groups. The rats receiving TAA plus MEGG exhibited significant increases in hepatic and renal antioxidant activities including GSH, GST, GR, GPx and CAT levels. Quantification of histopathological changes also supported the dose dependent protective effects of MEGG. These observations suggest that MEGG has dose dependent hepatoprotective and antioxidant effect against TAA induced oxidative stress

Anti-inflammatory and wound healing activity:

Gardenia gummifera L.f is a medicinal plant that belongs to the Rubiaceae family. The plant is well known for its traditional practice. The current investigation was to evaluate the wound healing and anti-inflammatory capabilities of *Gardenia gummifera* fruit methanol extract (GFME) and *Gardenia gummifera* stem bark methanol extract (GSME). The acute dermal toxicity, wound healing activity of excision, and incision in rat models were evaluated. Wound contraction and epithelialization periods were measured by the excision model and tensile strength was evaluated from the incision model. Similarly, denaturation of protein, human red blood cell (HRBC) membrane stabilization, proteinase inhibitory action was evaluated for the inflammatory action. In vivo, acute dermal toxicity of extracts did not show any toxicity. GFME and GSME treated wounds showed effective healing capability as proven by increased wound contraction and less epithelialization period and higher tensile strength. The anti-inflammatory studies revealed that GFME and GSME had significant anti-inflammatory capacity. The outcome of this present study displayed remarkable wound healing and anti-inflammatory action.

Neuropharmacological activities:

The biological activities of Dikamaliartane-A, a cycloartane isolated from gum resin Dikamali of *Gardenia gummifera*/Gardenia lucida was screened for some pharmacological actions. The study was carried out using albino mice (20-25gr). It reduced locomotor activity and potentiated pentobarbitone-induced sleeping time in mice indicating Central Nervous System depressant activity. It protected mice from strychnine and electro shock-induced convulsions indicating that it has anti-convulsant activity. All these activities were statistically significant.

The LD50 (Lethal Dose) was carried out in mice according to Organization for Environmental Cooperation and Development (OECD) Guidelines 423. The LD50 was tested in three mice for each dose with doses from 5mg/kg, 50mg/kg, 300mg/kg and 2000mg/kg. The LD50 was found to be 500mg/kg.

Cardioprotective effect:

The cardioprotective effect of methanolic extract of *Gardenia gummifera* root (MEGG) on Isoproterenol (ISO) induced myocardial infarction (MI) in rats was done. LCMS analysis of MEGG was done for the identification of cardioprotective constituents. Myocardial infarction was induced by the subcutaneous injection of ISO (6mg/100g body weight) at an interval of 24 h for 2 days. MEGG (125 and 250 mg/kg, p.o) was given to rats once daily for 45 days before the ISO challenge. The myocardial damage was assessed by quantifying the serum levels of cardiac marker enzymes (LDH, AST, ALT, CK-MB), serum iron and iron binding capacity, uric acid, and ceruloplasmin. Antioxidants such as catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GST) and reduced glutathione (GSH) were altered in MI rats. The level of lipid peroxidation was measured as malondialdehyde (MDA), triphenyl tetrazolium chloride (TTC) test, and quantification of histopathological changes also supported the dose-dependent protective effects of MEGG. MEGG significantly ($p \leq 0.05$) protected the above-mentioned parameters from falling from normal levels. LCMS analysis of MEGG revealed the presence of cardioprotective constituents such as erythrodiol, lupeol, epicatechin, β - sitosterol, asiatic acid, myricetin, oleanolic aldehyde, vernolic acid, chlorogenic acid, and dicaffeoylquinic acid. Results suggest that MEGG affords a dose dependant cardioprotection against isoproterenol-induced myocardial infarction.

