ISBN: 978-93-91768-01-0

Frontiers in Life Science
Volume VI

Editors

Dr. Shakun Mishra

Dr. Balaji Rajbhoj

Dr. Suchita C. Warangkar

Dr. Sujith R.

Bhumi Publishing

First Edition: 2022

Frontiers in Life Science Volume VI

(ISBN: 978-93-91768-01-0)

Editors

Dr. Shakun Mishra

Department of Botany,

Govt. S. N. P. G. College,

Khandwa (M.P.)

Dr. Balaji Govindrao Rajbhoj

Department of Botany,

Sundarrao More Arts Commerce and

Science College Poladpur,

Dist Raigad. Maharashtra

Dr. Suchita C. Warangkar

Department of Microbiology,

Netaji Subhash Chandra Bose Arts,

Commerce and Science College,

Nanded 431605, MS, INDIA

Dr. Sujith R.

Department of Microbiology,

SRM Institute of Science and Technology,

Potheri, Chengalpattu Dt., Tamilnadu

Bhumi Publishing

2022

First Edition: June, 2022

ISBN: 978-93-91768-01-0

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 VI"****. 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.

Editor

CONTENT

INNOVATIONS, TECHNIQUES AND MODERN BIOTECHNOLOGY INCLUDING GENETIC ENGINEERING PLANT TISSUE CULTURE IN *MENTHA ARVENSIS*

Mandaloju Venkateshwarlu

Department of Botany, Kakatiya University,

Warangal – 506 009.T.S. India

Corresponding author E-mail: drvenkat6666@gmail.com

Abstrat:

Plant tissue culture was exploited for both basic and applied aspects such as haploidy, mutagenesis, somatic embryogenesis, somaclonal variation, selection of cell lines resistant to antibiotics and antimetabolites, protoplast fusion, genetic manipulation and molecular biology (Dix and Street, 1975; Bajaj *et al.*, 1977; Lindsey and Yeomann, 1983). The concept of cellular totipotency i.e. ability to regenerate and entire organism from a single cell and plant part (explant) proposed by Haberlandt (1902) emerged as corollay of the cell theory proposed. According to this concept, the genetic information necessary for the development of the entire organism is contained in all living cell. Consequently when the cells are released from the developmental controls, they are able to divide and differentiate into specific organs and whole organisms.

Keywords: Innovations, Techniques, Biotechnology, Genetic Engineering, Plant Tissue Culture

Introduction:

Mentha is a genus of aromatic perennial herbs belonging to the family Lamiaceae, distributed mostly in temperate and sub-temperate regions of the world. The number of taxonomically valid species in the genus remains a matter of speculation as members freely cross amongst themselves, producing many intermediary forms. Polyploidy has also played an important role in the process of speciation in this genus. Most of the commercially important mints are hybrids or amphiploids. *M. piperita*, the peppermint, is a sterile first generation hybrid between *M. spicata* and *M. aquatica* (Hefendehl and Murray, 1972). The spearmint, *M. spicata*, is a hybrid of *M. longifolia* and *M. rotundifolia*. Morphological, cytological and biochemical data have shown that the tetraploid species of *M. spicata* (2n-48) originated by chromosomal doubling of hybrids between the two closely related and inter-fertile diploids, *M. longifolia* and *M. suaveolens* (Harley and Brighten, 1977). The bergamot mint, *M. citrata*, is considered to be a variety of *M. aquatica*. It is believed that *M. arvensis var*. piperascens, Japanese mint, is a hybrid

between *M. arvensis* and *M aquatica*. Mint cultivation is widely distributed. The United States of America is the main producer of peppermint and spearmint oils. The peppermint (*M. piperita*) is cultivated on a large scale in the states of Oregan, Indiana, Idaho, Ohio and Michigan (Chambers and Hummer, 1992), whereas spearmint cultivation is localized in Indiana and Michigan. Spearmint is also cultivated in France, the United Kingdom, Italy, Yugoslavia, Hungary, Bulgaria, Russia, South Africa, Thailand and Vietnam. Bergamot mint is commercially cultivated in China, Taiwan and India, and menthol or Japanese mint in India, China, Taiwan, Thailand, Japan and Brazil.

The first complete chemical defined nutrient medium was developed by Murashige and Skoog (1962). A standard culture medium consists of a balanced mixture of macro and micro nutrients, vitamins and plant growth regulators which play an important role in cell metabolism and cell membrane synthesis (Cocking, 1978). From the time Haberlandt presented his paper in 1902 until about 1934 hardly any progress was made in the field of plant tissue culture.

Plant scientists took advantage of totipotency concept and developed media and methods for tissue culture. The nutrient media used for most of the cultures were the formulations developed in the early 1930's by white (1934). The other media widely used for plant tissue culture studies were MS medium B5 medium (Gamborg *et al.,* 1968), SH medium.

Steward *et al.,* (1958) from Cornell University USA reported roots, then shoots and ultimately whole plants from culture of carrot roots in cell suspension. The rise of whole plant from a free cultured cell was first in tobacco plant by Vasil and Hilderbrandt (1965). the present tissue culture revolution began in 1969 when the Rockfellor Foundation sponsored a small conference on crop improvement through plant cell and tissue culture at the Villa Sebellani on Lake Como, Italy. Tissue from almost any organ can prove to be totipotent under appropriate condition. The mode of regeneration depends upon the species, the organ used and level of growth regulatory substance in the medium. The ability of shoots, roots and flowers to riseadventiously is being exploited in tissue culture (Flick *et al.,* 1983).

Plant genetic transformation involves the transfer of foreign genes into the genome of host plant cells and recovery of whole plants in which the transferred genes are expressed. This technology allows introduction of new trails into plants while presenting the original desired phenotype of clones. Classical potato breeding programs can potentially benefit from genetic engineering to rapidly in corporate or modify particular traits such as insert resistance herbicide tolerance, and modified metabolomics into commercially appreciated cultivars. Genetic transformation can reduce the time necessary to obtain improved varieties. Provided that too many genes do not control the intended character. Transformation also makes it possible to

transfer specific traits into selected genotypes without affecting their desirable genetic back ground.

Agrobacterium mediated gene transfer into plastme encoded streptanycin resistant plants (Double markers selection).

A dream of every plant breeder is introduce a useful new character is an already established cultivars, without any change, except for the correct expression of the desired trait. It is possible not only to transfer genes among plants, but also among other phyla, apparent thy without changing anything in the general expression of other genes of the "recipient" organism. Several reports have been published about genetic transformation of crops. With useful genes against insects, fugi, and viruses DNA can be forced into cell nuclei using two methods: direct and indirect DNA transfer. The former uses physical or chemical agents. While the latter uses biological carries. Such as Agrobacterium or virus (Potrykus. 1990).

The aim is not to just isolate transformed cell but to do with a reasonably high efficiency and to obtain transformed plants that are in distinguish able form untransformed plants with the exception of the introduced characters.

In this context the Labiatue family is particularly rich of iodiversity and since ancient times it was the object of a great variety of uses by humans. Because of their traditional use by Mediterranean communities, the most quoted genuses in the literature are: Hyssopum, Lavandula, Majorana, Melissa, Mentha, Ocimum, Origanum, Rosmarinus, Salvia, Thymus, and, to a minor extent: Acinos, Ajuga, Ballota, Calamintha, Clinopodium, Coridothymus, Dorystaechas, Galeopsis, Glechoma, Lamium, Leonurus, Lycopus, Marrubium, Melittis, Micromeria, Nepeta, Phlomis, Prunella, Satureja, Scutellaria, Sideritis, Stachys, Teucrium, Thymbra, and Zitiphora. Different kinds of traditional uses are documented and among others the following are cited: as human food or livestock feeding, aromatic, for liqueur production, as medicinal or veterinary plants, to combat pests and parasites, as cosmetic, for house decoration, as fuel or illumination, in the handicraft, to produce soap, to tan the leathers, to dye the cloths, in religious, magie or superstition practices.

Highly variable are also the forms of application or processing to which raw materials are subjected in order to permit their use: fresh, dry, pulverised, in water, oil or alcoholic infusion, as ointment, burned and eventually smoked, and sniffed as powder. A synthesis of the main uses and some information obtained from the literature databases are reported.

Lamiaceae is one of the large plant families used as a framework to evaluate the occurrence of some typical secondary metabolites (Wink 2003). The typical secondary

metabolism of Lamiaceae includes various terpenoids and phenolic compounds (Hegnauer 1989). Lamiaceae is subdivided in to two major groupings:

Mints are herbaceous plants and perennial aromatic herbs that are cultivated for their essential oils used both for medicinal and culinary purposes. These plants belong to the genus Mentha L. (Lamiaceae), which is a native from north temperate regions and occur in all five continents. According to a high polymorphism in morphology and a great diversity in essential oil composition, the number of species in the genus *Mentha* L. has been a matter of speculation for many years described the species of the genus based on inflorescence morphology. Several features have been used in the past to examine the diversity of Mentha using morphological (Malinvaud, 1880), cytological Sharma and Bhattacharyya, 1959; and Harley and Brighton (1977) published a critical review of the chromosome numbers in relation to the taxonomy of the genus. They estimated that there are probably only 25 species and rather fewer hybrids. They recognized five sections (*Audibertia, Eriodontes, Pulegium, Preslia, Mentha*) on the basis of basic chromosome numbers and morphological features. There is no problem of identification for the first four sections because no example of natural interspecific hybridization exists. The fifth section, Mentha, includes five species: *M. suaveolens M. longifolia (L.) Hudson, M. spicata L., M. arvensis L., and M. aquatica L.* Chromosome counts for this section suggest a basic number of x 12 with a range of numbers from diploid to octoploid. Plants of this section have a vigorous rhizome system as a means of spreading and dispersal and are self-compatible, and a high level of out breeding is assured due to gynodioecy. Natural interspecific hybridization occurs with high frequency in section Mentha, both in wild populations and in cultivation. Most hybrids are sterile or sub feraile, but vegetative propagation enables them to persist. Complex hybrid populations may arise, and if they are sub fertile, may cross with parental or non-parental species.

Mentha has been complicated by hybridization, by a high morphological polymorphism, as well as polyploidy and vegetative propagation. The best known hybrids are *M. piperita (peppermint) and M. spicata L.* (native spearmint), which are intensively cultivated for their essential oils. Mentha piperita results from a cross between *M. aguation and M. spicata; M. spicata is the hybrid between M. suaveolens and M longifolia* (Harley and Brighton, 1977) the great variability of *M spicata* led several workers to establish a subdivision of this hybrid, and two subgroups were described based upon two features. Cytological studies (Ruttle, 1931; Morton, 1956) led to the conclusion that two M. spicatacytotypes exist, with 2 36 and 2n 48 chromosomes respectively. According to the cytotype implied in the cross with *M aquatica*, two *M. Piperita* cytotypes result, with 2n 66 or 2 72 chromosomes, respectively Moreover,

morphological and chemical data divide M. spicata into two different subgroups according to the presence or absence of non-secretingtrichomes and the essential oil composition. Wild *M. spicata* is nearly always hairy, like its diploid parents, and can contain other terpenes that are found commonly in its diploid progenitors. (a) Relationships between species and hybrids based upon karyological data (Harley and Brighton, 1977).

(b) *Mentha arvensis* var. piperascens could have resulted from two crosses an asterisk indicates species implied in the cross that led to *M. gracilis*. Solid line Spicatae; dashed line Capitae; dashed-and-dotted line Verticillatae. spicata became glabrous with a characteristic odor due to carvone and menthone as the prevailing terpenes. *Mentha spicata* plants, introduced and distributed throughout the world, are often found as garden escapes. According to Lebeau (1974), it was essential to distinguish two *M. spicata* subspecies, *M. spicata* sub sp. spicata and *M. spicata* subsp. glabrata, with and without non secreting trichomes, respectively, for the following reasons:

(1) The presence or absence of non-secretingtrichomes led to a different aspect,

(2) Wide difference in perfume, and

(3) Difference in habitats.

Mentha spicata propagates almost entirely by vegetative means. Harley and Brighton (1977) described some individuals of *M. spicata* that were close in appearance to its progenitor diploid species. They noted that *M. spicata* segregates parental characters in its progeny by selfing, which was impossible to distinguish from the hybrids it often forms with either *M. suaveolens or M. longifolia*. In some cases, such hairy *M. spicata* plants were confused with *M. longifolia*. Over the past decade, several molecular techniques have been developed to provide information on diversity and genetic relationships. Two attempts to assess genetic relationships (Khanuja *et al.,* 2000) and cultivar identity (Fenwick and Ward, 2001) based on RAPD markers have been undertaken in Mentha species. The complex systematics of section Mentha led us to choose amplified fragment length polymorphism (AFLP), based on the selective polymerase chain reaction (PCR) amplification of restriction fragments from a total digest of genomic DNA (Vos *et al.,* 1995), to assess genetic diversity. It combines the specificity of whole-genome restriction fragment analysis and the selectivity of high stringency PCR amplification without prior knowledge of primer target sequences.

A large number of markers can be obtained quickly compared to restriction fragment length polymorphism (RFLP), randomly amplified polymorphic DNA (RAPD), or simple sequence repeat (SSR) Pejic *et al.,* 1998; Garcia- Mas *et al.,* 2000). Because of its high repeatability and resolution, the AFLP technique has been used successively to assess genetic

diversity, for example, in *Brassica juncea* (Srivastava *et al.,* 2001) and to resolve phenetic relationships in Dactylorhiza (Orchidaceae) and in Lactucasensulato (s.1.) (Lactuceae, Asteraceae) (Koopman, Zevenbergen, and Van den Berg 2001). The goal of the present study was to genotype diploid and polyploid species of section Mentha by AFLP to (1) assess the relationships among accessions of an extended collection representing legitimate species and the hybrids *M. piperita* and *M. spicata*; and (2) check the existence of two *M. spicata* groups with molecular markers.

Peppermint (*Mentha* X *piperita L*.) oil is one of the most popular and widely used essential oils, mostly because of its main components menthol and menthone. Peppermint oil is used for flavouring pharmaceuticals and oral preparations. Corn mint is the richest source of natural menthol. Carvonescented mint plants, such as spearmint (*M. spicata*), are rich in carvone and are widely used as spices, and they are cultivated in several countries. Studies were made into the yield and essential oil content of several mint species and the original.

The general aim the work was to xamine the optimal conditions for cultivating mint in Northern Finland. The specific aims of the study were (first) to investigate the differences in the oil content for several mint species and (secondly) to compare the effect of various factors such as plant spacing (10, 20 and 30 x 50 cm), liming (0, 4, 8, 12 and 16 tons ha-1), propagation methods (micropropagated and conventionally propagated plants) and harvest date (once at the end of August in comparison with first cut at the beginning of August and second cut in mid-September) on the cultivation success, quality and quantity of the plants. The constituents of the essential oil were analyzed from leaf samples using GC-MS. Among the peppermints of different origins studied, peppermint of USA and Egypt origin ('Black Mitcham') contain the highest menthol and optimum oil yield. Corn mint and Sachalin mints both had high menthol content. Due to several reasons, such as no significant differences between the different densities and oil composition, markedly higher amount of weeds at 30 x 50 cm than at 10 * 50 and 20 x 50 cm spacing and the high seedling costs and the danger of fungi and disease at a 10 x 50 cm spacing, a plant optimum of 20 x 50 cm spacing is recommended for Northern Ostrobothnia. If the pH value is lower than 6, or levels of Mg and Ca are low, liming at a rate of 4-8 t ha 1 for sandy soils in Finland is recommended in order to achieve higher fresh and oil yields.

In the first year, there were no differences in the dry leaf yield of micropropagated and conventionally propagated plants, but the menthol content was significantly higher in conventionally than in micropropagated plants. In the second year, only the dry leaf yield of micropropagated plants was higher than that of their conventionally propagated counterparts. Cutting peppermint only once during full bloom (the end of August) gives the maximum oil

yield of good quality. In conclusion, it is possible to achieve as high as or even higher oil quality and dry yield in North Ostrobothnia than it is in central Europe or south Asia. However, this requires observing certain cultivation factors such as having the right type of mint, soil pH, planting density, harvesting time and propagation method In addition, mints must be cultivated in the same place for only two and a maximum for three years.

Secondary metabolites in plants

Secondary metabolites are present in all higher plants, usually in high structural diversity. Many metabolites have been found to protect plants against viruses, bacteria, fungi, and most importantly against herbivores. Many secondary metabolities such as cyanogenic glycosides, glucosinolates, terpenes, saponins, tannins, anthraquinones, and polyacetylenes also act as allelochemicals, influencing the growth and development of neighbouring plants (Wink, 2003). For example, monoterpene limonene has shown deterrent and insecticide properties and carvone is used as sprouting inhibitors (Aflatuni, 2003).

Essential oils are complex and highly variable mixtures of constituents that belong to two groups: terpenoids and aromatic compounds. Hydrocarbons are almost always present in monoterpenes. Essential oils accumulate in all types of vegetative organs: flowers (bergamot tree), leaves (mint, eucalyptus), barks (cinnamon), woods (sandalwood), roots (vetiver), rhizomes (ginger), fruits (anise), and seeds (nutmeg). Essential oils are usually associated with specialized storage in plants.

Although essential oils are comprised of many types of compounds, the major ones are monoterpenes (Seigler, 1998). The synthesis and accumulation of essential oil structures are located near the surface, glandular trichomes, secretory cavities or secretory canals of the plants.The impact of environmental factors such as temperature, relative humidity, irradiance, photoperiod and cultivation practices influence the composition of essential oils. The influence of the method of extraction on oil composition and the labiality of the constituents of essential oil explains why the composition of the product obtained by steam distillation is most often different from that which is initially present in the secretary organs of the vegetable. In this context the Labiatae family is particularly rich of iodiversity and since ancient times it was the object of a great variety of uses by humans. Because of their traditional use by Mediterranean communities, the most quoted genus in the literature are: Hyssopum, Lavandula, Majorana, Melissa, Mentha, Ocimum, Origanum, Rosmarinus, Salvia, Thymus, and, to a minor extent: Acinos, Ajuga, Ballota, Calamintha, Clinopodium, Coridothymus, Dorystaechas, Galeopsis, Glechoma, Lamium, Leonurus, Lycopus, Marrubium, Melittis, Micromeria, Nepeta, Phlomis, Prunella, Satureja, Scutellaria, Sideritis, Stachys, Teucrium, Thymbra, and Ziziphora.

Highly variable are also the forms of application or processing to which raw materials are subjected in order to permit their use: fresh, dry, pulverized, in water, oil or alcoholic infusion, as ointment, burned and eventually smoked, and sniffed as powder. A synthesis the main uses and some information obtained from the literature data bases are reported.

Several mints of Mentha species are industrial crops, a source of essential oils enriched in certain monoterpenes that are widely used in food, flavour, cosmetic and pharmaceutical industries. Different species of mint are used across the globe for their medicinal and culinary properties. Mint is usually taken after a meal for its ability to reduce indigestion and colonic spasms by reducing the gastrocholic reflux. Less well recognized is peppermint's potential role in the management of numerous medical problems, including colonoscopy. The genus Mentha has a large number of species that differ widely in their characteristics and ploidy level. Since they are often perennial and produce suckers, Mentha species reproduce both by reproductive and vegetative means. In Mentha crops, the purity of cultivars is maintained by vegetative means of propagule generation. Mint species are widely distributed and are prone to attack by a variety of diseases and pests. Many laboratories worldwide are carrying out research and development work on mint species towards improvement in the yield and quality of essential oils, and incorporation of disease and pest tolerance and increasing the propagule productivity. Peppermint oil is the major constituent of several over the counter remedies for symptoms of irritable bowel syndrome (IBS). Results of clinical trials indicated that it could be efficacious for relief in IBS (Pittler and Ernst, 1998). In this review, the authors aim to correlate various aspects of in vitro regeneration and genetic transformation in Mentha species.

Mint essential oil compositions

Mints are cultivated for their essential oils, yielded by distillation of over-ground herbaceous foliage. The oil contains a large variety of aromatic chemicals in varying composition. The oils and their fractions are in great demand world-wide.

Uses of mint herbage and essential oils

Mints are extensively cultivated for their oils and terpenoid components of the oil, such as menthol, carvone, linalyl acetate and linalool, for use in pharmaceutical, cosmetic, food, flavour, beverage and allied industries (Chaddha and Gupta, 1995). Table 3 gives the various applications of important mint species.

Genetic information in terpene biosynthesis in mints

The genetics of biosynthesis of chemical constituents of all the important species of Mentha has been studied (Hefendehl and Murray, 1972). Utilizing crosses between Mentha species, it has been shown that single Mendelian gene(s) control presence or absence of major

compounds such as carvone, menthone, menthol and piperitenonepiperitone. Hefendehl and Murray (1972) suggested that from the biogenetic viewpoint, *M. arvensis* and *M. piperita* perform the same conversions: The monogenic basis for the conversion of menthone to menthol showed that gene R, either in homozygous (RR) or in heterozygous (Rr) form, was responsible for the reduction of menthone to menthol or carvone to carveol The dominant A gene allowed the conversion of piperitenone into pulegone. The gene P either in homozygous dominant (PP) or in heterozygous condition (Pp) caused the conversion of pulegone into menthone. It may be pointed out here that gene P is controlled indirectly by the gene F, responsible for the conversion of pulegone into menthofuran. There is so far no evidence of linkage between these genes. In *M. spicata* the dominant gene C was responsible for production of carvone from limonene, while the dominant gene Lm prevented the conversion of limonene to piperitenone. There is little conversion of carvone into its alcohol carveol since the species does not have the dominant gene R that allows this conversion. Hefendehl and Murray (1972) concluded that a genotype having dominant Lm and recessive cc resulted in accumulation of limonene whereas the genotype with both genes in the recessive form (Im, Im, cc) contained only 3-oxygenated compound and no 2-oxygenated compound. The complementary genotype, possessing both dominant genes (Lm, Lm, CC), produced only the 2-oxygenated compound, carvone. The lavender odour of *M. citrata* is caused by 60 to 70% linalool and linalyl acetate. Chemical and genetic analysis revealed that the dominant gene I in *M. citrate* resulted in production of 60-70% linalool/linalyl acetate but only 1-1.5% isopinocamphone and 0.5-2.5% -pinene. All individuals of linalool chemotype must have one dominant I gene to produce linalool and 4 dominants are possible since this gene is present on two different homologous pairs of chromosomes.

Enzymes involved in monoterpene biosynthesis

The physiology and carbon metabolism of glandular cells are best studied in mint (McCaskil and Croteau, 1999). The pathway originates in the plastids (leucoplasts) of the secretary cells of these highly specialized non photosynthetic glandular structures (Turner *et al.*, 1990). The monoterpene family of natural products therefore is derived from the plastidial, mevalonate-independent pathway for isoprenoid metabolism, which provides isopentenylbisphosphate (and, by isomerization, dimethylallylbisphosphate) as the universal precursor of the terpenoids. Aspects of monoterpene production in callus cultures of mint also have been studied in relation to its production in whole plant. A recently discovered pathway and the enzymes involved in monoterpene biosynthesis in the mint is summarized in The oxygenation pattern of monoterpenoids of mint has been determined by regiospecific cytochrome P450-catalysed hydroxylation of the common olefinic precursor, (-) limonene. A

PCR product of limonene-0-6-hydroxylase was used as a probe to isolate full length cDNA clone which provided the tool for isolating the homologous cDNA from peppermint and related Mentha species. The pathway of monoterpene biosynthesis in mint has been well established by in vivo and cell-free studies, and all of the enzymes involved have been described Monoterpene biosynthesis and accumulation has been specifically localized to the glandular trichomes (McCaskill *et al.,* 1992).

Pests and Pathogens of mints

The polyphagoussemiloopers also voraciously devour the tender foliage. White flies lay eggs on the ventral surface of the mint leaves, and the nymphs suck the sap resulting in arrested growth. Mint crops are associated with large numbers of pests and pathogens that cause substantial damage to crops and considerable loss of oil yield. Termites attack the underground parts and cause serious damage to the root system and main stem of the plants. Cutworms damage the young shoots of mint plants after they have sprouted Polyphagous caterpillars often cause severe damage to the crop by defoliating it within a few days.

A number of microbes have been observed to cause diseases in mint crops. The stolon rot that occurs in the rainy season is caused by Macrophomiaphascoli and Pythium, In the affected plants, leaves wilt and turn yellow and growth is stunted. Rust is caused by Pucciniamenthae; orange colour rust postules appear on leaves, which afterwards turn yellow and ultimately get shed. The powdery mildew caused by Erysiphecichoracearum results in circular white powdery patches on the leaves, which subsequently spread to the stem and other parts of the plant; the severely affected leaves drop off. Leaf blight caused by Alternaria species during summer months results in the appearance of irregular dark brown spots with concentric zones surrounded by a pale yellow margin on the upper surface of the leaves; heavy defoliation occurs subsequently. There is a need for genetically engineered important mint cultivars with heterologous genes for rendering them pest or disease resistant. The leaf spots caused by Corynespora spp. also inflict considerable damage on mint crops sudharsan (2000).Several types of control measures, including the use of insecticides and disease-inhibiting chemicals, and breeding of pest and pathogen resistant cultivars have been followed in mints but limited success has been obtained in controlling both pest and pathogens.

Conventional pesticides used on mint crops

Applications of gondal mixtures (gardenia gummifera, asafoetida, aloes, rosin in the ratio of 1:2:2:2 diluted in water) and insecticides like chlordane, dieldrin, heptachlor and toxaphene have been recommended for controlling termites and must be applied at 3 stages, before sowing, at planting, or after planting of suckers. Soil application of aldex or chlorodane before planting

or spraying with metasystox, malathion or thiometan gives effective control against cutworms. For the early stage attack of caterpillars, 5% diptrex or 2% folidol, and for advanced stage, spraying with thiodon or endrin gives effective control. Semilooper infection can be controlled by spraying with endosulfan or carbayi mixed in water. Leaves badly infested with white flies have to be collected and burnt or the crops sprayed with rosin compounds to destroy the insects. Waterlogging promotes infection of pathogens, viz. Macrophomia and Pythium. This can be avoided by planting Mentha on ridges. The treatment of planting stock with fungicides effectively controls the pathogens. Hot water treatment, by steeping the stolons in water or spraying with copper fungicide (0.3% containing 50% metallic copper) or sulfur powder (0.5% containing 50% elemental sulfur) in water, controls rust. Powdery mildew disease can be controlled by spraying with copper fungicides and lime sulfur. Spraying of copper fungicide also helps in checking blight disease caused by Alternaria.

Results and Discussions:

The result of present investigation show that the leaf explants from mature plants of ***Mentha arvensis*** could be induced to produce multiple shoots *in vitro* maximum number of shoots were (2-4 to 4-6) induced on MS medium fortified with various concentrations of BAP, NAA and Kn.The present study demonstrates the successful shoot regeneration from the

in vitro cultured leaf explants of ***Mentha arvensis*** and the efficacy of the plant growth regulators was assessed by counting the number of shoots per leaf callus as well showed that 3.0 mg/l NAA and 2.0 mg/l BAP was found best for callus induction and growth. But in the present experiment, a higher level of NAA (3.0 mg/l) and BAP (3.0 mg/l) was found best for callus induction. *In vitro* regeneration trails followed by *in vivo* plant shoottips acclimatization. The results showed a variable shoot forming capacity depending on the combination of growth regulators used in the culture medium. The number of shoots produced increased with the concentration of BAP and Kn until 1.5 mg/l or 0.5 mg/ l of the cytokinin and showed high frequency of explants exhibiting compact green callus with shoots (4-6), growth and also for shot induction. MS medium supplemented with 2.0 or 1.0 mg l^{-1} of BAP and Kn shoot regeneration was obtained within 15-20 days and proliferation was also observed in the same concentration of medium Upadhyay *et al* has also showed that 1.0 mg/l of cytokinin (BAP and Kn) was found best for shoot regeneration and shoot proliferation. MS medium supplemented with different concentrations of BAP, NAA, IAA and Kn is presented. (Table –I, plate-I, fig -1 leaf explants, fig-2 callus, fig -3 plant lets).

Table 1: Innovations, Techniques and Modern Biotechnology including Genetic Engineering Plant Tissue Culture in *Mentha arvensis*

Growth regulators (mg/ l)	Leaf explants showing callus response	No. of leaf explants
NAA (1.0) + BAP (2.0)	30	Callus
NAA (2.0) + BAP (3.5)	25	Callus
NAA (3.5) + BAP (2.0)	20	Shoots(2-4)
NAA (4.0) + BAP (3.0)	15	Shoots(4-6)
IAA (3.0) + BAP (2.0)	10	Shoots+Callus
2,4,D (3.0) + BAP (2.0)	05	Shoots (1-2)
Kn (2.0) + BAP (1.0)+IAA	25	Green Callus
Kn (3.0) + BAP (2.0)+IAA	20	Callus with shoot
Kn (4.5) + BAP (3.0)+IAA	15	Shoots (2-4)+roots
Kn (3.0) + BAP (4.0)	10	Shoots (2-3)+roots

Fig -1

Fig -2

Fig -3

Plate 1: Innovations, Techniques and Modern Biotechnology including Genetic Engineering Plant Tissue Culture in *Mentha arvensis*

Concussion:

Several types of control measures, including the use of insecticides and disease-inhibiting chemicals, and breeding of pest and pathogen resistant cultivars have been followed in mints but limited success has been obtained in controlling both pest and pathogens. There is a need for genetically engineered important mint cultivars with heterologous genes for rendering them pest or disease resistant.

References:

Bajaj Y.P.S. Reinert J. and Haberle E. (1977).Factors enhancing in vitro production of haploid plants in anther and isolated microspores". In: R.J. Gautheret (Ed) La culture des Tissus et-des cellules des vegetaux. Masson, Paris, pp. 47- 58.

Boxus.P.H., Quoirin M. and Kaine J.M. (1977). Large scale propagation ofstrawberry plants from tissue culture. In: Applied and Fundamental Aspects of Plant cell, Tissue and Organ Culture, (eds.) J.Reinert and Y.P.S. Bajaj, Springer-Verlag, Berlin, Heidelberg, New York, pp. 130- 143.

Bruneton J (1995).Pharmacognosy, Phytochemistry, Medicinal Plants. Lavoisier Publ. Londres, New York, Paris, 405-466. 3: 244-252.

Chambers, H.L., and Hummer, K.E. (1992). Chromosome counts in the mentha collection at USDA ARS NCGR. Taxon 43: 423-432.

Cocking E.C. (1978)."Selection and somatic hybridization ". In: Frontiers of Plant Culture. ED. T. A.Thorpe. Academic Press pp. 151-158.

Colby, S.M., Alonso, W.R., Katahira, E.J., McGarvey, D.J., and Croteau, R. (1993). 4S-Limonene synthase from the oil glands of spearmint (Mentha spicata): cDNA isolation, characterization, and bacterial expression of the catalytically active monoterpenecyclase. J Biol Chem. 268: 23016-23024.

Dix P.J. and Strect H.E. (1975). "Sodiucm chloride- resistant cultured cell lines from hicotianasylvestris and Capscicumannuum" Plant SciLett. 5: 231-237.

Fenwick, a. L. and s. M. Ward. (2001). Use of random amplified polymorphic DNA markers for cultivar identification in mint. HortScience 36: 761-764.

Flick C.E., Evans D.A. and Sharp W.R. (1983). "Organogenesis", In Hand book of plants cell culture, Vol. I. C.D.A. Evans W.R. Sharp P.V..Ammirato and Y.Yamada (eds), Mc. Millan, New York, pp:13-81.

Gamborg O.L., Miller R.A. and Ojima K. (1968). "Nutrient requirements of suspension cultures of soyabean root cells" Expcell Res 50: 151- 158

Garcia-mas, J., M. Oliver, H. Go' mez-paniagua, and M. C. De vicente. (2000). Comparing AFLP, RAPD and RFLP markers for measuring genetic diversity in melon. Theoretical Applied Genetics101: 860-864.

Good R (1974).The Geography of the Flowering Plants, 4th ed. London,Longman, 518 pp.

Haberlandt G. (1902).Culture versuchemitisoliertenPflanzanZellen.Math Naturwiss K.L. Kais Akad.Wriss Wien, 111: 69-92.

Harley, R.M., and Brighton, C.A. (1977).Chromosome no. in the genusmentha. Bot. J. Linn. Soc. 74: 71-96.

Hedge C (1992)A global survey of the biogeography of the Labiatac. In Harley RM & Reynolds T (eds) Advances in Labiatae Science. Royal Botanic Gardens, Kew 7-17.

Hefendehl, F.W., and Murray, M.J. (1972). Changes in monoterpene composition in Mentha aquatica produced by gene substitution.Phytochemistry 11: 189-195.

Hefendehl, F.W., and Murray, M.J. (1973). Monoterpene composition of a chemotype of Mentha piperita having high limolene.Planta Med.23: 101-109.

Hegnauer R (1989). Chemotaxonomie der Pflanzen.EineÜbersichtüber die Verbreitung und die systematic Bedeutung der Pflanzenstoff. Basel, Boston, Berlin.IISBN 3-7643-1895-3.

Horsch R.B., Fry J., Hoffmann N.L., Eicholtz D., Rogers S.G. and Fraley (1985).A simple and general method for transferring genesinto plants. Science 227: 1229-1231.

Khanujasps, shasanyak, srivastava a &kumar s (2000). Assessment of genetic relationships in Mentha species. Euphytica 111: 121 125.

Koopman, W. J. M., M. J. Zevenbergen, And R. G. Van Den Berg.(2001). Species relationships in Lactuca s. 1. (Lactuceae, Asteraceae) inferred from AFLP fingerprints. American Journal of Botany 88: 1881-1887.

Lebeau, J. (1974). Nouvellesmises au point dans le genre Mentha.NaturaMosana 27: 109-141.

Lindsey K. and Yeoman M.M. (1983)."Noval experimental system forstudying the production of secondary metabolities by plant tissue cultures. In plant Biotechnology" (Eds.). S.H. Maritell and H. Smith Cambridge University, Cambridge. pp. 39-66. Linnaeus. 1767. SystemaNaturae. Stockholm. Tomus II: 391–392.

Malinvaud, E. (1880). Simple aperc,u des hybridesdans le genre Mentha. Bulletin de la Socie'teBotanique de France 27: 332-347.

McCaskill, D.G., Gershenzon, J., and Croteau, R. (1992).Morphologyand monoterpene biosynthetic capabilities of secretary cells.clusters isolated from glandular trichomes of peppermint. Planta 187: 445-454.

Morton, J. K. (1956). The chromosome numbers of the British Menthae.Watsonia

Murashige T. and Skoog F. (1962)."A revised medium for rapid growthand biassay with Tobacco tissue culture physiol., 159: 47 497.

Pattnaik S.K. and Chand.P.L. (1996). In vitro propagation of medicinal herb Ocimumamericanim L. synOcimum.canumsims (holy absil) and Ocimumsantum L. (holy basil) plant cell Rep. 15: 846-850.

Pejic, I., P. Ajmone-marsan, M. Morgante, V. Kozumplick, P. Castiglioni, G. Taramino, and M. Motto. (1998). Comparative analysis of genetic similarity among maize inbred lines detected by RFLPS, RAPDs, SSRS, and AFLPS. Theoretical Applied Genetics 97: 1248-1255.

Potrykus I. (1990). Gene transfer to cereals: Biotechnology, 8: 535-542. An assessmentPowell, W., M. Morgante, C. Andre, M. Hanafey, J. Vogel, F. Tingey,and A. Rafalski. 1996. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Molecular Breeding 2: 225-238.

Seigler DS (1998). Plant Secondary Metabolism.Kluwer Academic Publishers.Boston/ Dordrecht/London, pp 759.

Sharma, A. K., and N. K. Bhattacharyya, (1959). Cytological studies on different species of Mentha with special reference to the occurrence of chromosomal biotypes. Cytologia 24: 198-212.

Srivastava, A., V. Gupta, D. Pental, and A. K. Pradhan. (2001). AFLPbased genetic diversity assessment amongst agronomically important natural and some newly synthesized lines of Brassica juncea. Theoretical Applied Genetics 102: 193-199.

Steward F.C., Mepes M.G. and Mears K. (1958). Growth & organized development of cultured cells organization in cultured grown from freely suspended cells. Amer. J. Bot., 445: 705-708.

Turner, G., Gershenzon, J., Nielson, E.E., Froehlich, J.E., and Croteau, R. (1990). Limonene synthase responsible for monoterpene biosynthesis in peppermint is localized intoleucoplast of oil glands secretary cells. Plant Physiol. 120: 879-886.

Vasil V. and Hilder brand A.C. (1965). Differentiation of tobacco plants from single isolated cells in micro culture" Science 150:889-892.

White P.R. (1939).Potentially unlilmilted growth of excised plant callus artifivial medium. Amer. Bot., 26: 59-64.

Wink (2003). Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. Phytochemistry 64: 3-19.

A STUDY ON IMPACT OF STRESS ON EATING BEHAVIOUR AMONG IT EMPLOYEES

M. Sivasakthi* and G. Ramya

Department of Nutrition & Dietetics,

PSG College of Arts and Science, Coimbatore

*Corresponding author E-mail: sivasakthiphd2015@gmail.com

Abstract:

Stress is one of the common prevailing factors faced by every individual. Work stress, it is all involved in all fields but when compared to the employees of other fields the stress faced by the IT professionals are comparatively large. The people who are employed in IT sector faces a lot of health problems due to work over load. Stress appears to alter the eating behavior of the individual, either over eating or under eating. The present study was carried out with 115 respondents form the IT profession and was focused on knowing their stress level, eating behavior, anthropometric status and physical activity status. **Perceived stress scale (PSS-10)** was used to assess the stress level. These results suggested that female respondents were more prone to stress than the male respondents. Around 58% of the respondents falls under normal weight category and 16% of the respondents do regular physical activities and relation between stress level and physical activity was assessed through correlation technique and the results obtained were significant i.e., stress level decreases with increase in physical activity. Around 52% of the respondents preferred eating foods when they are stressed. Nutrition education in the workplace is important to prevent increased lifestyle-related diseases among employees. **Nutrition assistance** was provided to all the respondents through e-posters on importance of physical activity, stress relieving foods and increasing foods, stress mechanism. In summary, proper physical activity and food practices highly helps in relieving and managing stress.