Uses:

Resin from the leaf buds is used in healing wounds, indigestion, gas trouble, ulcer and cardiac problems. The resin is acrid, bitter, thermogenic, cardiotonic, antioxidant, antihyperlipidemic, carminative, antispasmodic, stimulant, diaphoretic, antihelminthic, antiseptic and expectorant. It is traditionally used in conditions of cardiac debility, obesity, lipolytic disorders, bronchitis, dyspepsia, flatulence for cleaning foul ulcers and wounds, neuropathy, splenomegaly and is given to children in nervous disorders and diarrhoea due to dentition. It is also used in veterinary practice to keep off flies from wounds. Paste obtained from the bark is used as an antiplasmodic and expectorant.

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EXOENZYME PRODUCTION BY BACTERIA ISOLATED AND IDENTIFIED FROM INDUSTRIAL WASTE

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Abstract:

Bacteria producing exoenzymes are of great importance considering their contribution to the host metabolism as well as for their various applications in industrial bioprocesses. In this work bacteria were isolated from the fly ash samples and physicochemical parameters of fly ash were measured by standard protocols. Morphologically five different colonies were isolated and taken for biochemical identification. Based on the results of phenotypic and biochemical tests, the strains were identified as *Bacillus sp*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Pseudomonas putida* and *Staphylococcus aureus*. While screening for exo-enzyme production all the isolates were found to be effective in production of exoenzymes.

Keywords: Fly ash, bacteria, characterization, exoenzyme production

Introduction:

The increasing use of fossil fuels has raised concerns about possible deleterious health effects of the final combustion product. Fly ash, also known as flue-ash, is one of the residues generated in combustion, and comprises the fine particles that rise with the flue gases and depending upon the source and makeup of the coal being burned, the components of fly ash vary considerably, includes substantial amounts of silicon dioxide (SiO₂) (both amorphous and crystalline) and calcium oxide (CaO) and other elements include arsenic, beryllium, boron, cadmium, chromium, hexavalentchromium, cobalt, lead, manganese, mercury, molybdenum, selenium, strontium, thallium, and vanadium, along with dioxins and PAH compounds, which contaminants ground and surface through metal leaching in and around fly ash settling ponds (Banker *et al.*, 2012). Microbes are interminable throughout the atmosphere. Numerous bacteria had been identified so far but those accounts only 2% of the total Bacteria present in the atmosphere. Microorganisms survive in contaminated habitat because they are metabolically capable of utilizing its resources and can occupy a suitable niche and contaminants are often potential energy sources for microorganisms (Ilyina *et al.*, 2003). The impacts of elevated heavy metal levels on the size and activity of natural soil microbial communities have been well

documented (Sharma and Kalra, 2006). Moreover, based on the previous results the effect of soil fertilization with fly ash has been quite explored. The microbes are the important elements of the soil environment as they participate in the degradation of the organic matter and make the nutrients available to other soil organisms. This favors the formation of soil aggregates and immobilizes the heavy metals and stimulate the activity of soil enzymes viz., dehydrogenase, urease and phosphatases etc., (Pati and Sahu, 2004). Furthermore, there are no such biomacromolecules in fly ash and the presence of microorganisms in the fly ash using some ingredients as sole source which has be dealt separately as a study. Keeping in view, the present study was aimed to isolate the bacteria from the fly to check their multi enzyme production.

Materials and Methods:

Collection of sample

The fly ash Samples were collected from NLC (Neyveli Lignite Corporation Limited), Neyveli, Tamil Nadu, India.

Physico chemical parameters of fly ash

The Physico chemical parameters of fly ash were measured by following the standard protocols (Yeledhalli *et al.*, 2007; Mlitan *et al.*, 2013).

Isolation and identification of bacteria from fly ash

1 ml of processed sample was taken and serially diluted up to 10⁻⁵ and 0.1 ml sample from respective dilutions were placed on nutrient agar plates and the plates were incubated for 24- 48 hours at 37°C. After the incubation period the colonies with different morphology were streaked sub cultured on nutrient agar plates for further studies. The phenotypic and biochemical tests were performed to identify the strains, according to the Bergey's Manual of Systematic Bacteriology (1984) guidelines.