Key words: Perceived stress scale, eating behavior, nutrition assistance

Introduction:

Stress is one of the common prevailing factors faced by every individual. Work stress, it is all involved in all fields but when compared to the employees of other fields the stress faced by the IT professionals are comparatively large. The IT sector is the fast-developing sector among all field in the country (Andrew *et al.,* 2008). Usually, the stress arises in software companies because of employee's nature of work night shifts, achievements, targets and work overload (BusharaBano *et al.,* 2012).

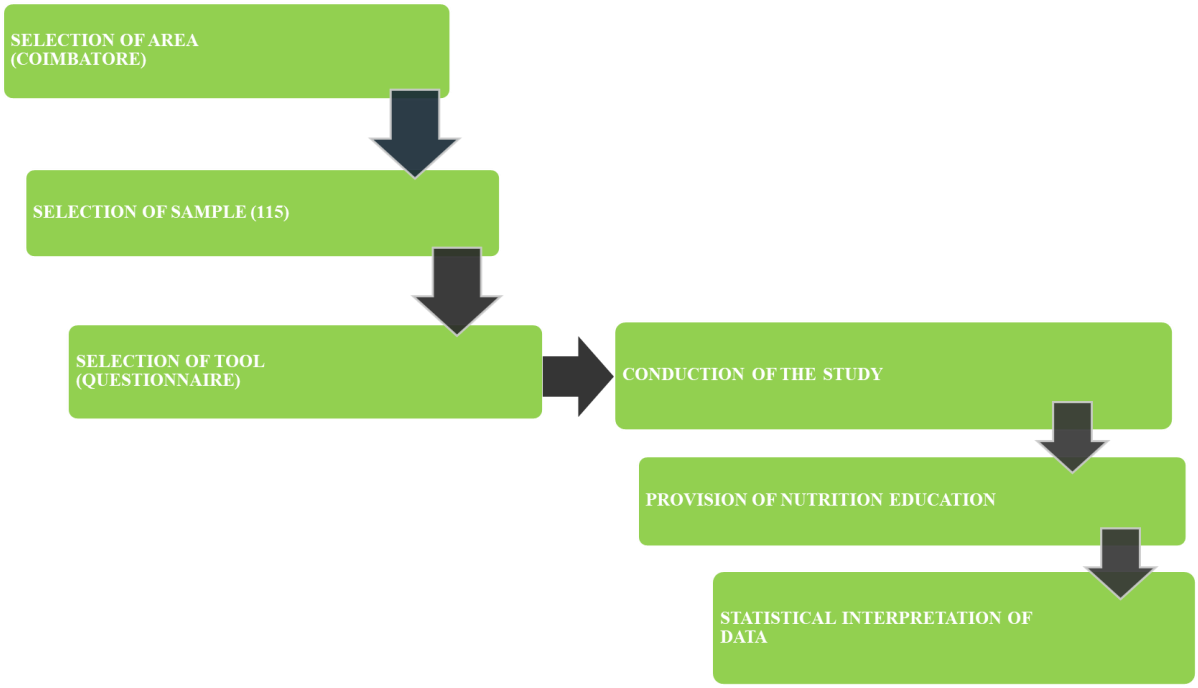
The people who are employed in IT sector faces a lot of health problems due to work over load as a result they face a lot of physical and mental stress. Epel, Jimenez *et al.* (2004) stated that stress related eating may have the potential to significantly contribute to unhealthy eating behaviors.

If stress-eating takes place often or even daily, these eating behaviors can result in unhealthy weight change and changes in physiologic measures such as nocturnal levels of insulin, cortisol, and blood levels of total/ HDL cholesterol ratio all of which effect onset of chronic disease.

Stress management training makes people more aware of stressors and their effects. Techniques of stress reduction are taught to try to decrease the individual's personal vulnerability to stressors. These techniques included relaxation, meditation and various methods with a cognitive focus, which get the person to appraise their environment in a different way.

Employee assistance programs generally work through prevention and/ or confrontation. Prevention works through the concept of wellness and is basically educational and encouraging. They offer health screening and fitness programs, social activities and try to build increased personal awareness regarding physical and mental well-being.

Methodology:



Selection of area

The study was conducted in Coimbatore city. Coimbatore is the second largest software producer in Tamil Nadu. The investigator was familiar with the township of Coimbatore. Hence Coimbatore was selected as the area of study to conduct the online survey.

Selection of sample

The IT and BPM industry contributes to 7.9 percent of India's GDP and employs around 41 lakh professionals. Employees working in IT industry are prone to develop a lot of health problems due to continuous physical and mental stress of their work. In the present study around 115 employees were selected who is working in various IT companies located at Coimbatore region.

Selection of tools

A questionnaire is a research instrument that consists of a set of questions or other types of prompts that aims to collect information from a respondent. The questionnaire used in the study need to elicit information from the respondents such as stress level, anthropometric status and their physical activity status. Among the stress assessment techniques, perceived stress scale is the tool employed in the study to gather details from the respondents.

Perceived stress scale

Perceived stress is the most commonly used measurement of stress. Perceived stress refers to one's own appraisal that stress exists. The perceived stress scale (PSS) is a 10-item instrument that was developed in 1983 to measure perceived stress (Cohen, Kamarck, Mermelstein 1983). The PSS is the most commonly used measure of perceived stress in the stress-eating literature (Griffin, Friend *et al.,* 1983). The PSS-10 has the most satisfactory psychometric properties (Maroufizadeh *et al.,* 2018). It is a validated measure of recent stressful experiences and is correlated with other measures of stressful life events. PSS-10 consists of six positively worded items and four negatively worded items. Each item in the PSS-10 is rated on a 4-point Likert scale ranging from never (0 points) to very often (4 points). The last part of the questionnaire sought to obtain socio-demographic status, and collect weight and height data to calculate the BMI of each participant and some questions were asked regarding physical activity and eating behaviour.

Conduction of the study

Google form is a free online tool that allows us to collect information easily and efficiently. The questionnaire was formed using the google form. The link of the google form was posted online through E-mail ID to the selected samples of IT sector in Coimbatore.

Provision of nutrition education

Nutrition programs in the workplace offer a direct opportunity to workers and employers; they have the potential to improve worker's physical and mental health, and thereby improve work attendance, productivity and employer reputation.

Statistical interpretation of data

SPSS version 20 statistical tool was used to analyse the data. The tests include, cross-tabulation, correlation, regression was performed to interpret the results.

Results and Discussion

Table 1: Anthropometric status of the selected IT employees

Sr. No.	**Anthropometric status**	**Reference range**	**Number of samples (n=115)**			
			Female (n=68)		**Males (n=47)**	
			Numbers	**Percentage**	**Numbers**	**Percentage**
1	Underweight	<18.5	4	5.8	1	2.1
2	Normal	18.5-24.9	44	64.7	22	46.8
3	Overweight	25.0-29.9	17	25	20	42.5
4	Obesity grade I	30.0-34.9	2	3	31	65.9
5	Obesity grade II	35.0-39.9	1	2	-	-
6	Obesity grade III	≥40	-	-	-	-

BMI was calculated based on self-reported height (cm) and weight(kg). BMIs (kg/cm^2) <18.5, 18.5-24.9, 25-29.9, 30-39.9, ≥40 were defined as underweight, normal weight, overweight, obesity grade I, obesity grade II, obesity grade III, respectively. About 5.8%, 64.7%, 25%, 3% and 2% of the respondents were underweight, normal, overweight, grade I obese, grade II obese respectively.

It is recommended that the respondents should follow the life style modifications and proper dietary pattern laid down by ICMR (2010) would help them in keeping their physique in a well-balanced manner within the normal range of WHO classification rather result in either underweight or obese category. However, the overweight (female-25% and male-42.5%) people must follow regular physical activities like walking and any other form of physical activity and balanced diets, to avoid being entered into critical grade of obesity.

Physical activity status of the selected subjects

It was noted that, 16% of the respondents do regular physical activities, while 84% of the respondents do not prefer any physical activities.

Table 2: Preference of physical activity

Sr. No.	Type of Physical activity	Number of subjects (n=18)	
		No.	**%**
1	Walking	11	61.1
2	Walking, cycling	1	5.5
3	Walking, jogging	2	11.1
4	Walking, swimming	3	16.6
5	Walking, weight lifting	1	5.5
Total		**18**	**100**

It is revealed from the table that nearly 61.1 % of the respondents are aware of the importance of brisk walking, which eventually increases the strength of the body by balancing body metabolic activities and helps in reduction of obesity which is considered as the most vital cause for most of the degenerative diseases and stress linked diseases. Rest of the respondents were engaged themselves in other physical activities like weight lifting, jogging, cycling and swimming.

Table 3: Preference of food items during stress

Sr. No.	Preference of packed foods	Number of subjects (n=60)	
		No.	**%**
1	Beverages	27	45
2	High sugar foods	6	10
3	Junk foods	13	22
4	Processed foods	9	15
5	Fruits	3	5
6	Home foods	2	3
Total		**60**	**100**

From this table it is concluded that nearly 5% and 3% of the respondents found to consume fruits and home foods respectively, since most of the people devoid of time they depend only on convenience foods due to their busy schedule engaged in their employment activities. They are advised to spend their little time in selection of nutritious foods which requires less span of time to cook would really make them in maintaining their good health and mental health.

Table 4: Cross- tabulation of gender and stress level

Gender * Stress level Crosstabulation

Count

		Stress level			Total
		High	Low	Moderate	
Gender	Female	8	2	58	68
	Male	4	4	39	47
Total		12	6	97	115

Cross- tabulation was performed to compare the variables (stress level and gender) and to analyse the results. From the above table it was inferred that, female respondents were more prone to stress in comparison with the male respondents.

Table 5: Correlation between stress level and physical activity

Correlations

		Stress level	Physical activity
stress level	Pearson Correlation	1	-.827**
	Sig. (2-tailed)		.000
	N	115	115
Physical activity	Pearson Correlation	-.827**	1
	Sig. (2-tailed)	.000	
	N	115	115

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

Correlation was performed to assess the relationship between stress level and physical activity. The Pearson correlation between stress level and physical activity is -0.827. This shows that there is negative correlation between stress level and physical activity. Hence, stress level decreases with increase in physical activity.

Conclusion:

In the present study among 115 respondents, 59% (68) were female and 41% (47) were male and their stress level was assessed using perceived stress scale **(PSS- 10).**

It was noted that, 16% of the respondents do regular physical activities, while 84% of the respondents do not prefer any physical activities.

It was reported that majority of the respondents i.e., 61% preferred walking, 5% of the respondents preferred walking and cycling, 11% of the respondents preferred walking and

jogging, 16% of the respondents preferred walking and swimming and 5% of the respondents preferred walking and weight lifting.

From the selected subjects 27% of the respondents do physical activity daily, 16% of the respondents twice in a week, 27% of the respondents thrice in a week and 27% of the respondents weekly once respectively.

It was noted that 52% of the respondents preferred to eat when they are stressed while 48% of the respondents do not prefer to eat.

About 45% of the respondents preferred beverages, 10% of the respondents preferred high sugar foods, 22% of the respondents preferred junk foods, 15% of the respondents preferred processed foods, 5% of the respondents preferred fruits, while 2% of the respondents preferred home foods.

It was observed that, 62% of the respondents skip the meal when they are stressed while 38% of the respondents do not skip the meal.

Nutrition education on stress mechanism, stress relieving foods and stress increasing foods, importance of physical activity in reducing stress, were provided to all the selected respondents through e-posters via mail.

In conclusion, IT employees are more prone to stress due to their work load and proper health education/ assistance can pave the way for improvement.

Recommendations:

Employee assistance programs:

Nutrition guidance to the employees in the work place can be experimented and lively results can be obtained and analyzed.

Acknowledgement:

The author thanks PSG College of Arts and Science for having rendered laboratory facilities to carryout the research.

References:

Andrew J Noblet and john J Rodwell, Integrating the job stress, journal of public administration and research and theory, 2016, 19(3).

BusharaBano and Rajiv Kumar Jha, Organizational stress among public and private sector employees, The Lahore journal of business, 2012, 1(1), 23-36.

Epel, Jimenez et al, “Are stress eaters at risk for the metabolic syndrome” Ann N Y Acad sci, 2004, 1032:208-10.

Kamarck, T., and Mermelstein. R. “A global measure of perceived stress”

J health soc behave, 1983, 24: 385-396

Griffin, friend et al, “Effects of environmental demands, stress, and mood on health practices.” J Behav Med, 1983, 16(6):643-61.

RECENT TRENDS AND ROLE OF CHOLINERGIC TRANSMISSION IN ADVANCED PHARMACOLOGY

Anamika P. K.*, Deborshi Nath, Fahad Aziz Bhat, Jubilee Santhosh and Jayanth N

College of Pharmaceutical Sciences,

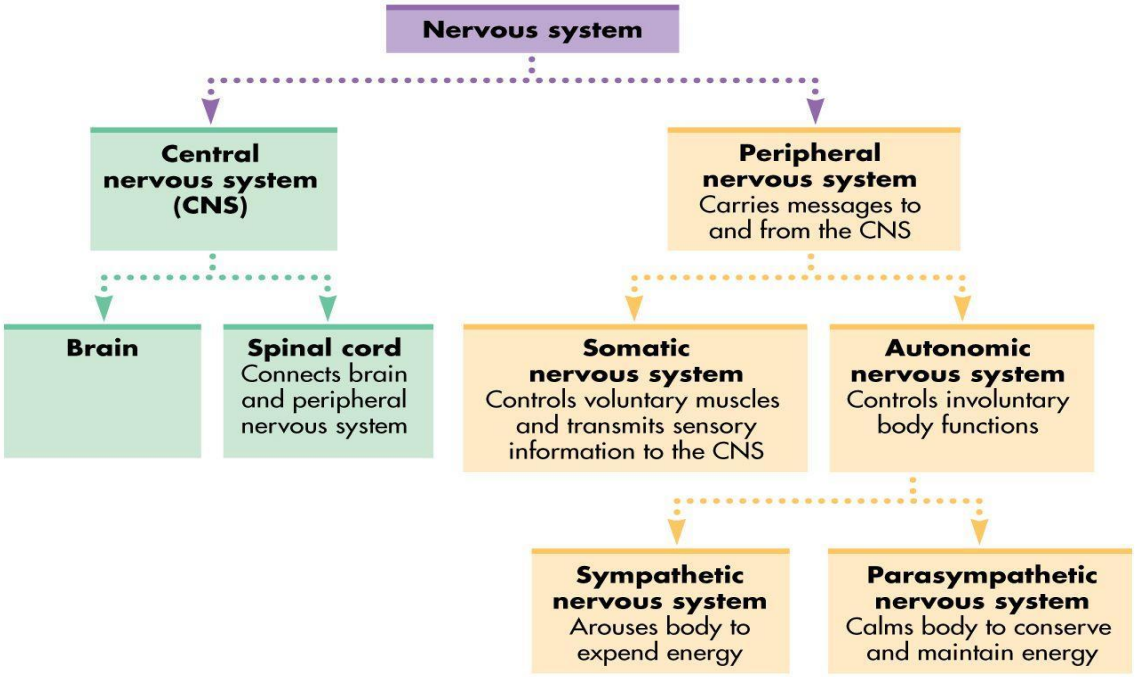
Dayananda Sagar University, Bengaluru

*Corresponding author E-mail: anamikapk2191@gmail.com

Introduction:

The autonomic nervous system (ANS) is the portion of nervous system that control visceral function of the body such as cardiac function, blood pressure etc. Animal nervous system can be divided into two parts:-

The central nervous system (CNS) consisting of brain and spinal cord. The peripheral nervous system (PNS) consists of cranial and spinal nerves and their branches.



Division of Autonomic nervous system

The two division of the autonomic nervous system are the Sympathetic division and the parasympathetic division

The sympathetic system is associated with the **fight or fright** response and the parasympathetic activity is referred by **rest or digest.**

E.g. - The heart receives connections from both the sympathetic and parasympathetic division. One cause heart rate to increase and the other cause heart rate to decrease

Function of sympathetic nervous system

- Active, even at rest, however it assumes dominant role when the body becomes stressed (trauma, fear, exercise, cold etc.)
- Fight or fright – Protect mechanism designed to help person to cop up with the stress or get away from it
- Danger heart rate increases, BP rises, eyes dilates, blood flow shifts from skin to skeletal muscles

Function of parasympathetic nervous system

- Rest and digest
- Saves energy
- Dilation of blood vessels in skin
- Decrease heart rate
- Increase the secretion of digestive enzyme

Acetylcholine and Norepinephrine are the major neurotransmitters in autonomic nervous system.

- Sympathetic – most releases norepinephrine - Parasympathetic – release acetylcholine

Acetylcholine

- All parasympathetic postganglionic neurons
- All preganglionic neurons
- Blood vessels, muscles and sweat glands

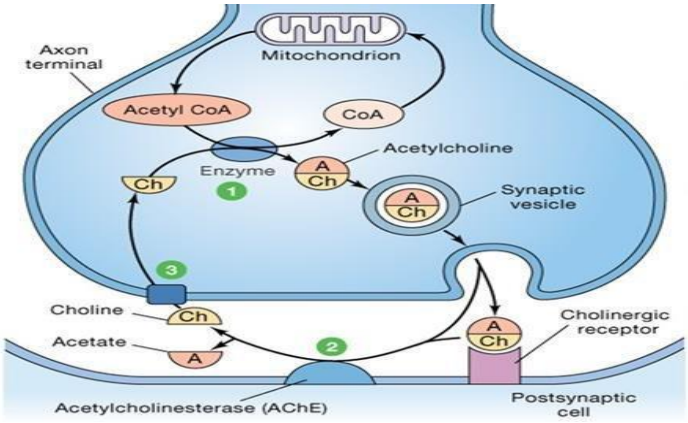
Norepinephrine

- Most sympathetic postganglionic neurons
- Epinephrine (adrenaline)
- Adrenal medulla

Cholinergic System Synthesis of Acetylcholine

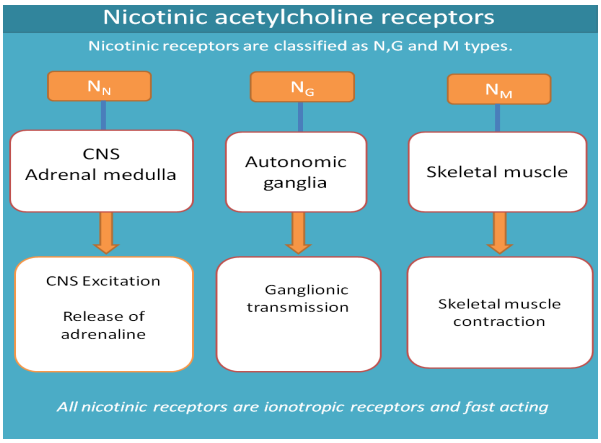
1) The initial substrates for the synthesis of acetylcholine are glucose and choline. Both enters into the neurons through facilitated or active transport
2) Pyruvate derivative from glucose is transported into mitochondria and converted to Acetyl coenzyme A (acetyl-coA).
3) With the help of enzyme called *acetylcholine transferase*, acetylcholine is synthesized from acetyl-CoA and choline
4) The acetylcholine is then transported into and stored within the storage vesicles

5) Conduction of action potential cause depolarization which results in the release of transmitter via exocytosis (entry of calcium).
6) In the junctional extracellular space (bio phase) acetylcholine interacts with cholino receptor
7) The interaction between transmitters and their receptors are reversible
8) In the bio phase or junction there is an enzyme called *Acetyl cholinesterase* which rapidly hydrolyse acetylcholine
9) This hydrolysation of transmitter is required for the control of neurotransmitter.



Muscarinic Receptor subtypes

M-receptors subtypes	M1	M2	M3	M4	M5
Other name	neural	Cardiac muscarinic receptors	Glandular muscarinic receptors		
Location	Exocrine glands and autonomic ganglia	Atria and conducting tissue of the heart	Exocrine glands and smooth muscle	CNS	Substantia nigra (cns)
Function	Affects arousal attention, REM, emotional response, affective disorder	Cardiac inhibition	Lacrimal, salivary Mostly stimulatory effect	Direct regulatory action on K and Ca ion channels	May regulate dopamine release at terminals within the striatum



1) Direct Acting

- Esters of choline Acetylcholine Carbachol Bethanecol
- Cholinomimetic alkaloid Pilocarpine Muscarine

2) Indirect acting (Anticholinesterase)

- Reversible Neostigmine Physostigmine Pyridostigmine
- Irreversible

* Organophosphorus compounds – Echothiophate

* Organophosphorus compounds – Echothiophate

Cholinomimetic choline esters and natural alkaloids

- Muscarinic cholinergic receptors agonists can be divided into two groups a) synthetic choline esters b) Natural occurring alkaloids
- Methacholine differs from Ach in its greater duration and selective of actions. Its action is prolonged because the added methyl group increase its resistance to hydrolysis by cholinesterase.
- Carbachol and Bethanecol which are unsubstituted carbamoyl esters are completely resistant to hydrolysis by cholinesterase.
- The three major natural alkaloid in this group are pilocarpine, Muscarine and arecoline and have the same principle site of action

Pharmacological actions

1) **Gastrointestinal Tract**: - All muscarinic agonists can stimulate the smooth muscle of the GI and motility because it activates M1 receptor. It increases the peristaltic movement.
2) **Urinary tract**: - Agonistic drugs can cause the contraction of detrusor muscles, increase voiding pressure because it activates M3 receptor. Due to the contraction capacity of urinary bladder decreases.
3) **Respiratory System**: - Agonistics drugs acts on M1 receptor and cause contraction in smooth muscles hence bronchial smooth muscle gets stimulated by muscarinic agonists' drugs.
4) **Eye**: - Muscarinic agonists stimulate the pupillary constrictors and ciliary muscles due to M1 receptors when applied in to the eye can cause pupil constriction and one can't focus properly
5) **Cardiovascular System**: - It effects the vagal stimulation into the heart. It stimulates M2 receptors and cause decrease in heart rate sometimes cause hypotension.
6) **Exocrine glands**: - All the glands gets activates by natural alkaloids and esters of choline. Here M3 receptors gets activates and increases all the secretion of various glands like Salivary glands, Sweat glands etc.

Therapeutic uses

1) **Gastrointestinal disorder**: - Muscarine agonist's drugs are helpful in constipation condition as it increases the motility because it increases the peristaltic movement by stimulating M1 receptors which is present on smooth muscle of GI.
2) **Urinary Bladder Disorder**: - It stimulates M1 receptor which in turn increases or enhance the contraction of detrusor muscles of urinary bladder. Thus it is helpful for the patients having urinary retention.

3) **Xerostomia**: - Some drugs like pilocarpine which is given during xerostomia i.e. dryness of mouth. It acts on M3 receptors which in turn increases the secretion of glands. In xerostomia it is given because it can produce more saliva and helps to get rid of dryness of mouth.
4) **Ophthalmological**: - Some drugs are there like pilocarpine which activates the M1 receptors and increase the pupillary contraction. This makes more secretion of tears and helps in the treatment of various types of glaucoma. It helps in overcoming the mydriasis which is produced by atropine.
5) **CVS**: - It helps in decreasing blood pressure and also helps in relieving the vascular constriction by activating M2 receptor. It helps in the treatment of cardiac arrest or heart attack.

A. Acetylcholine

Acetylcholine is an ammonium compound which cannot penetrate membranes. Therapeutically it don't have any effect because it rapidly get deactivated by cholinesterase. Acetylcholine has both muscarinic and nicotinic activity.

Actions

1) Decrease in heart rate and cardiac output: Acetylcholine on the heart stop the effects of vagal stimulation. Hence it decreases the cardiac rate and helps to reverse Tachycardia

2) Decrease in blood pressure: Injection of acetylcholine can causes vasodilation and lowering of blood pressure. Acetylcholine activates M3 receptors present in smooth muscles of blood vessels which in turn results in the production of NO from arginine.

Actions in GI, **Urinary part and eye**: In GI it increases the peristaltic movement due to the activation of M1 receptors. It increase the motility and hence it is used during constipation.

In the urinary tract, it increases the tone/contraction of detrusor muscles causing expulsion of urine and is used during urinary retention.

In the eye, it increase the ciliary muscle contraction and cause mitosis (Pupillary contraction).

B) Bethanecol

Actions: Bethanecol stimulates the muscarinic receptors which cause increased intestinal motility and tone by stimulating M1 receptor. It also stimulates the detrusor muscles of the bladder which cause expulsion of urine.

Therapeutic applications: Bethanecol is used to stimulate the atonic bladder which helps in no obstructive urinary retention.

Adverse effects:

Adverse effects includes sweating, salivation, decreased blood pressure, nausea, abdominal pain, diarrhoea, and bronchospasm.

c) Pilocarpine

Actions:

When applied to the eye pilocarpine produces a rapid meiosis and contraction of the ciliary muscle. Pilocarpine stimulates M3 receptor and cause more secretions such as sweat, tears, and saliva but this drug has some selectivity issues.

Therapeutic use:

Pilocarpine is used mostly during glaucoma. It decrease the intra-ocular pressure of both narrow and open angle glaucoma.

Adverse effects:

Pilocarpine can cause CNS disturbances as it can enter the brain. It cause more sweating and salivation.

Indirect-Acting Cholinergic Agonists:

Indirect acting cholinergic (Reversible) acts by inhibiting *Acetylcholinesterase* enzyme. It's an enzyme which hydrolyse acetylcholine into acetate and choline. Inhibiting this enzyme can increase the amount of acetylcholine which is produced in synapse

Physostigmine

Actions:

Physostigmine has multiple actions. It acts in both muscarinic and nicotinic sites. It has intermediate kind of action which lasts for 2-6 hours. This drug can enter CNS and stimulate the cholinergic site.

Therapeutic uses:

Urinary tract: - The drug increases **intestinal and bladder motility**, which helps in more urination

Eye: - When applied in the eye, it produces meiosis (Pupillary contraction) which leads to more secretion of tears and helps in treatment of glaucoma but pilocarpine is more effective. It is also helpful during Atropine

Adverse effects:

The effects of physostigmine on the CNS may lead to ***convulsions*** when high doses are used. ***Bradycardia, fall in cardiac output, paralysis of skeletal muscle***. However, these effects are rarely seen with therapeutic doses.

Indirect-Acting Cholinergic Agonists (Irreversible):

Anticholinesterases (Irreversible) basically the organophosphorus compounds bind covalently to acetylcholinesterase and cause irreversible action. This results in long-lasting increase in acetylcholine at all sites where it is released.

Mechanism: - Echothiophate binds covalently with receptors by removing the hydrogen group of active acetylcholinesterase and makes it inactive

Actions: Echothiophate produces intense meiosis and, thus, has found therapeutic use.

Therapeutic uses:

An ophthalmic solution is used for the patients having glaucoma mostly open angle glaucoma. However it's not the first line treatment for glaucoma

Adverse effect:

Long term use can cause Cataract

Cholinergic Antagonist

1) Anti-muscarinic agents –Atropine, Scopolamine, Ipratropium

2) Ganglionic Blockers- Mecyclamine, Nicotine

3) Neuromuscular Blockers

Atracurium, Tubarcurarine

Anti-muscarinic Agents

These are commonly known as Antimuscarinic, these agents (for example, atropine and scopolamine) which block the muscarinic receptors and mimic all the action produced by cholinergic agonistics drugs.

A. Atropine

Atropine is a tertiary amine alkaloid, which has more affinity to muscarinic receptors. It competes with acetylcholine and binds with the receptors and does not allow acetylcholine to bind with the receptors. It can act both centrally and peripherally. The duration of action can be there for 4 hours to one day (sometimes)

Actions:

a) Eye: Atropine blocks all cholinergic activity on the eye by blocking M1 receptors, which can cause mydriasis, no response to light and can't focus near vision (cycloplegic).

b) Gastrointestinal (GI): Atropine block M1 receptor present in GI and reduce the peristaltic movement and motility which in turn reduce activity of the GI tract. This drug only reduce the GI motility but it don't have any effect on HCl.

c) Urinary system: It reduces the contraction of detrusor muscles as it blocks the M1 receptor present in Urinary tract. It reduces the hyper motility of urinary bladder

d) Cardiovascular: Atropine in heart dual effect. At low dose it cause decreased cardiac rate because of M1 receptor at pre junction is blocked. At high dose SA node gets blocked which results in increased heart rate.

E) Secretions: Atropine blocks all the secretion. It blocks the M3 receptors which in turn block all the secretion of body, like Saliva, sweat and cause xerostomia (dryness of mouth).

Therapeutic uses:

a) Ophthalmic: In the eye, topical atropine cause both mydriasis and cycloplegic effects. It is also used during the pain on eye due to spams or contraction of ciliary muscles.

b) Antispasmodic: Atropine is used as an antispasmodic agent to relax the GI tract and bladder. It is helpful curing diarrhoea as it blocks M1 receptors and reduce the motility and peristaltic movement.

c) Antidote for cholinergic agonists: Atropine is used for the treatment of overdoses of cholinesterase inhibitor.

d) Antisecretory: It acts as Antisecretory agent which block secretions in the upper and lower respiratory tract before surgery.

Adverse effects:

It is dose dependent, atropine may cause **dry mouth**, **blurred vision tachycardia**, and **constipation**. Effects on the CNS include **restlessness**, **confusion, hallucinations, and delirium**, which may progress to depression.

B. Scopolamine

Scopolamine belladonna alkaloid. It also produces CNS ad peripheral effect. Scopolamine has greater action on the CNS and has longer duration than atropine.

Actions: Scopolamine is one of the most effective antimotion sickness drugs available. Scopolamine also has the unusual effect of blocking short-term memory. Scopolamine can produce sedation at therapeutic dose and at higher doses it produces excitement.

Therapeutic uses: Although similar to atropine, therapeutic use of scopolamine is limited to prevention of motion sickness (for which it is particularly effective) and to blocking short-term memory

Adverse effect: Adverse effect is as same as Atropine

C) Ipratropium

Inhaled ipratropium is a derivative of atropine, is useful in treating asthma in patients who are contradicted to adrenergic drug. Ipratropium is also used in the management of chronic obstructive pulmonary disease.

D) Tropicamide

These agents are used as ophthalmic solutions for similar conditions as atropine (mydriasis and cyclopegia). Their duration of action is shorter than that of atropine Tropicamide produces mydriasis for 6 hours.

Ganglionic Blockers

Ganglionic blockers specifically act on the nicotinic receptors of both parasympathetic and sympathetic autonomic ganglia. Some also block the ion channels.

A. Nicotine

Nicotine is a major constitute of cigarette. It blocks the cholinergic responses mediated by nicotinic acetylcholine receptors **(nAchRs).**

It also blocks ganglionic stimulation which results in blocking of persistent depolarization and cause relaxation.

B) Mecyclamine

Mecyclamine produces a competitive nicotinic blockade of the ganglia. The duration of action is about 10 hours after a single administration. The uptake of the drug via oral absorption is good, in contrast to that of trimethaphan. As with trimethaphan, it is primarily used to lower blood pressure in emergency situations.

Neuromuscular Blocking Drugs

These drugs block cholinergic transmission between the junction of nerve and muscles. These neuromuscular blockers have similar structure like acetylcholine and they act both agonistic and antagonistic.

A. Nondepolarizing (competitive) blockers

Curare was the first drug that was found which has the ability to block the neuromuscular junction. But Tubarcurarine is considered to be the prototype agent in this class.

Mechanism of action:

At low doses: At low dose these agents interact with the receptors and binds with receptor and prevent acetylcholine to bind with receptor. It competes with the acetylcholine so it is also called as competitive blockers. These agents prevents the depolarization and hence caused in the prevention of muscle contraction.

At high doses: Non depolarizing blockers have same effect like at low dose but along with that it block the ion channels of the end plate. This leads to further weakening in the transmission to neuromuscular junction.

Actions:

All the muscles do not block in the same way. First the muscles of the face will be blocked then neck limbs are paralyzed, finger and finally diaphragm will be paralyzed.

Therapeutic uses:

These are used along with anaesthesia during surgery to relax skeletal muscle. These agents are also used in orthopaedic surgery.

Adverse effects: In general, agents are safe with minimal side effects

Depolarizing agents

Mechanism of action:

Succinylcholine is a drug which is similar to acetylcholine and binds with the receptors and mimic the function of acetylcholine. Like acetylcholine it do not get hydrolysed by acetylcholinesterase. It works in 2 phases

In **first phase** it cause opening of sodium channel which results in depolarization of the receptor.

In **second phase**, continuous binding leads to incapable of more transmission and along with time this depolarization leads to the closing of sodium channel. The receptor becomes resistant to depolarization.

Actions

Succinylcholine first produces short-lasting muscle fasciculation and then it cause paralysis. At high dose it may cause respiratory depression.

Therapeutic uses:

As it has rapid onset and short duration of action, succinylcholine is useful when rapid endotracheal intubation is required during the induction of anaesthesia so that GI content won't come out because of GI Contraction.

References:

Aigner TG. (1995) Pharmacology of memory: cholinergic—glutamatergic interactions. Current opinion in neurobiology, 5(2)

Chavda N, Yadav P, Chaudhari *et al* (2011)Second year student's feedback on teaching methodology and evaluation methods in Pharmacology. National Journal of Physiology, Pharmacy and Pharmacology. 1(1):23.

Cheng Q, Yakel JL (2015) the effect of α7 nicotinic receptor activation on glutamatergic transmission in the hippocampus. Biochemical pharmacology. 2015 Oct 15; 97(4):439-44.

Fibiger HC. (1991) cholinergic mechanisms in learning, memory and dementia: a review of recent evidence. Trends in neurosciences. Jun 1; 14(6):220-3.

Goodman LS. (1996) Goodman and Gilman's the pharmacological basis of therapeutics. New York: McGraw-Hill

Hunter AJ, Murray TK, Jones JA, Cross AJ, Green AR. The cholinergic pharmacology of tetrahydroaminoacridine in vivo and in vitro. British journal of pharmacology. 1989 Sep; 98(1):79.

Idiaquez J and Benarroch E (2018) Anatomy, physiology, and pharmacology of the autonomic nervous system (ANS). In Evaluation and Management of Autonomic Disorders Springer, Cham. (pp. 3-18).

Kleinz MJ, Spence I. (2008) The pharmacology of the autonomic nervous system. Small animal clinical pharmacology. 1; 5:59.

Musial A and Bajda *et al.* (2007) recent developments in cholinesterase's inhibitors for Alzheimer's disease treatment. Current medicinal chemistry, 14(25):2654-79.

Sarter M and Parikh V. Choline transporters, cholinergic transmission and cognition. Nature Reviews Neuroscience. (2005) Jan; 6 (1):48-56.

Silman I and Sussman JL. (2005) Acetylcholinesterase: 'Classical 'and 'non-classical 'functions and pharmacology. Current opinion in pharmacology, 293-302.

Tripathi KD. (2013) Essentials of medical pharmacology. JP Medical Ltd.

Westfall TC. (1977) Local regulation of adrenergic neurotransmission. Physiological Reviews. 57(4):659-728.

Whalen K (2018) Lippincott Illustrated Reviews: Pharmacology. Wolters Kluwer India Pvt Ltd

Wonnacott S, Bermudez I, (2018) nicotinic acetylcholine receptors. British journal of pharmacology. 1785-8.

PANDEMIC OF COVID-19 AND THE SERICULTURAL ENTERPRISE

Vitthalrao B. Khyade

[1]Sericulture Unit, Malegaon Sheti Farm, Agricultural Development Trust Baramati, Shardanagar, (Malegaon Khurd) Post Box No - 35, Baramati, Pune 413 115, Maharashtra

[2]Department of Zoology, Shardabai Pawar Mahila Mahavidyalaya, Shardanagar, Tal. Baramati, Dist. Pune – 413115 (India)

Corresponding author E-mail: vbkhyade.2016@gmail.com

Abstract:

The pandemic through corona virus (COVID-19) made the lockdown protocol all over the world, which affected most of the sectors of human life including the economic growth and development. The sectors of the economy are the recipients of the hard hit through COVID-19 and sericultural sector is not an exception for this influence. The present attempt deals with assessment of the economic influence of COVID-19 on usefulness (profitability)in sericultural practices in Pune District of India. The preliminary data on cultivation of mulberry and rearing of larval instars of silkworm, *Bombyx mori* (L) were elicited from the sample farmers selected randomly from selectedtaluka places of Pune districts of Maharashtra state. The schedule of semi-structure was followed for the collection of the primary data (number of farmers and area of mulberry cultivation) through the discussion through the google meet. The secondary data on quantitative status (yield of the silk cocoon and prices) was collected through silk board (central and state) for the year: 2018; 2019; 2020 and 2021. Statistical analysis of the data revealed that, the cost on mulberry cultivation and rearing of silkworm larvae for commercial silk has remained same for the year: 2018; 2019; 2020 and 2021 (during pre-COVID-19 and COVID-19 periods). The drastic variations in gross returns (in the form of income) accrued was exhibited during the respective periods. This was due to drastic changes, such as closing the significant cocoon markets (of higher prices, like Ramanagara cocoon market of Karnataka state), decreased price)for the silk cocoon and inconvenience in travelling to reach market. The lockdown made to stop the reeling the silk from cocoon by the commercial reeling units. This was resulted into incurring double loss. Sericulture farmers have not recovered the cost of production of Rs. 19377.60 and forgone Rs. 12621.99 per crop of hundred disease free laying (DFLs).

Keywords: COVID-19, Silk Cocoons, Moriculture, Sericulture.