Exo-Enzyme analysis

Starch hydrolysis

Nutrient starch agar plates were prepared and sterilized. Then the medium was poured in to plates. After solidification of medium the culture was streaked on a single line and incubated. A positive test is indicated by the clearance of the medium around the Colonies, which was further visualized by addition of Lugol's iodine.

Casein hydrolysis

Skim milk agar plates were prepared and sterilized. The plates were inoculated with the Actinomycetes isolate in a single line and incubated at 30° C for 72 hours. The zone of clearance around the colonies indicated a positive result.

Gelatin test

Nutrient gelatin agar plates were prepared and sterilized. Then the medium was poured into plates. After solidifying the agar was streaked on a single line Actinomycetes isolate and

incubated. A positive test was indicated by the clearance of the medium around the colonies. This was further visualized by flooding cultures with acidified HgCl_2

Lipid hydrolysis

Spirit blue agar was prepared and tributrin was added as the substrate for lipase activity. The substrate mixture was homogenized in the magnetic thermal stirrer and sterilized. The medium was then inoculated with the Actinomycetes isolate in a zigzag manner and incubated. A positive lipase activity was determined by the reaction of dye round the colonies and on further incubation a zone of clearance around the colonies with dye concentrated around the colonies.

Lecithinase test

Preparation of agar plates: The basal medium was autoclaved in batches of 95 ml before poured into plates 5 ml of egg yolk emulsion was added to each batch. Test: Agar plates were inoculated spot wise with 3 or 4 organisms. After 3, 5, and 8 days, the plates were checked in transmitted light for the occurrence of opaque zones around the colonies these may have more than 10nm; the precipitated vary considerably in strength lipase activity was indicated by the formation of a poorly layer in LV-positive cultures mostly at the edge of the opaque zone, in LV negative cultures around the colony. A strong lipase reaction was difficult to distinguish from a weak LV reaction.

Pectinase assay

400 ml of dissolved agar powder was taken and sterilized. Pectin and yeast extract in another 100ml of distilled water was prepared. 500ml of mineral salt solution was dissolved and divided into 2 equal parts and set up at the pH of 5 and 7 of both the solutions were autoclaved at 121°C for 15mins. After sterilization inoculated the organisms and incubated the plates at room temperature for 5 days. After incubation, the plates were flooded with 1% aqueous solution of hexadecyltrimethyl ammonium bromide (Sigma).

Results and Discussions:

The Physico-chemical parameter of the collected fly ash samples was found be, pH (9.6), Electrical Conductivity (EC (dSm-1) (8.6), Total Nitrogen (%) (0.002), Available Phosphorous ($\mu\text{g g}^{-1}$) (0.75), Organic Carbon (%) (0.566), Zinc (82), Fe (4150), Ni (204), Mn (70), Cu (58.6), Cd, (42.3), Pb (40.1), B (29.0), Al (4615), Si (5600) (Table : 1). Morphologically five different colonies were isolated and taken for biochemical identification. Based on the results of phenotypic and biochemical tests, the strains were identified as *Bacillus sp*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Pseudomonas putida* and *Staphylococcus aureus* (Table 2). The microbes are the important elements of the soil environment as they participate in the degradation of the organic matter and make the nutrients available to other soil organisms. (Tiwari *et al.*, 2008). This favors the formation of soil aggregates and immobilizes the heavy metals and stimulate the activity of soil enzymes viz., dehydrogenase, urease and phosphatases

etc., (Pati and Sahu, 2004). A great amount of elements (C, K, Ca, Mg, Cu, Zn and Mn) get into the soil as a result of ash used at different doses and may probably change the chemical as well as physicochemical soil properties which intern may determine the biological properties irrespective of the crop (Yelledhalli *et al.*, 2007).