Introduction:

The pandemic of COVID-19 is viral disease caused by corona virus. The corona virus is scientifically recognized as, "Severe-Acute-Respiratory-Syndrome-2" (SARS-CoV-2). This viral pathogen was identified firstly through the outbreak in Wuhan city of the country China, in the month of December of the year: 2019. All the attempts to control COVID-19 there are reported as failed. This situation was allowed the corona virus to spread worldwide with significant speed. On the day 30 January, in the year: 2020 the "World Health Organization" (WHO) used to declare a "the Public Health Emergency of International Concern". On the day 11 March, in the year: 2020 the "World Health Organization" (WHO) used further to declare a "the COVID – Pandemic". As per the record of 7 April, 2022, the pandemic of COVID – 19had caused more than four hundred ninety-five million persons affected by the disease of COVID – 19 and about six million deaths through the disease of COVID – 19. Isn't it a deadliest in history? The symptoms of corona disease (COVID -19) appeared to range from undetectable to deadly. The fever, cough of dry nature and fatigue are common symptoms of disease of COVID – 19. There was severe illness more likely in elderly patients of COVID – 19. The pathogens of disease COVID – 19 deserve capacity of fast transmission through the breathing of the patients.When the people are in close proximity, the risk of breathingsin close proximities of theair contaminated with "COVID – 19" was themost significant. The contaminated fluid reaching close to the eyes, nose and mouth of healthy person was also responsible for the transmission of "COVID – 19" disease. Infected persons are typically contagious for 10 days, and can spread the virus even if they do not develop symptoms. Mutations have produced many strains (variants) with varying degrees of infectivity and virulence (Zoumpourlis, *et al*., 2020).

Since December, 2020, the vaccines against "COVID – 19" disease have been approved. The vaccines against "COVID – 19" disease have been widely distributed all over the world. The preventive measures for the "COVID – 19" disease include: masking over mouth and nose; social distancing; improved ventilation; filtration of the air; etc. The quarantining the symptomatic persons serve to control the transmission of "COVID – 19" disease. Use of monoclonal antibodies and novel antiviral medicines serve to treat the patients of "COVID – 19" disease.

According to the Office of the Commissioner (23 November 2020), the treatment for the COVID-19 disease include: use of "Monoclonal Antibody", Antiviral drugs of novel quality and the efforts towards controlling the symptoms of this disease. Interventions through the government include: restrictions on travelling, lockdown protocol, restrictions on the business, controlling the hazards at work places and to trace the contact with infected persons.

The pandemic of the COVID – 19 exerted the triggered and severe disruption for the social and economic sectors all over the world. Thelargest global-recession, disruption in chain of supply, panic buying, shortage of widespread supply (example: food supply) were the significant influences caused through the pandemic of COVID – 19- disease. The agriculture-based practices like sericulture are also influenced by the pandemic of COVID – 19 – disease.

The sericulture is integrated well with the system of farming. It has the capabilities of continuous (throughout the year) earning or generating significant income for the farmers. According to Hanumappa and Erappa (1985), the sericulture requires minimum capital investment. Sericulture provides the employment for a complete year (continuous twelve months). China is the first country for the highest production of raw silk. India stands second in raw silk production. Rearing of silkworm larvae through the use of leaves of mulberry in India is for about agriculture land of about 1, 91,893 ha. For the year: 2006 – 2007, the Indian mulberry silk production was reported 16525 MT. There was export of silk goods for the year: 2006 – 2007 and earned US$ 737.76 in year 2006-07 (Anonymous, 2007). The advent of new bivoltine double hybrid (double cross breed) race (variety) of silkworm and innovative technology of moricultural practices and rearing methods made the sericulture productivity changing at fast rate with an upward trend. Changes in the nature of commercial views, influence of global warming, pandemics of diseases like COVID – 19 are influencing the cost of production, quantity of production and quality of production are the major factors to be considered for all the sectors of occupations. Inthis regard, it is imperative to understand the economics of sericulture. Awareness and motivation among the sericulture farmers is highly essential. Such type of catalysis is going to motivate the new farmers to take up sericulture and increase their income. Some of the earlier studies tried their best to find out the economic prospects in sericulture (Anonymous, 1989, Ravindran *et al*., 1993, Lakshmanan *et al*., 1996). Thepresent attempt was aimed to carry out the analysis of sericultural economics with reference to the influence of pandemic COVID -19 – disease.

Material and Methods:

The study was completed through the steps like, selection of region, collection of the data and statistical analysis of collected data. The agricultural land under the cultivation of mulberry and number of farmers busy in the rearing of silkworm larvae for commercial silk, both are significant in Pune districts of Maharashtra state at Indian national level, which made to select purposely for the present attempt. Pune districts of Maharashtra state stands first both in mulberry acreage and production of mulberry silk cocoon in the country (Anonymous, 2019). For the purpose to assess the influence of COVID-19 induced lockdown on usefulness (in the

form of profit) of sericultural enterprise, the essential primary data with reference to: resources used in the cultivation, production of silk cocoon through the rearing the silkworm larval instars, number of crops (harvesting the leaves of mulberry, *Morus alba* L. and number of cycles of the rearing the silkworm larval instars) taken per year, number of DFLs (Disease Free Layings) used for each crop and yield (in the form of quantity of silk cocoons)were obtained from farmers (group of selected farmers. The sample size: 100 farmers) through focus tele discussion through the google meet through the Krishi Vidnyan Kendra, Baramati, office of Central Silk Board and Maharashtra State Silk Board (at Malegaon Tal. Baramati Dist. Pune – 413115). The basic tools of statistics (Zulfiqar Ali and Bala Bhaskar, 2016; Manjunatha*et al*., 2018) and the technique for budgeting the enterprise were employed to assess the profitability of sericulture enterprise (Chinnappa*et al*, 2020). The primary data collected were relevant (or pertinent) to the crops of mulberry, *Morus alba* (L.) and production of silk cocoon through rearing of larval instars of silkworm, *Bombyx mori* (L.) for the year: 2018; 2019; 2020 and 2021 (during pre-COVID-19 and COVID-19 periods). In order to measure (or to gauge) the influence of pre-COVID-19 and COVID-19 periods, data on cost of production (in the form of money) and the returns (in the form of money) during the same months of the agricultural year: 2018; 2019; 2020 and 2021was elicited from the same sample of selected farmers of Pune districts of Maharashtra state. Further, the secondary data on adventsof silk cocoons and prices of the silk cocoons of bivoltine (pure race) and double hybrid (race of crossbreed) in Maharashtra state and the market at Ramanagaram of Karnataka state was collected through the Krishi Vidnyan Kendra, Baramati, office of Central Silk Board and Maharashtra State Silk Board (at Malegaon Tal. Baramati Dist. Pune – 413115) Central Silk (Silk Bulletin, 2020). For the purpose to obtain consistency in the results, attempt on collection of the data was in triplicate set (three sets of samples for present attempt include: Baramati Taluka, Daund Taluka and Indapur Taluka).

Results and Discussion:

The results on the attempt to analyse the influence of pandemic of COVID – 19 on sericultural enterprise are summarised in tables (Table- 1, 2 and 3) and presented in fig. 1. The variable cost and fixed costs for the mulberry cultivation are the two different types of expenditure considered in present attempt. The variable cost (expenditure in Rs) against human labour, bullock labour, use of farm yard manure (FYM), use of fertilizers and the use of plant protection chemicals in present attempt of analysis was found recorded Rs. 15850; Rs. 5700; Rs. 24000; Rs. 12000 and Rs. 1600 respectively (Table-1 A). The total variable cost (TVC) for the mulberry cultivation in the region selected in the attempt was Rs. 69150 (Table-1 A).The heads of expenditure with reference to fixed cost for the production of leaves of mulberry, *Morus alba*

(L) considered in present attempt include: Depreciation (Rs); Interest on Fixed Capital (Rs); Rental Value of land (Rs); Total Fixed Cost (TFC) (Rs); Total Cost (TC) (Rs); Total Yield of Mulberry Leaves (Kg) and Cost Per Kg of Mulberry leaves (Rs) and found recorded: Rs. 8089; Rs. 12220; Rs. 16800; Rs. 37109; Rs. 93309; 24656 Kg and Rs. 3.75 respectively (Table-1 B). In the irrigated region (Baramati Taluka and Indapur Taluka), the cost of production (both, variable and fixed) of leaves of mulberry was lower in comparison with rainfed region (Daund Taluka), sample selected for the study in present attempt.The unit cost of production (both, variable and fixed) of leaves of mulberry was lower under the irrigated condition as compared to the rainfed condition. However, the gross returns, net returns were more under irrigated condition over rainfed condition with higher being among big farmers over medium and small farmers. In cocoon production, the total cost of cocoon production was lower with rainfed farmers as compared to irrigated farmers with lesser among small farmers category over medium and big farmers category. The unit cost of cocoon production was lower under irrigated farmers over rainfed farmers with least being among medium farmers over big and small.The heads of expenditures for labours for the rearing the larval instars of silkworm, *Bombyx mori* (L) considered in the present attempt include: expenditure for rearing the third instars; expenditure for rearing the fourth instars; expenditure for rearing the fifth instars; Transfer of mature fifth instar on mountage (chandrake) (OR Spreading the Mountage on mature fifth instar; Harvesting the silk cocoon and grading; Cleaning the bed; Disinfection of the Rearing House; Total Cost of Production for Labour and recorded Rs. 540; Rs. 1080; Rs. 2700; Rs. 360; Rs. 720; Rs. 270; Rs. 270; Rs. 5940 respectively Table- 2 A).The heads of expenditures for the rearing the larval instars of silkworm, *Bombyx mori* (L) considered in the present attempt include: Larvae after the Second moult (Chawki worms) (DFL); Bleaching powder (Kg); Detol (Lit); Astra (gm); Vijetha (Kg); Leaves of mulberry (Kg); Lime (Kg) and total expenditure and recorded Rs 2300; Rs. 225; Rs. 600; Rs. 600; Rs. 600; Rs. 2486; Rs. 75 and Rs. 6886 respectively (Table-2B).

Total cost of production in sericulture enterprise for the pre-pandemic period (Year: 2018); pandemic period (Year: 2019 and 2020) and post pandemic period (Year: 2021 and 2022) for Pune district was found reported Rs. 29066. The cost of production was found remained constant for all the three periods (pre-pandemic; pandemic and post-pandemic) of the study. The yield of silk cocoon (Kg) for the pre-pandemic period (Year: 2018); pandemic period (Year: 2019 and 2020) and post pandemic period (Year: 2021 and 2022) for Pune district was found reported 120 Kg. The yield of silk cocoon (Kg) was found remained constant for all the three periods (pre-pandemic; pandemic and post-pandemic)of the study. The price of silk cocoons per Kg was found fixed in Maharashtra (Rs. 400 per Kg silk cocoon). The price of silk cocoons per

Kg was found fixed in Karnataka state was variable. It depends on the quality of the silk cocoon. The price of silk cocoon in Karnataka state isranging from Rs. 200 to Rs 1600 (per Kg silk cocoon). The well esteemed Ramanagarasilk cocoon market is one of the significant markets for silk cocoon in Asia. Location of this Ramanagara silk cocoon market is 40 km away from Bangalore. The Ramanagara silk cocoon market is towards Mysore.The record of the silk cocoons sold each day in this market is about 40,000 Kg to 50,000 kg (on an average). This is the silk market of authority of Karnataka government. This market use to welcome the silk city to Ramanagaram, between Sholay Hill and another Sri SriRavanaSiddheshwara Betta between the two hills, on the banks of the Arkavati river. It deserves the historical background, which appears to be the due to the traditional silk industries established during the political period of Emperor TipuSultan of Mysore.The Farm of Coconuthas been established here in about 2.00 acresof land. The significant feature of this farm of coconut lies in nurturing the farmers of silk production and the reelers of the silk. This market availed the silk work for many more families. Therefore, the Ramanagar silk market gained the accreditation of the most significant commercial centre in Asia (Anonymous, 2020).The average price of selling the silk cocoons by the farmers during the pre-COVID-19 period was Rs.400.00 per Kg. The pandemic of COVID – 19 made to lower this price to Rs. 200 per Kg of silk cocoons. while that of COVID-19 period was Rs.200.00. This was evident with attempt of studies byNiyati and Vijayamba (2020). The gross return accrued to farmers during the pandemic of "COVID-19- induced lockdown period" was the most insignificant. This situation made not even covered the total cost incurred by silk farmers and leading to face a double loss.Constraints faced by Indian farmers of sericultureduring "COVID-19 lockdown period" include: (1) Crashes in price of silk cocoon due to negative psychology (unwillingness) of the reelers to purchase the silk cocoons; (2) Locks to the silk cocoon markets (The closure of silk cocoon market; (3) Closure of the rearing of silkworm larvae by the farmers. This was due to non-availability of chawki worms (2nd instar) at the government farms. Due to non-availability of the human resource (labour), the government farms of chawki rearing were closed the work during lockdown period of pandemic of COVID - 19; (4) Inconvenience in the transport of silk cocoon from the rearing farm to the market; (5) Lockdown period during pandemic of COVID – 19 resulted into non-availability of critical inputs for moriculture and appliances essential for silkworm. rearing appliances as majority of shops were closed during lockdown (Kumaresan*et al*, 2020 & Anonymous, 2020).

Table 1 (A): Variable Cost of Production of Mulberry leaves through the Maintenance of Garden of Mulberry, *Morus alba* (L).

Sr. No.	Particulars of variable cost (expenditure)	Quantity	Rate (Rs)	Total Variable Expenditure (Rs)
1	Human Resource as Labours	54	300	16200
2	Resources of Bullock as Labours	06	950	5700
3	Input – Farm Yard Manure (FYM) (Quintal)	06	4000	24000
4	Input– Fertilizers (Quintal)	06	2000	12000
5	Input- Plant Protection Chemicals (Lit.)	28.125	400	11250
6	Total Variable Cost (Rs)	-	-	69150

Table 1 (B): Fixed Cost of Production of Mulberry leaves through the Maintenance of Garden of Mulberry, *Morus alba* (L).

Sr. No.	Particular of Fixed Cost	Total Expenditure (Fixed Cost) (Rs)
1	Depreciation (Rs)	-08089
2	Interest on Fixed Capital (Rs)	12220
3	Rental Value of Land (Rs)	16800
4	Total Fixed Cost (Rs)	37109
5	Total Cost (TC) (Rs)	93309
6	Yield of Leaves of Mulberry (Kg)	24856
7	Cost of Production of Leaves of Mulberry per Kg	03.75

Table 2 (A): The Expenditures for Labours for the Rearing the Larval Instars of Silkworm, *Bombyx mori* (L)

Sr. No.	Expenditure Particulars	Quantity	Rate (Rs)	Total Expenditure (Rs)
1	Labour Expenditure for Rearing the Third Instar Larvae	2	300	0600
2	Labour Expenditure for Rearing the Fourth Instar Larvae	3.6	300	1080
3	Labour Expenditure for Rearing the Fifth Instar Larvae	9	300	2700
4	Transfer of the mountage (Chandrika) on mature larvae for spinning	2.4	150	360
5	Harvesting the Cocoons and their grading	4.8	150	720
6	Disinfection of Rearing House	0.9	300	270
7	Bed cleaning	0.9	300	270
8	Total Expenditure for labour for rearing silkworm larvae	-	-	5940

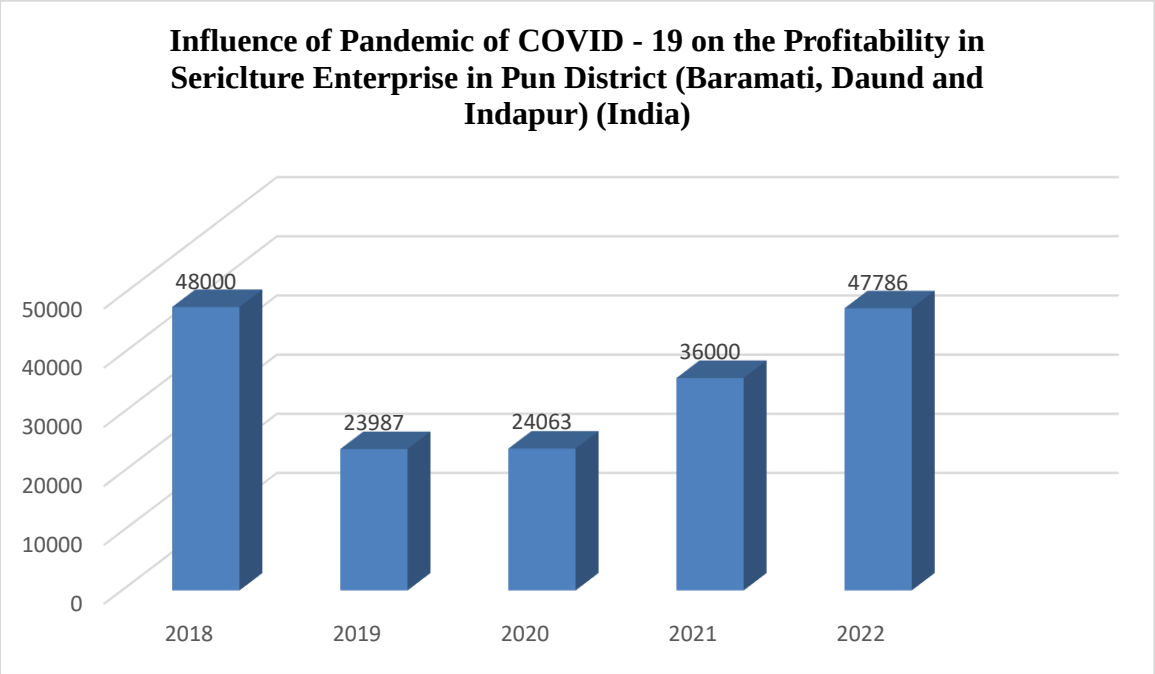
Table 2 (B): The Expenditures towards the Inputs for the Rearing the Larval Instars of Silkworm, *Bombyx mori* (L)

Sr. No.	Inputs Particulars	Quantity	Rate (Rs)	Total Expenditure (Rs)
1	Larvae after the second moult (Chawki worms)	100	34.5	3450
2	Bleaching Powder (Kg)	7.5	30	0225
3	Lime (Kg)	7.5	10	0075
4	Decol (Lit)	3	200	0600
5	Astra (gm)	150	200	0600
6	Vijetha (Kg)	6	100	0600
7	Mulberry leaves (Kg)	2486	3.75	9322.50
8	Total Variable Cost (Rs)	-	-	20812.50
9	Depreciation (Rs)	-	-	1497
10	Interest on Fixed Capital (Rs)	-	-	6757
11	Total Fixed Cost (Rs)	-	-	8254
12	Total Cost (Rs)	-	-	29066.50

Table 3: Influence of Pandemic of COVID – 19 on Profitability (Advantageousness) of Production of silk cocoons

Sr. No.	Particulars	Year: 2018 (Pre-COVID-19)	Year: 2019 (COVID-19)	Year: 2020 (COVID-19)	Year: 2021 (Post-COVID-19)	Year: 2022 (Post-COVID-19)
1	Total Cost of Production (Rs)	29066.50 (±476.87)	29066.50 (±715.31)	29066.50 (±597.08)	29066.50 (±538.48)	29066.50 (±521.69)
2	Yield of Silk cocoon (Kg)	120 (±1.968)	120 (±6.891)	120 (±8.429)	120 (±19.786)	120 (±21.173)
3	Price (Rs) (per Kg Silk Cocoon)	400	200	200**	300*	400
4	Gross Return (Rs)	48000 (±779.49)	24000 (±391.74)	24000** (±489.68)	36000* (±447.71)	48000 (±721.84)
5	Net Return (Rs)	18933.50	-5066.50	-5066.50**	-3799.50*	18933.50
6	Cost per Kg	242.22	242.22	242.22	242.22	242.22
7	Gross Return per Kg Profit per Kg	400	200	200	300	400
8	Profit per Kg	157.78	-42.22	-42.22**	118.33*	157.78

**: P≤0.01; *: P≤0.05



Conclusion:

The attempt of studies on the analysis of the influence of pandemic of the COVID – 19 on sericulture enterprise coincided with two crops each of hundred DFLs (Disease Free Laying) concluding leading to double loss. The silk farmers, in addition to economic loss have encountered personal inconveniences at the time of marketing of silk cocoon the local market as well as to Ramanagara market. This inconvenience was due tolack of transport and frightened psychology towards the possibility of getting infected with corona virus. The farmers busy in mulberry cultivation and rearing the silkworms during the pandemic of the COVID – 19 in Pune district (Baramati taluka, Indapur taluka and Daund taluka) faced the strategy of greater expenditure and insignificant returns. Looking into the magnitude of loss, in future, the government (or the associations of silk farmers) (or both)should plan on the reasonable relief/ compensation to sericulture growers to retain their interest in sericulture enterprise. Besides, the government (or the associations of silk farmers) (or both) should plan for covering such unforeseen (unexpected) situations having negative (or non-significant) repercussions on- silk farming community under the coverage of insurance.

Acknowledgement:

This work would not have been possible without the support of the Managing Editor, Bhumi Publishing (Nigave Khalasa, Kolhapur 416207, Maharashtra, INDIA) and the office staff members at the silk board (state government and central government) at the Vidyanagar of Malegaon (Tal. Baramati district Pune). Teachers of commerce faculty, Sharadabai Pawar Mahila Arts, Commerce and Science College, Sharadanagar helped a lot (more than the expectation of author). The efforts of Managing Editor, Bhumi Publishing (Nigave Khalasa, Kolhapur 416207, Maharashtra, India) deserve appreciations and exert a grand salutary influence.

References:

Anonymous (2019) Seri-States of India- A Profile, Central Silk Board. pp.1-8.

Anonymous (2020) Udhampur's sericulture farmers demand opening of Cocoon Auction Market amid COVID-19 crisis. ANI News. Updated on 9 June 2020. (https://www.aninews.in/news/national/general-news/udhampurs-sericulture-farmers-demand- opening-of-cocoon-auction-market-amid-covid-19-crisis20200609103806/)

Chinnappa B., Kiran Kumar R Patil, Manjunatha G. R and Soumya H. S. (2020) Economic Utilisation of Areca Leaf Sheaths for Rural Livelihood. Indian Journal of Agricultural Economics, 75(1), 90-110

CSRTI-Mysore (2019) Annual Report of Central Sericultural Research & Training Institute (2018-19), pp.1-126.

Kumaresan P., Nanjunda S., Jaganathan K., Kumar Santosh Gopal, Rudranna Gowda H., Ravikumar D., Geetha G. S., Joycy Rani Dasari, Halagunde Gowda G. R. and Shambhulingappa H.H. (2020) An Impact Analysis of Coronavirus Crisis on Indian Silk Industry, Central Silk Board, pp:1-39.

Laiqh A. Khan (2020) Ban sought on import of silk from China, The Hindu, updated on 16 July 2020 (https://www.thehindu.com/news/national/karnataka/ban-sought-on-import-of-silkfrom-china/article32094830.ece)

Manjunatha G. R., ShafiAfroz, Dipesh Pandit, Biswas T. D. and Chanda S. (2018) MTS-3599: Study on mulberry sericulture production in West Bengal: A Statistical Approach, Annual report (2018-19) of CSRTI-Berhampore, pp. 133.

Niyati, S and Vijayamba, R (2020) COVID-19 in Rural India–II: I Had to Sell Cocoon @Rs 150/kg, Instead of Rs 500/kg, NEWS CLICKS updated on 7 April 2020 (https://www.newsclick.in/COVID-19-Rural-India-Sell-Cocoon-Rs-150-kg-Instead-%20Rs%20500-kg)

Rajit Ranjan Okhandiar and Kumaresan, P. (2019) Indian Sericulture Industry leaps ahead, 6 th Asia-Pacific Congress of sericulture & Insect Biotechnology (APSERI-2019), CSB, Mysore during 2-4 March 2019, pp.7-11.

Silk Bulletin (2020) Silk flash of Central Silk Board, Browsed on March-Aug 2020 (http://csb.gov.in/statistics/prices/silk-flash/).

WHO (2020) Coronavirus Disease (COVID-19) Dashboard of WHO, browed on 17 Aug 2020 (https://covid19.who.int)

Zoumpourlis V, Goulielmaki M, Rizos E, Baliou S, Spandidos DA (October 2020). "[Comment] The COVID-19 pandemic as a scientific and social challenge in the 21st century". Molecular Medicine Reports. 22 (4): 3035–3048. doi:10.3892/mmr.2020.11393. PMC 7453598. PMID 32945405.

"WHO-convened global study of origins of SARS-CoV-2: China Part". World Health Organization. 30 March 2021. Retrieved 31 March 2021.

COVID-19 Dashboard. ArcGIS. Johns Hopkins University. Retrieved 7 April 2022.

"The pandemic's true death toll". The Economist. Retrieved 28 March 2022.

CDC (11 February 2020). "COVID-19 and Your Health". Centers for Disease Control and Prevention. Retrieved 6 January 2022.

Clinical Questions about COVID-19: Questions and Answers US Centers for Disease Control and Prevention

Office of the Commissioner (23 November 2020). "Coronavirus (COVID-19) Update: FDA Authorizes Monoclonal Antibodies for Treatment of COVID-19". FDA. Retrieved 19 December 2021.

"The Great Lockdown: Worst Economic Downturn Since the Great Depression". IMF Blog. Retrieved 23 April 2020.

"Coronavirus - The latest news on the COVID-19 outbreak". Sky News. Retrieved 20 July 2021.

Anonymous (2019) Seri-States of India- A Profile, Central Silk Board. pp.1-8.

Anonymous (2020) Udhampur's sericulture farmers demand opening of Cocoon Auction Market amid COVID-19 crisis. ANI News. Updated on 9 June 2020. (https://www.aninews.in/news/national/general-news/udhampurs-sericulture-farmers-demand- opening-of-cocoon-auction-market-amid-covid-19-crisis20200609103806/)

Chinnappa B., Kiran Kumar R Patil, Manjunatha G. R and Soumya H. S. (2020) Economic Utilisation of Areca Leaf Sheaths for Rural Livelihood. Indian Journal of Agricultural Economics, 75(1), 90-110.

CSRTI-Mysore (2019) Annual Report of Central Sericultural Research & Training Institute (2018-19), pp.1-126.

Kumaresan P., Nanjunda S., Jaganathan K., Kumar Santosh Gopal, Rudranna Gowda H., Ravikumar D., Geetha G. S., Joycy Rani Dasari, Halagunde Gowda G. R. and Shambhulingappa H.H. (2020) An Impact Analysis of Coronavirus Crisis on Indian Silk Industry, Central Silk Board, pp:1-39.

Laiqh A. Khan (2020) Ban sought on import of silk from China, The Hindu, updated on 16 July 2020 (https://www.thehindu.com/news/national/karnataka/ban-sought-on-import-of-silkfrom-china/article32094830.ece)

Manjunatha G. R., ShafiAfroz, Dipesh Pandit, Biswas T. D. and Chanda S. (2018) MTS-3599: Study on mulberry sericulture production in West Bengal: A Statistical Approach, Annual report (2018-19) of CSRTI-Berhampore, pp. 133.

Zulfiqar Ali, S Bala Bhaskar (2016). Basic statistical tools in research and data analysis. Indian Journal of Anaesthesia Vol.60, Issue 9 Pages: 662-669. www.ijaweb.org

Niyati, S and Vijayamba, R (2020) COVID-19 in Rural India–II: I Had to Sell Cocoon @Rs 150/kg, Instead of Rs 500/kg, NEWS CLICKS updated on 7 April 2020 (https://www.newsclick.in/COVID-19-Rural-India-Sell-Cocoon-Rs-150-kg-Instead-%20Rs%20500-kg)

Rajit Ranjan Okhandiar and Kumaresan, P. (2019) Indian Sericulture Industry leaps ahead, 6 th Asia-Pacific Congress of sericulture & Insect Biotechnology (APSERI-2019), CSB, Mysore during 2-4 March 2019, pp.7-11.

Silk Bulletin (2020) Silk flash of Central Silk Board, Browsed on March-Aug 2020 (http://csb.gov.in/statistics/prices/silk-flash/).

WHO (2020) Coronavirus Disease (COVID-19) Dashboard of WHO, browed on 17 Aug 2020 (https://covid19.who.int)

Zoumpourlis V, Goulielmaki M, Rizos E, Baliou S, Spandidos DA (October 2020). "[Comment] The COVID-19 pandemic as a scientific and social challenge in the 21st century". Molecular Medicine Reports. 22 (4): 3035–3048. doi:10.3892/mmr.2020.11393. PMC 7453598. PMID 32945405.

WHO-convened global study of origins of SARS-CoV-2: China Part". World Health Organization. 30 March 2021. Retrieved 31 March 2021.

"COVID-19 Dashboard by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University (JHU)". ArcGIS. Johns Hopkins University. Retrieved 7 April 2022.

Office of the Commissioner (23 November 2020). "Coronavirus (COVID-19) Update: FDA Authorizes Monoclonal Antibodies for Treatment of COVID-19". FDA. Retrieved 19 December 2021.Anonymous. 1989. An economic survey of sericulture in Tamil Nadu. Deptt. Of Agricultural Economics, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.

Anonymous. 2007. Annual Report. DGCIS, Ministry of commerce, Govt. of India.

Hanumappa, H.G. and Erappa,S. 1985. Economic issues in sericulture: study of Karnataka. Economic and Political Weekly 20(31):3122-3224.

Jayaram, H., R. Ganapatathi Rao, S. Lakshmanan and B. Mallikarjuna. 1996. Role of input delivery system in sericulture-An empirical study. Central Silk Board, Bangalore.

Kulkarni, V.S. 1993. Yield gaps analysis of bivoltine (NB4D2) cocoon production in Mandya district. CSRTI, Mysore.

Lakshmanan, S., B. Mallikarjuna, H. Jayaram, R. Ganapathy Rao, M. R. Subramanian, R.G. Geeta Devi and R. K. Dutta. 1996. Economic issues of production of mulberry sericulture in Tamilnadu-Micro-economics study. Indian J. Seric. 35(2): 128-131.

Marihonnaiah, Y. 1986. Studies resource productivity in cocoon production in tumkur district of Karnataka. M. Sc. (Agril.) Thesis, University of Agricultural Sciences, Bangalore, India.

Neelakantasastry, T.V. 1982. Cost structure of sericulture industry in Chittor district of Andhra Pradesh. M. Sc. (Agri.) Thesis. Andhra Pradesh Agricultural university, Hyderabad, India.

Ravindran, N., S. Anita, B. Parthipan and S. Elangovan. 1993. Sericulture: A profitable farm venture. Agricultural Situation in India 18 (3): 23-26.

POST-TRANSLATIONAL MODIFICATIONS (PTMS)

Surendra Kumar*, Aranav Yadav and Ankitesh Kumar
Department of Genetics and Plant Breeding,
Chandra Shekhar Azad University of Agriculture and Technology,
Kanpur – 2080 02, Uttar Pradesh, India
*Corresponding author E-mail: skcsauk@gmail.com

What does PTM mean?

Changes in internal and external environment must be detected and responded to by cells. Chemically altering proteins is one approach for adapting to these changes. Reversible post-translational modifications (PTMs) of proteins transfer conditional chemical changes from sensors to effectors. PTMs play a critical role in many cellular processes such as cellular development, protein degradation, signalling and regulatory processes, gene expression control, and protein-protein interactions, as well as contributing to biological processes and pathological situations.

Introduction:

Post-transcriptional modification, also known as co-transcriptional modification, is a set of biological processes that occur in most eukaryotic cells and involve chemically altering an RNA primary transcript after it has been transcribed from a gene to produce a mature, functional RNA molecule that can then leave the nucleus and perform a variety of functions in the cell. There are many types of post-transcriptional modifications achieved through a diverse class of molecular mechanisms. The conversion of precursor messenger RNA transcripts into mature messenger RNA, which can then be translated into protein, is one example. The addition of a 5' cap, the addition of a 3' polyadenylated tail, and RNA splicing are three important stages that drastically alter the chemical structure of the RNA molecule. Because the initial precursor mRNA produced by transcription often contains both exons (coding sequences) and introns (non-coding sequences), splicing removes the introns and directly links the exons, while the cap and tail facilitate the transport of the mRNA to a ribosome and protect it from molecular degradation.

Following protein production, post-translational modification (PTM) refers to the covalent and generally enzymatic modification of proteins. Ribosomes convert mRNA into polypeptide chains, which may later proceed through PTM to generate the mature protein product. When prohormones are transformed to hormones, PTMs are crucial components in cell

signalling. Post-translational modifications (PTMs) involve the addition of a chemical group following protein translation (Walsh *et al.*, 2005), regulating, stability and function.PTMs are essential for a variety of cellular processes and provide another level of protein regulation, which is usually reversible. There are a large number of PTMs that take place in the cell such as phosphorylation (Burnett and Kennedy, 1954), methylation (Grewal and Rice, 2004), acetylation (Glozak *et al*., 2005) and glycosylation (Spiro, 2002); regulating various biological activities such as transcriptional regulation (Waby *et al.,* 2008) and protein degradation (Orford *et al.,* 1997.Post-translational modifications can occur on the side chains of amino acids or at the C- or N-termini of proteins. They can alter an existing functional group or introduce a new one, such as phosphate, to expand the chemical repertory of the 20 conventional amino acids. Phosphorylation is the most prevalent post-translational modification and is a typical technique for modulating enzyme activity. Many eukaryotic and prokaryotic proteins have carbohydrate molecules linked to them, a process known as glycosylation, which can help increase protein folding and stability while also having regulatory functions. Lipidation is the process of attaching lipid molecules to a protein or part of a protein that is linked to the cell membrane.Cleaving peptide bonds, as in converting a propeptide to a mature form or deleting the initiator methionine residue, are two further types of post-translational modification. Disulfide bond production from cysteine residues is referred to be a post-translational modification, for example, once disulfide links are created, the peptide hormone insulin is cut twice and a propeptide is removed from the middle of the chain; the resulting protein is made up of two polypeptide chains joined by disulfide bonds.

Oxidative stress causes some types of post-translational modifications. Carbonylation is one example of a modification that causes the changed protein to be targeted for breakdown, which can lead to the creation of protein aggregates. Specific amino acid modifications can be used as biomarkers indicating oxidative damage. Sites with a functional group that can act as a nucleophile in the reaction, such as the hydroxyl groups of serine, threonine, and tyrosine; the amine forms of lysine, arginine, and histidine; the thiolate anion of cysteine; the carboxylates of aspartate and glutamate; and the N- and C-termini, are frequently modified post-translationally. Furthermore, while being a poor nucleophile, asparagine's amide can function as a glycan attachment site. Rarer modifications can occur at oxidized methionines and at some methylenes in side chains.

The most common PTMs are the specific cleavage of precursor proteins, formation of disulfide bonds, or covalent addition or removal of lowmolecular-weight groups, thus leading to modifications such as acetylation, amidation, biotinylation, cysteinylation, deamidation,

farnesylation, formylation, geranylgeranylation, glutathionylation, glycation (nonenzymatic conjugation with carbohydrates), glycosylation (enzymatic conjugation with carbohydrates), hydroxylation, methylation, mono-ADP-ribosylation, myristoylation, oxidation, palmitoylation, phosphorylation, poly(ADP-ribosyl)ation, stearoylation, or sulfation. All amino acid side chains are known to undergo chemical diversification due to various PTMs.

How does Post Translational Modification work?

PTMs can occur at any time during the life cycle of a protein. Many proteins are changed immediately after they are translated to help them fold correctly or to direct the nascent protein to certain cellular locations (such as the nucleus or membrane). More changes are made after folding and localisation to activate or inactivate catalytic activity. Tags that target a protein for breakdown are also covalently bonded to proteins. They are modified via a step-by-step procedure of protein maturation or activation that combines post-translational cleavage and the addition of functional groups.

Where does Post Translational Modification occur?

PTMs are mediated by enzymatic activity and occur at different amino acid side chains or peptide links. Enzymes that perform more than 200 types of PTMs account for 5% of the proteome. Kinases, phosphatases, transferases, and ligases, which add or remove functional groups, proteins, lipids, or sugars from amino acid side chains, and proteases, which cleave peptide bonds to remove specific sequences or regulatory subunits, are examples of these enzymes. Many proteins, such as autokinase and autoprotolytic domains, can change themselves via autocatalytic domains. Depending on the nature of the alteration, PTMs may also be reversible. Phosphates, for example, hydrolyze the phosphate group of a protein to remove it and reverse its biological action.

Types of PTMs analysis

- Phosphorylation
- Glycosylation
- Ubiquitination
- S-Nitrosylation
- Biotinylation
- Methylation
- N-Acetylation
- Lipidation
- S-myristoylation
- S-prenylation
- Alkylation
- Glutamylation

Most common Post-Translational Modifications:

Recent developments in mass-spectrometry (MS) methods have enabled the identification of thousands of PTM sites. Consequently, novel enrichment strategies have uncovered the global cellular importance of several types of modifications (e.g., acetylation, ubiquitylation, O-GlNac,

N-linked glycosylation). More than 200 diverse types of PTMs are currently known, ranging from small chemical modifications (e.g., phosphorylation and acetylation) to the addition of complete proteins (e.g., ubiquitylation). Some are as follows:

- **Phosphorylation**

Phosphorylation of proteins is the most researched post-translational modification. Protein function, enzymatic activity, protein–protein interactions, and protein localisation are all regulated by phosphorylation on serine, threonine, and tyrosine residues. Phosphorylation is a reversible process that is performed by phosphatases. Phosphorylated proteins can be dephosphorylated by protein dephosphatases.

- **Glycosylation and Glycanation**

Glycosylation occurs on the majority of proteins synthesised on ribosomes linked with the endoplasmic reticulum. That is, sugar moieties are covalently attached to the polypeptide chain. N-linked glycosylation of asparagine and O-linked glycosylation of serine and threonine are the two most common kinds of glycosylation in Eukaryotes.