Bacteria are known to excrete protons, organic acids, enzymes and siderophores to enhance the mobilization of metals which boosted the phytoextraction of metals from fly ash (Kumari and Singh, 2011). Though, the isolates were subjected to analysis on the production of exoenzymes such as Amylase (Starch hydrolysis), Gelatinase (Gelatin hydrolysis) Lipase (Lipid hydrolysis) a Protease called caseinase (Casein hydrolysis) pectinase (Pectin hydrolysis). Though all the isolates were found to be effective exoenzyme producers, their role in the transformation and or degradation of fly ash is negligible (Table: 3). Because, there are no such biomacromolecules such as carbohydrate, protein, lipid etc., in fly ash But the presence of these organisms shows that they are able survey in the fly ash using some ingredients as sole source which has be dealt separately as a study.

The presence of these organisms' shows that they are able survey in the fly ash using some ingredients as sole source which has be dealt separately as a study. Keeping in view, the survival of these species suggest the detoxification potential and so is suggested to attain positive interactions at the landfill remediation i.e. one species showing lesser tolerance might help reduce the future growth limiting factor or enhance ecological interactions forming a self-sufficient stable ecosystem. As a future studies, the focus should be shifted more on anaerobic bacteria and special enzymes reacting to metallic and mineral compounds.

Table 1: Physicochemical characteristics of fly ash

Sr. No.	Physicochemical parameters	
1	pH	9.6 ± 0.42
2	Electrical Conductivity EC (dSm-1)	8.6 ± 0.38
3	Total Nitrogen (%)	0.002 ± 0.001
4	Available Phosphorous ($\mu\text{gg-1}$)	0.75 ± 0.006
5	Organic Carbon (%)	0.566 ± 0.059
6	Zinc ($\mu\text{gg-1}$)	82 ± 3.1
7	Fe($\mu\text{gg-1}$)	4150 ± 207
8	Ni ($\mu\text{gg-1}$)	204 ± 10.2
9	Mn ($\mu\text{gg-1}$)	70 ± 3.4
10	Cu ($\mu\text{gg-1}$)	58.6 ± 2.38
11	Cd ($\mu\text{gg-1}$)	42.3 ± 2.12
12	Pb ($\mu\text{gg-1}$)	40.1 ± 2.00
13	B ($\mu\text{gg-1}$)	29.0 ± 1.26
14	Al ($\mu\text{gg-1}$)	4615 ± 230
15	Si ($\mu\text{gg-1}$)	5600 ± 280

Table 2: Biochemical characteristics of the isolates

Biochemical tests	<i>Bacillus sp</i>	<i>Pseudomonas aeruginosa</i>	<i>Pseudomonas fluorescens</i>	<i>Pseudomonas putida</i>	<i>Staphylococcus aureus</i>
Gram's staining	Gram positive rods in chains	Gram Negative rods	Gram Negative rods	Gram Negative rods	Gram positive cocci in bunches
Motility	Motile	Motile	Motile	Motile	Non-Motile
Spore staining	Endospore	-	-	-	-
Nutrient Agar	White colour rhizoidal colony	Large colour colony with diffused pigmentation	Large opaque, irregular colony with diffused pigmentation	Small opaque, irregular colony with diffused pigmentation	Golden yellow colonies
MacConkey agar	Large, irregular pale pink colour colonies	Non-Lactose fermenting colonies	Non-Lactose fermenting colonies	Non-Lactose fermenting colonies	Lactose fermenting colonies
Blood agar	Haemolytic colonies	Beta haemolytic colonies	Beta haemolytic colonies	Beta haemolytic colonies	Golden yellow colonies
Catalase	Positive	Positive	Positive	Positive	Positive
Oxidase	Negative	Positive	Positive	Positive	Negative
Coagulase	-	-	Not done	Not done	Positive
Indole	-	Negative	Positive	Positive	Negative
Methyl Red	-	Positive	Positive	Positive	Positive
Voges Proskauer	-	Negative	Negative	Negative	Positive
Citrate	-	Positive	Positive	Positive	Negative
Nitrate reduction test	-	Positive	Negative	Negative	-
Triple Sugar Iron test	-	Alkaline butt- Alkaline slant	Acid butt- Alkaline slant	Acid butt- Alkaline slant	-
Mannitol	-	-	-	-	Positive (Acid)