- **Ubiquitination**

Protein ubiquitination occurs when a covalent ubiquitin is attached to lysine, cysteine, serine, threonine, or the N-terminus of a protein. Ubiquitin is a tiny protein with a molecular weight of +/-8.6 kDa that is found in practically all tissues. A three-enzyme cascade catalyses ubiquitination, an enzymatic reaction (E1, E2, and E3). This ensures substrate specificity as well as the procedures of activation, conjugation, and ligation.

Techniques to detect PTMs

Post-translational modification of proteins can be experimentally detected by a variety of techniques, including:

- Mass spectrometry
- Eastern blotting
- Western blotting

Function of PTMs

Category of PTMs and their functions:

- Phosphorylation - Change protein comformation; Activate/inactivate catalytic activity
- Ubiquitination - Target protein for degradation
- Glycosylation - Direct a protein to its destination
- Acetylation - Affect protein comformation and its affinity to other proteins
- Lipidation- Affect the activity and subcellular location of a protein

- Methylation - Affect protein comformation and its affinity to other proteins
- Proteolysis - Remove peptide sequences or regulatory domains

Workflow of PTMs analysis

- Digestion of proteins into small fragments
- Protein separation and analysis using LC/MS/MS
- Database search
- PTM mapping
- Full protein annotation

Technological platform

- 2D Fluorescence Difference Gel Electrophoresis (DIGE)
- Liquid Chromatography (LC)
- High Performance Liquid Chromatography (HPLC)
- Matrix Assisted Laser Desorption Ionization Mass Spectrometry (MALDI-MS)

PTMs impact on health and disease

The study of heart disease, cancer, neurodegenerative illnesses, and diabetes necessitates the examination of proteins and their PTMs. The development of specific detection and purification procedures are the key hurdles in investigating post-translationally changed proteins. Fortunately, a range of new and enhanced proteomics technologies are overcoming these technological challenges.

Conclusion:

Despite considerable efforts to understand the relevance ofposttranslational modifications in the cellular context, weare still in the process of unraveling the complexity of thesemodifications and their tremendous impact. For the discovery and characterization of these site-specific protein changes, sophisticated technological developments like as high resolution mass spectrometry and dependable in silico methods are becoming more available. The involvement of glycosylation in the creation of disordered or fundamentally unstructured proteins is one such innovative use (IUPs). Newly discovered proteins that have been substantially changed by post translational processes, resulting in non-functional or defective protein molecules are known as disorderly proteins.

These proteins have been demonstrated to be important in gene transcription, protein expression, enzyme activity, cell signalling, and other processes. These protein molecules are actively sought after molecular targets for developing medicines for cancer treatment and other chronic diseases due to their significance in disease pathogenesis and cellular homeostasis. Other

well-known protein changes, such as phosphorylation, are important players in developing the field of translational medicine for diseases that are diverse, such as cancer. With the continual addition of novel post-translational modifications, determining the biological importance of newly found proteins using traditional approaches is a difficult challenge.

PTMs play an important role in the functions of proteins. N-linked glycans are a large portion of the protein mass and have a profound effect on the shape of the mature S protein and its binding to surface receptors, in addition to promoting its folding and intracellular trafficking. N-linked glycans may contribute to the antigenicity of S protein, and glycosylation may also aid in the activation of an innate immune response, altering viral pathogenesis. N protein phosphorylation increases its preferential binding to viral RNA and may control the uncoating and assembly process during replication.

Future perspective:

The systematic identification of conventional and novel PTM will be substantially expedited over the next decade, thanks to the introduction of revolutionary labelling techniques (such as H2O18 labelling) and the ever-growing capacity of mass spectrometry. Furthermore, as we gain a better understanding of the basic molecular mechanisms behind PTMs, functional studies will transition away from less selective inhibitors and toward targeted depletion of key modifying enzymes employing CRISPR-based gene knockdown/knockout techniques.

Currently it is playing important role identify protein modifications of SARS-CoV-2. In terms of translational applications, PTMs of coronavirus proteins and the interference of PTMs of host proteins by coronavirus proteins may be attractive targets for therapeutic intervention. Carbohydrate binding agents, for example, that engage directly with glycans on the virion surface, may be able to prevent virus attachment and entrance. Multiple mutations will be necessary for the virus to develop medication resistance because there are multiple N-linked glycosylation sites present. Recombinant coronaviruses with the DUB/deISGylation activity selectively removed from PLPro, on the other hand, could be promising vaccine candidates since they retain the protease activity essential for replication but are deficient in subverting the host innate immune response. A greater understanding of the PTMs of coronavirus proteins will bring fresh insights into the creation of more efficient vaccines and innovative antivirals, given their importance in both veterinary and public health settings.

We're only scratching the surface of the field's vastness and its impact on normal development and disease pathology. The successful development of novel prognostic markers as well as therapeutic targets for cancer, severe neurodegenerative diseases, and other debilitating

genetic diseases depends on the continued search and evaluation of various functional modifications of proteins and understanding their interaction in various biological pathways.

References:

Berger, S. L. (2007). Nature 447, 407–412.Creasy, D. M., and Cottrell, J. S. (2004). Proteomics 4, 1534–1536.

Burnett, G., Kennedy, E.P., 1954. The enzymatic phosphorylation of proteins. J. Biol. Chem. 211, 969–980.

Cohen, P. (2001). Eur. J. Biochem. 268, 5001–5010.

De la Fuente van Bentem, S., Anrather, D., Roitinger, E., Djamei, A., Hufnagl,

Fuchs, S. M., Laribee, R. N., and Strahl, B. D. (2009).Biochim.Biophys. Acta 1789, 26–36.

Glozak, M.A., Sengupta, N., Zhang, X., Seto, E., 2005.Acetylation and deacetylation of non-histone proteins. Gene 363, 15–23.

Godde, J. S., and Ura, K. (2008). J. Biochem. 143, 287–293.

Goodlett, D. R., Keller, A., Watts, J. D., Newitt, R., Yi, E. C., Purvine, S., Eng, J. K., von Haller, P., Aebersold, R., and Kolker, E. (2001). Rapid Commun.Mass Spectrom. 15, 1214–1221.

Grewal, S.I.S., Rice, J.C., 2004. Regulation of heterochromatin by histone methylation and small RNAs.Curr.Opin. Cell Biol. 16, 230–238.

Gygi, S. P., Rist, B., Gerber, S. A., Turecek, F., Gelb, M. H., and Aebersold, R. (1999). Nat. Biotechnol. 17, 994–999.

Hannoun, Z., Greenhough, S., Jaffray, E., Hay, R. T., & Hay, D. C. (2010). Post translational modification by SUMO. *Toxicology*, *278*(3), 288-293.

Hubbard, M. J., and Cohen, P. (1993).Trends Biochem. Sci. 18, 172–177.

Issad, T., and Kuo, M. (2008).Trends Endocrinol.Metab. 19, 380–389.

Jones, A. M., Mansfield, J., & Grant, M. (2007). Considerations on post-translational modification and protein targeting in the arabidopsis defense proteome. *Plant Signaling & Behavior*, *2*(3), 153-154.

Khainovski, N., Pillai, S., Dey, S., Daniels, S., Purkayastha, S., Juhasz, P., et al. (2004). Mol. Cell. Proteomics 3, 1154–1169.

Kiss T (July 2001). Small nucleolar RNA-guided post-transcriptional modification of cellular RNAs, *The EMBO Journal.* **20** (14): 3617–22

Oda, Y., Huang, K., Cross, F. R., Cowburn, D., and Chait, B. T. (1999).Proc. Natl. Acad. Sci. USA 96, 6591–6596.

Olsson, A., Manzl, C., Strasser, A., and Villunger, A. (2007). Cell Death Differ. 14, 1561–1575

Ong, S. E., Blagoev, B., Kratchmarova, I., Kristensen, D. B., Steen, H., Pandey, A., and Mann, M. (2002). Mol. Cell. Proteomics 1, 376–386.

Orford, K., Crockett, C., Jensen, J.P., Weissman, A.M., Byers, S.W., 1997. Serine phosphorylation of Arabidopsis proteins involved in RNA metabolism. Nucleic phosphorylation-regulated ubiquitination and degradation of beta-catenin. J. Biol. Chem. 272, 24735–24738.

Reid, G., Gallais, R., and Metivier, R. (2009). Int. J. Biochem. Cell Biol. 41, 155–163.

Ross, P. L., Huang, Y. N., Marchese, J. N., Williamson, B., Parker, K., Hattan, S., ... & Pappin, D. J. (2004). Multiplexed protein quantitation in Saccharomyces cerevisiae using amine-reactive isobaric tagging reagents. *Molecular & cellular proteomics*, *3*(12), 1154-1169.

Roth, S. Y., Denu, J. M., and Allis, C. D. (2001). Annu. Rev. Biochem. 70, 81–120.

Spiro, R.G., 2002. Protein glycosylation: nature, distribution, enzymatic formation,

T., Barta, A., et al. (2006). Phosphoproteomics reveals extensive in vivo the defence phosphoproteome of Arabidopsis thaliana using differential mass tagging. Proteomics 6, 4155–4165.

Vidal CJ (ed.) (2010) Post-Translational Modifications in Health and Disease. New York: Springer.

Waby, J.S., Bingle, C.D., Corfe, B.M., 2008. Post-translational control of sp-family transcription factors.Curr. Genomics 9, 301–311.

Walsh C (2005) Postranslational Modification of Proteins: Expanding Nature's Inventory. Englewood, CO: Ben Roberts and Company.

Walsh CT, Garneau-Tsodikova S, and Gatto GJ, Jr. (2005) Protein posttranslational modifications: The chemistry of proteome diversifications. Angewandte Chemie International Edition 44: 7342–7372.

Walsh, C.T., Garneau-Tsodikova, S., Gatto, G.J., 2005. Protein posttranslational modifications: the chemistry of proteome diversifications. Angew.Chem. Int. Ed. Engl. 44, 7342–7372.

Wu, X., Sklodowski, K., Encke, B., and Schulze, W. X. (2014). A kinasephosphatase signaling module with BSK8 and BSL2 involved in regulation of sucrose-phosphate synthase. J. Proteome Res. 13, 3397–3409.

Zhang, H., Zhou, H., Berke, L., Heck, A. J.,Mohammed, S., Scheres, B., et al. (2013). Quantitative phosphoproteomics after auxin-stimulated lateral root induction identifies an SNX1 protein phosphorylation site required for growth. Mol. Cell. Proteomics 12, 1158–1169.

Zhang, Y., Wolf-Yadlin, A., Ross, P. L., Pappin, D. J., Rush, J., Lauffenburger, D. A., and White, F. M. (2005). Mol. Cell. Proteomics 4, 1240–1250.

FRESH WATER FISH FAUNA OF PANZARA AND KAN RIVERS OF SAKRI TAHSIL, MAHARASHTRA (INDIA)

S. S. Bhoi

Department of Zoology,

Uttamrao Patil Arts and Science College Dahiwel. (M. S.) 424304.

Corresponding author E-mail: bhoiss16@gmail.com

Abstract:

The present study has shown that Panzara and Kan rivers supported 41 fish species belonging to 7 orders, 13 families and 29 genera. The order Cypriniformes was dominant with 23 (56%) species followed by Perciformes with 7 (17%) species, order Suliformes with 5 (12%) species, Synbranchiformes 3 (7%) species and Osteoglossiformes, Clupeiformes and Beloniformes with single species each. Fish diversity was assessed by calculating the various indices such as Shannon-Weiner index (H), Simpson's Dominance Index (D), Simpson's index of diversity (1-D) and Pielou's Evenness index of species richness. It can be concluded that Panzara and Kan rivers flourish with rich fish fauna. It requires proper management and conserves this fish health.

Keywords: Biodiversity indices, Fish fauna, Simpson's index, Conservation etc.

Introduction:

Panzara Kan (Panzara+ Kan) rivers are life line of Sakri tahasil, these acts as important fish reserves (Patole and Patil, 2009). Fish is sensitive to changes in water chemistry due to different anthropogenic activities from their catchments. Fish responses to environmental disturbances, including hydro-morphological factors are different in time and space in comparison to simpler organisms, as they tend to be integrated over larger intervals. Fish has been identified as suitable for biological assessment due to its easy identification and economic value (Goswami and Mankodi, 2010). Fish assemblages have widely been used as ecological indicators to assess and evaluate the level of degradation and health of water bodies at various spatial scales (Mandal, 2010). Earlier workers like Madhusudan et al. (2011) studies on diversity of fish in Gondoor and Nakane lakes in Dhulia (M. S.). Jaiswal and Ahirrao (2012) studied on ichthyofaunal diversity of Rangavali dam, Navapur district Nandurbar (M.S.).

Fish diversity comprises of species richness (number of species in a defined area), species abundance (relative number of species) and phylogenetic diversity i. e. relationships between different groups of species (Kharat *et al.,* 2012). Narsimha and Banergee (2013) observed that

there are many advantages of using fish assemblage as biological indicator. Therefore, it is essential to conserve the diversity of fish from freshwater reservoirs and tanks (Khodake *et al.*, 2014). Patole (2014) reported ichthyofaunal diversity of Nandurbar district (Northwest Khandesh region) of Maharashtra (India). Fish diversity is also a good bioindicators of water quality like zooplankton and phytoplankton species considered as biological tool for further bio-monitoring and assessing trophic status of water bodies (Kawade and Pandarkar, 2015). Recently Patole (2015) noted ichthyofaunal diversity of Tapi River flows through Dhule and Nandurbar district of Northwest Khandesh (Maharashtra). Aquatic ecosystems consist of a biotic and biotic component which directly affects the diversity of flora and fauna of water bodies (Borane, 2015). Very recently, Kawade and Pandarkar (2016) studies diversity indices of fish Heterogenesity of Kalu dam, Ahmednagar, Maharashtra. Fishes are one of the good and cheapest sources of protein food for all classes of people. Fishes are the important vertebrate group of animal's world contributing to the biodiversity of animals (Rawal and Deshmukh, 2016; Deepali Sonawane and Patole, 2017).

In the present investigation fish diversity were studied in Kan and Panzara River located at Sakri Tahsil of Dhule District in Maharashtra state, India. We also studied different indices of fish diversity.

Material and Methods:

For the study of fish diversity, fishes were purchased from fisherman they were collected in Panzara and Kan rivers of Sakri tahsil. Fishes brought to laboratory and preserved in 4% formalin solution in separate specimen jar according to the size of species. Then photographs were taken with the help of digital camera. The fishes were identified by local name named by local fisherman. The scientific identification and classification of fish species were made by using standard book and keys. Specimens with doubtful identifying characters were identified from zoological survey of India (ZSI) Pune.

Study Area:

Panzara River

The Panzara is the largest river in length of Sakri Tahasil (Dhule). It is life line of Dhule district and one of the tributaries of interstate river Tapi that flow eastward in to Arabian Sea. It lies between 20^0 to 52^0 North latitude and 73^0 to 55^0 North longitudes in North west side of Maharashtra. It originates at Hanuman near Pimpalner, Tal. Sakri Dist. Dhule (M. S.) and meet to Tapi River near Mudavad Tal. Shindkheda, Dist. Dhule (M. S.). Its total length is about 136 km.

Kan River

Kan River flows through the Dhule district and its confluence with Panzara River is near the Sakri Taluka. It is the tributary of Panzara. It lies between 21^0-3' North latitude and 730-59^0 East longitude in North West. It originates near Dhaner Tal. Sakri Dist. Dhule (MS) meet to Panzara at Datarti (Sakri), Dist. Dhule (MS). Total length of Kan River is about 54 km. These rivers are only one main source for water in Dhule dist. of Maharashtra. The water of these rivers is used for drinking, irrigation and aquaculture.

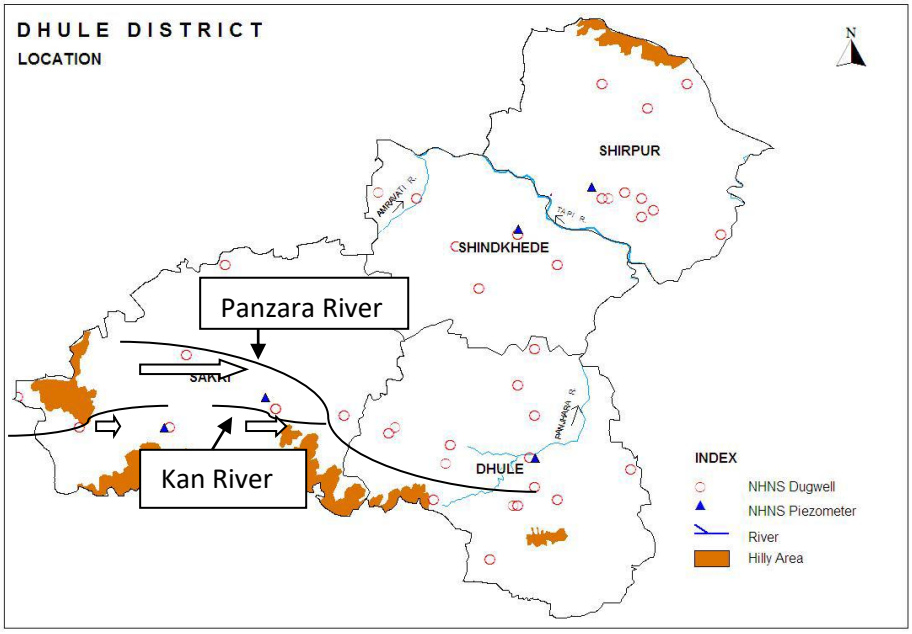


Figure 1: Dhule district Map shows Sakri Tahasil (Panzara and Kan rivers)

For determination of diversity indices, total number of species, total number of individuals in a sample and total number of individuals of a species were determined. From these data Shannon - Weiner Index (H), Simpson's Dominance Index (D) and Simpson's Index of Diversity (1-D) and Pielou's evenness Index (J) were determined using the following equations.

1. **Shannon - Weiner Index (H).** It depends on both the number of species present and the abundance of each species. H = -ΣPi (lnPi), where Pi is the proportion of each species

Pi = A/T where A is number of each species in the sample, and T is the total number of individuals of all species in the sample.

2. **Simpson's Dominance Index (D)** is determined using the following equations.

$$D = \frac{n_1 (n_1 - 1) + n_2 (n_2 - 2) + \text{--------} + n_{20} (n_{20} - 1)}{N (N - 1)}$$

Where n is the total number of individual of a particular species and N is the total number of Individuals of all species.

3. **Simpson's Index of Diversity** = 1- D

H

4. **Pielou's evenness Index (J*):*** ----------

Ln* S

Where H is the Shannon - Weiner Index and S is the number of species.

Results and Discussion:

The fresh water fish diversity of Panzara and Kan rivers of Sakri Tahasil and their abundance is shown in table-1. This showed that most of the fish species recorded were widely distributed in the water bodies of Sakri Tahsil. In the present investigation the fish collected from Panzara and Kan Rivers by making two collection stations centre. We collected 41 species (29 genera) belong to 7 different orders and 12 families.