Table 3: Degradation activity of isolates

Sr. No.	Name of the Test	Bacterial Strain				
		1	2	3	4	5
1	Strach hydrolysis	+	+	-	-	+
2	Gelatin hydrolysis	+	+	+	+	+
3	Lipid hydrolysis	-	-	+	+	+
4	Casein hydrolysis	+	-	-	-	-
5	Pectin hydrolysis	+	-	+	-	+

[+] : Positive Reaction , [-] : No Reaction



Figure 1: Growth of *Bacillus sp.* on Nutrient Agar

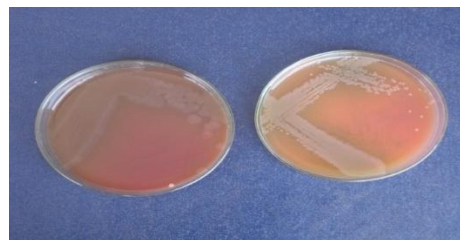


Figure 2: Growth of *Pseudomonas aeruginosa*, *Staphylococcus aureus* in Blood agar



Figure 3: Growth of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in MacConkey agar and Mannitol salt agar respectively

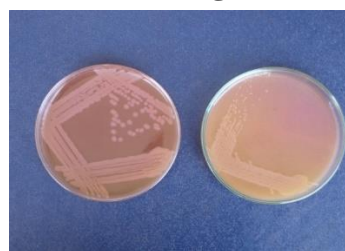


Figure 4: Growth of *Bacillus sp* in MacConkey & Nutrient agar

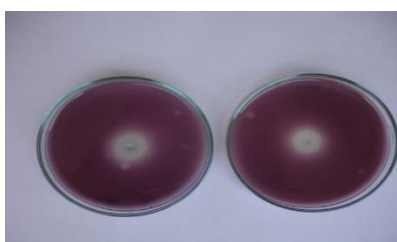


Figure 5: Starch hydrolysis of *Bacillus sps* (Left) *Pseudomonas aeruginosa* (Right)



Figure 6: Protease by *Pseudomonas putida*



Figure 7: Pectinase by *Bacillus sp*



Figure 8: Lipolysis by *P. aeruginosa*



Figure 9: Starch hydrolysis by *Bacillus sp*

Conclusion:

Characterization of the tolerance against fly ash toxicity is the key feature in selection of locally growing microorganisms. The essential recommendations by various researchers include fast growth, high biomass and active detoxification mechanism. Fly ash collected from Neyveli

(NLC) was subjected to Physico-chemical parameter analysis. Then the same samples were processed for bacterial screening. *Bacillus sp*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Pseudomonas putida* and *Staphylococcus aureus* were isolated.. The isolates were subjected to analysis on the production of exoenzymes such as Amylase (Starch hydrolysis), Gelatinase (Gelatin hydrolysis) Lipase (Lipid hydrolysis) a Protease called caseinase (Casein hydrolysis) pectinase (Pectin hydrolysis). Though all the isolates were found to be effective exoenzyme producers, their role in the transformation and or degradation of fly ash is negligible. Because, there are no such biomacromolecules such as carbohydrate, protein, lipid etc., in fly ash But the presence of these organisms shows that they are able survey in the fly ash using some ingredients as sole source which has be dealt separately as a study. Keeping in view, the survival of these species suggest the detoxification potential and so is suggested to attain positive interactions at the landfill remediation i.e. one species showing lesser tolerance might help reduce the future growth limiting factor or enhance ecological interactions forming a self-sufficient stable ecosystem. As a future studies, the focus should be shifted more on anaerobic bacteria and special enzymes reacting to metallic and mineral compounds.

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Frontiers in Life Science Volume IX

ISBN: 978-93-91768-16-4

About Editors



Dr. Amita Srivastava is working as an Assistant Professor at Department of Zoology, Dayanand Girls College, Kanpur, U.P., India. She has 22 years of teaching experience. She had been awarded with Ph. D. in 2003 on the topic "Bionomics and Control Measure of Citrus insects with special reference to *Papilio demoleus*, *Aonidiella* Sp. And *Tonica ziziphi*". Dr. Srivastava is member of many prestigious associations like Indian Science Congress Association, Kolkata, Entomological Research Association, Udaipur, Indian Society of Life Sciences, Kanpur, International Association of Zoologists, Asian Biological Research Foundation (ABRF), etc. She has published more than 17 research papers in various national and international journals. She received Excellence in Research Award at 2nd National Conference Cum Workshop On CTTABMMB-2019 organized by Department of Biotechnology and Microbiology, Kalp Laboratories, Mathura.



Dr. Sandip P. Patil, M.Sc., SET, Ph. D. is currently working as the Head of Microbiology department and IQAC coordinator at R. C. Patel Arts, Commerce and Science College, Shirpur-425405, India. He has teaching experience of 13 years to UG and PG classes of Microbiology and Biotechnology. He is the recognized research guide of KBC NMU, Jalgaon in the subject of Microbiology. Currently 04 students are pursuing their doctoral studies under his able guidance. He has completed 04 research projects from various national funding agencies. He has published 19 research papers in reputed international journals, 06 books and 02 Indian patents to his credit. He has won First Prize at State Level Avishkar-2017 and Best Innovation Award by GHR Research Labs, Nagpur. His research expertise is in the field of Microbial Biochemistry, Enzymology, Industrial Microbiology, Plant pathology and Plant-microbe interactions etc. He is the Life member of Association of Microbiologists of India (AMI) and Biotech Research Society of India (BRSI).



Dr. Dhvani Upadhyay is presently serving as an Assistant Professor in the Department of Microbiology at India's leading Parul University situated in western India region wherein guiding 05 PhD. Scholars. She is an adept researcher, diligent academician and an erudite educationalist with over 8.5 years of ingenious research experience in the field of microbiology and biotechnology. Having more than 3.5 years of delineating teaching experience, she has been expounding pragmatic know-how to the graduate, postgraduate, and Ph.D. scholars. Moreover, her conscientious research discoveries and interpretation of facts published through over 65 research papers and review articles in peer referred journals enumerated in internationally acclaimed platforms of Web of Science and Scopus. Being a GATE qualified researcher, she holds the authorship of sundry 16S rRNA FASTA sequence of multitudinous bacteria submitted to noteworthy establishment GenBank and NCBI. With profound knowledge and experiential assimilation, she has been selected as a life member of "Indian Science Congress Association". Government of Rajasthan bestowed her with DBT-Student Research Project in the course of her PhD. She has her prowess and radical pursuit in Microbial Enzymology, Environmental Microbiology, Protein Science and Molecular Biology. Her present research elucidates an endowed intramural empirical project with assiduous planning fixated on "water purification utilizing microorganisms" as a principal investigator. She has also devised digital learning E-course content on four disparate essential subjects of microbiology. Furthermore, under her meticulous guidance and superintendence 30 plus post graduate as well as beyond 40 graduate students have effectuated their dissertation projects scrupulously.



Dr. V. Vidhya has done her master degree & Ph.D in Zoology from S. T. Hindu College, Nagercoil, Kanyakumari District, Tamilnadu. She is presently working as an Assistant professor in Department of Zoology, St. Jude's College, Thoothoor, Kanyakumari District, Tamilnadu. She is the life member of Society of Innovative Researchers & Academicians (SIRA). She has attended more than 55 National, International conferences and workshops. She has published 19 research papers in many leading National and International journals including Scopus and UGC Care and presented more than 14 papers in National, International conferences and seminars. She has also published 11 chapters in edited book. Her area of research is environmental toxicology, fish biology and parasitology in fish.