Similar types of work were carried out by earlier workers like Patole and More (2010). They have been studied biodiversity of fresh water fishes from Sakri, Tahasil. They examined 221 specimens, among them 31 species (25 genera's) of 05 orders, also recorded 17 new species. Ubarhande et al (2011) observed 08 orders, 11 families, 22 genera and 27 species. Madhusudan et al (2011) have showed18 fish species in Gondoor and Nakana lakes where Cyprinidae was dominance over other families. Abu Hanif *et al.*, (2015) reported 26 species belonging to 5 orders from Sandha Rivers of South West Bangladesh. Sakhare (2001) noticed the occurrence of 23 fish species belonging to 7 orders in Jawalgaon reservoir in Solapur district of Maharashtra. Patole (2014) studied ichthyofaunal diversity of Nandurbar district of Maharashtra State; he reported 32 species from 24 genera. Where order Cypriniformes dominate over the other orders. Patole (2015) mentioned ichthyofaunal diversity of tapi river flows through Nandurbar and Dhule district. He reported 32 fish species belonging to 23 genera. He finds similar results i.e. order Cypriniformes where dominance. Deepali Sonawane and Patole (2017) observed 20 fish species belonging to 16 genera, 11 families and 06 orders in Nakane, Sulwade and Dedorgaon dams of Dhule district (M. S.) where Cypriniformes was dominant over other orders. Kawade and Pandarkar (2016) studied on diversity indices, Ahmednagar (M. S.). They reported 27 fish species where order Cypriniformes was dominated over other orders. Our work is corroborated with these earlier workers.

In the present study occurrence of 41 fish species from Panzara-Kan River showed that good fish diversity and their production. It might be suitable water quality of rivers that support proper breeding places for fish. The fishes belonging to order Cypriniformes was dominant.

Table 2 shows fish species richness and various diversity indices. It is observed that, in present study species abundance was 2478, Shannon- Weiner Index (H) recorded 3.264. The Simpson's Dominance Index (D) was recorded 0.037 and Simpson's Index of Diversity (1-D) was recorded 0.963. The Pielou's evenness (J) value was recorded 0.879.

Table 1: Fish abundance and diversity in Panzara and Kan rivers, Sakri (India)

Sr. No.	**Species**	**Order**	**Family**	**Common name**	**Abun-dance**
1.	*Xenentodon cacila*	Beloniformes	Belonidae	Vam	13
2.	*Tenualosa ilisha*	Clupeiformes	Clupeidae	Bhat-masa	148
3.	*Acanthocobitis botea*	Cypriniformes	Balitoridae	Mooree	112
4.	*Acanthocobitis mooreh*			Mooree	107
5.	*Oreonectus evezardi*			Mooree	35
6.	*Schistura denisoni*			Mooree	23
7.	*Barilius bendelisis*		Cprinidae	Zora	142
8.	*Crossocheius latius*			Regadi	42
9.	*Danio aequipinnatus*			Ger	37
10.	*Garra mullya*			Mhya	105
11.	*Hypophthalmichthys nobilis*			Silver	25
12.	*Labeo boggut*			Ger	23
13.	*Lepidocephalichthys guntea*			Mooree	98
14.	*Lepidocephalichthys themalis*			Mooree	88
15.	*Oteobrama cotio cotio*			Ger	26
16.	*Punctius amphibious*			Kanvar	68
17.	*Puntius conchonius*			Chhoti-Debri	73
18.	*Puntius sarana sarana*			Kunder	62
19.	*Puntius sophore*			Lal Dhebri	68
20.	*Puntius ticto*			Dhebri	80
21.	*Rosbora daniconius*			Zora	69

22.	*Salmostoma bacaila*			Mavala	32
23.	*Salmostoma clupiodes*			Chal	27
24.	*Salmostoma phulo phulo*			Chal	38
25.	*Tor khudree*			Khavalya	26
26.	*Notopterus notopterus*	Osteoglossiformes	Notopteridae	Patoda	21
27.	*Chanda nama*	Perciformes	Ambassidae	Kach-Masa	89
28.	*Parambasis ranga*			Dhebri	18
29.	*Channa guchua*		Channidae	Dok	139
30.	*Channa punctata*			Dok	142
31.	*Channa orientalis*			Dok	135
32.	Channa *marulius*			Dok	149
33.	*Glossogobius giuris*		Gobidae	Khavalya	42
34.	*Mystus bleekeri*	Silurifomes	Bagridae	Chichva	13
35.	*Rita pavimentata*			Sisava	09
36.	*Heteropneusta fossilis*		Clariidae	Tochya	15
37.	*Clupisoma garua*		Schilbidae	Vavadi	79
38.	*Ompok bimaculatus*		Silirudae	Papada	18
39.	*Macrognathus panicalus*	Synbranchiformes	Metacembelidae	Vam	18
40.	*Mastacembelus armatus*			Vam	09
41.	*Mastacembelus pancalus*			Vam	15

Table 2: The fish species richness and diversity indices

Sr. No.	**Index**	**Value**
01	Species Richness	41
02	Species abundance (N)	2478
03	Shannon- Weiner Index (H)	3.264
04	Simpson’s Dominance Index (D)	0.037
05	Simpson’s Index of Diversity (1-D)	0.963
06	Pielou’s evenness (J)	0.879

Conclussion:

These Ichthyofaunal studies suggest that this water body is rich in fish fauna. Therefore a sustainable strategies needs to raising awareness among the fishermen for conservation of fish diversity in the Panzara and Kan rivers of Sakri Tahsil.

Acknolwedgement:

The author is grateful to the Principal, S.G. Patil Arts, Science and Commerce College, Sakri Dist. Dhule (M.S.) for providing necessary laboratory facilities. Also thankful to Zoological Survey of India, Western Regional Station, Akurdi, Pune (M. S.) for the identification of fish.

References:

Abu Hanif, A., Muhammad, B. S., Reaz, C., Sultan M., Ashfaqun, S. H. and Sukham, M. (2015). Biodiversity and Conservation of Threatened Freshwater Fishes in Sandha River, South West Bangladesh, *World Appl. Sci. J.*, 33 (9): 1497-1510.

Borane, V. R. (2015). Study of ichthyofaunal diversity in relation to physico-chemical characteristics of Londhare dam water, Shahada taluka, Maharashtra, India, *J. Environ. Res. Develop.* 10(01), 59-64.

Deepali Sonawane and Patole, S. S. (2017). Studies on ichthyofaunal diversity of Nakane, Sulwade and Dedorgaon dam of Dhule Dist. (M. S.). *Gol. Res. Thots*. 6 (9): 1-9.

Goswami, A. P. and Mankodi, P. C. (2010). Diversity of fishes from fresh water reservoir Nyari - II Rajkot district, Gujarat, India. *Elect. J. Environ. Sci.*, Vol. (3) 23-26.

Jaiswal, D. P. and Ahirrao, K. D. (2012). Ichthyodiversity of the rangavali dam, Navapur distirct Nandurbar, Maharashtra state, *J. Res. Biol*. 3(1), 241-245.

Kawade, S. A. and Pandarkar, A. K. (2015). Study of Zooplankton diversity of Dnyaneshwar Sagar in relation to trophic status. *J. Exp. Zool. India*, 18(2): 807-810.

Kawade, S. A. and Pandarkar, A. K. (2016). Study of diversity indices fish Heterogeneity of Kalu dam, Ahmednagar, Maharashtra. *Int. J. Adv. Biol. Res.* 6(1): 75-78.

Kharat, S. S., Paingankar, M. and Dahanukar, N. (2012). Freshwater fish fauna of Krishna River at Wai, northern Western Ghats, India. *J. of Threat. Taxa*, 4(6): 2644-2652.

Khodake, S. P., Borale, P. R and Petare, R. K. (2014). Ichthyofaunal diversity in Jamkhedi reservoir in Dhule district of Maharashtra, India, *J. Environ. Res. Develop.* 9(1): 177-183.

Madhusudan, V. Amrutsagar, V. and Lohar, P. S. (2011). Diversity of Mollusca and Fish in Gondoor and Nakane Lakes in Dhulia, Northwest Maharashtra, India. *J. Ecobiotechnol*., 3(6): 16-2.

Mandal, D. K. (2010). Water quality parameters and fish biodiversity Indices as measures of ecological degradation: A case study in two floodplain lakes of India. 2 (1): 85-92.

Narasimha, R. K. and Benrgee, (2013). Fish species diversity of Nagaram Tank of Warangal Andhara Pradesh. *IOSR-JESTFT* Vol.3: 14-18.

Pandarkar, A. K, Pawar, B. A. and Shendge, A. N. (2014). Ichthyofaunal studies of Visapur reservoir in relation to fish culture, Ahmednagar district, Maharashtra. Flora and fauna. 20(2): 247-250.

Patole, S. S. and Patil, M. U. (2009). A note on ichthyofauna of Panzara River (Tah. Sakri), dist. Dhule, Maharashtra. *J. Aqua. Biol.* 24(2):61-66.

Patole, S. S. and More, B. C. (2010). Biodiversity of fresh water fishes from Sakri-Tahsil (Dist-Dhulia) of Maharashtra. Research Link, 75, (IX-4): 15-17.

Patole, S. S. (2014). Ichthyofaunal diversity of Nandurbar district (Northwest Khandesh region) of Maharashtra (India). *Int. J. Fisheries and Aqua. Studies*. 2(2):167-172.

Patole, S. S. (2015). Ichthyofaunal diversity of Tapi River flows through Dhule and Nandurbar district of Northwest Khandesh (Maharashtra), India. *Proc. Zool. Soc. India.* 14(1):43-50.

Rawal, R. and Deshmukh, P. S. (2016). Biodiversity of fish fauna in Lakhapura Reservoir, Bhikagaon Tehsil, Khargone District, M. P. *Int. J. of Inno. Res. and Adv. Stud.* 3(4):75-77.

Sakhare, V. B. (2001). Ichthyofauna of Jawalgaon reservoir in Solapur district of Maharashtra, *J. Aqua. Biol.*, 16 (1 and2): 31-33.

Ubarhande, S. B., Jagtap, J. T. and Sonawane, S. R. (2011). Ichthyofaunal diversity from Ambadi dam, Taluka Kannad, district Aurangabad (M.S.) *Rec. Res. In. Sci. Tech*., 3(6), 34- 37.

HYBRID RENEWABLE ENERGY SYSTEM FOR NATURAL RESOURCE CONSERVATION

K. Suganya*[1], R. Jayashree[2], R. Rizam[1] and P. Bhavani[1]

[1]Department of Environmental Sciences,

Tamil Nadu Agricultural University, Coimbatore

[2]Department of soils and Environment,

Agricultural College and research institute, Madurai

*Corresponding author E-mail: ksuganyaphd@gmail.com

Introduction:

Due to population growth, suburbanization, and industrial development, energy demand is steadily increasing. Due to the rapid depletion of fossil fuels such as coal, oil, and gas, traditional energy sources have a greater influence on the environment, resulting in an increase in CO_2 levels, which causes global warming. The use of renewable energy sources is required. Because of their unpredictable and intermittent character, individual energy sources cannot offer continuous power to the load. As a result, renewable energy sources such as wind, solar, hydro, biogas, and fuel cells can be combined to create a hybrid system that is more dependable and environmentally benign. Distributed energy source is the name given to this type of renewable energy sources and the generation is called as distributed generation.

However, most renewable energy, such as solar and wind energy, is inherently unstable and intermittent when compared to conventional energy sources. Over the course of hours or days, solar irradiance and wind speed can change dramatically. Furthermore, poor energy density is seen as a major disadvantage of renewable energy. A single renewable energy source is clearly insufficient to maintain a continuous energy delivery system. Integration of multiple renewable energy sources has been proposed to alleviate the difficulties.

India has held two auctions for hybrid wind/solar projects. Both auctions were under-subscribed, with SB Energy, Adani Green Energy, and ReNew Power winning bids totaling 1.56 gigawatts (GW) out of a total of 2.4 GW on offer. The discovered costs were just below the 2.70 Indian Rupee ceiling tariff. In May 2019, India had added 65-70 GW of wind and solar capacity, with wind and solar accounting for 9.5 percent of total energy output. This percentage might rise to 15-16 percent if the government's aim of 175 GW is met by 2022 (Khare *et al.*, 2013). For the past two decades, the Indian government has attempted to link rural areas utilizing national grid

extension. However, present power access is still less than 50%, with an actual connection of less than 14%. Rural populations with low load demand and dispersed settlements will not get energy in the foreseeable future in this scenario. Although India is a rapidly rising economy, with an annual GDP growth rate of roughly 6% over the last two decades, the development of rural areas remains worrying (Kumar *et al.*, 2018). Despite the country's tremendous hydroelectric potential, it cannot be really utilized due to massive river water distribution problems.

According to McKinsey's own modelling, wind-solar storage hybrid systems might generate round-the-clock power with cost and reliability levels comparable to existing coal-fired power plants in the next 4-5 years if the above improvements are taken into account. By 2025, a hybrid system capable of delivering a flat load of 250MW might be built by integrating solar, wind, and battery storage for a levelized cost of energy of 3-4/kWh. Of course, this is depending on the region specificity as well as seasonal variations. (Deshmuk *et al.*, 2015).

Hybrid systems:

A hybrid system is made up of two or more renewable and non-renewable energy sources. Energy sources (AC/DC), AC/DC power electronic converters, and loads are the essential components of the hybrid system as shown in Fig 2.

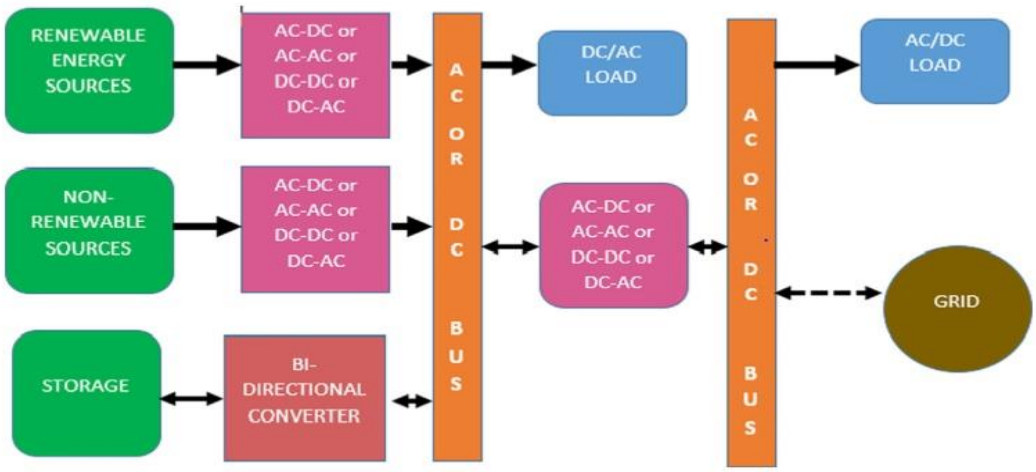


Figure 1: Hybrid Systems (Adapted from Shivarama Krishna and Sathish Kumar, 2015)

Hybrid renewable energy systems

Renewable energy output is currently increasing year after year, with most countries aiming for greater than 15% renewable energy generation by 2020. A hybrid energy system is made up of two or more renewable energy sources that are combined to boost system efficiency and provide greater energy supply balance (Neves *et al.*, 2014). Wind, photovoltaic, fuel cell, and micro-turbine generators are interconnected in hybrid energy systems to create power for

local loads and connect to grid/micro-grids, reducing reliance on fossil fuels (Negi and Mathew, 2014). It is necessary to establish a sustainable and efficient energy system to address the recurring electrical difficulties in remote locations in order to boost growth. The present generation capacity and estimated potential has been estimated in the table 1.

Table 1: Installed capacity of renewable energy sources in India (Dave and Kumar 2019)

Sr.No.	Source	Total installed capacity (MW)
1	Wind Power	34046
2	Solar Power	21651
3	Bimass Power	8701
4	Waste to Power	138
5	Small Hydropower	4486

Types of hybrid renewable energy systems

The different types of renewable energy systems includes

1. Biomass-wind-fuel cell
2. Photovoltaic-wind
3. Solar-Induced Hybrid Fuel Cell from Biomass
4. Completely Renewable Hybrid Power Plant
5. Hydro-wind

1. Biomass-wind-fuel cell

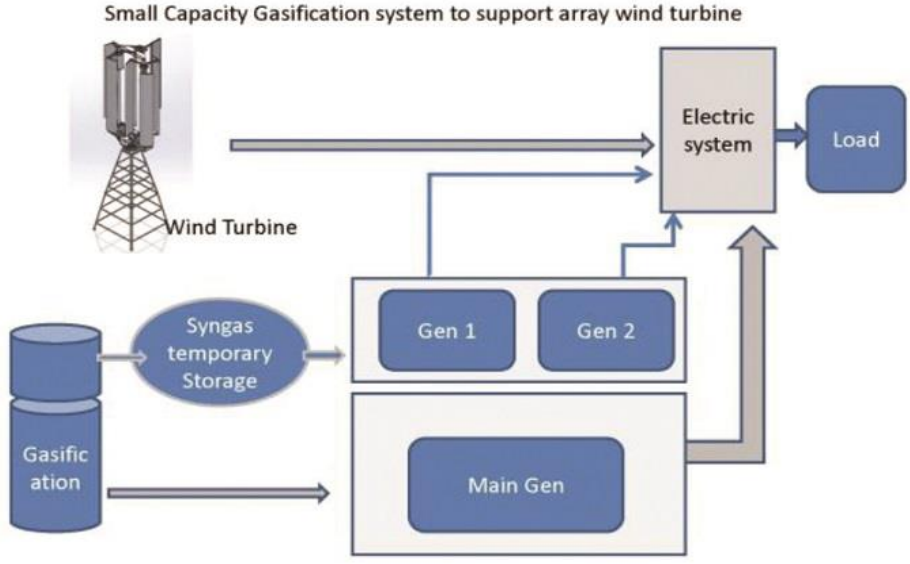


Figure 2: Biomass-wind-fuel Cell (Dave and Kumar, 2019)

Consider a load that requires 100% power supply; if no renewable energy system can meet this need, two or more renewable energy systems can be integrated. For instance, 60% from a biomass system, 20% from a wind energy system, and the remaining from fuel cells. As a result, integrating all of these renewable energy sources may be able to meet 100% of a load's power and energy requirements, such as a home or business.

2. Photovoltaic-wind:

A photovoltaic array combined with a wind turbine is another example of a hybrid energy system. The wind turbine would provide higher production in the winter, while the solar panels would produce their peak output in the summer. Wind, solar, geothermal, and trigeneration stand-alone systems can provide better economic and environmental returns than hybrid energy systems (Sawle *et al.*, 2016).

3. Solar-induced hybrid fuel cell from biomass:

Researchers have invented a new form of low-temperature fuel cell that uses a catalyst activated by solar or thermal energy to directly convert biomass to electricity. A new solar-induced direct biomass-to-electricity hybrid fuel cell that can run on a range of fuels has been developed. A polyoxometalate (POM) catalyst is used in the fuel cell, which changes colour as it reacts with light. Under sunlight irradiation, electrons in the biomass can be converted to polyoxometalate (POM), and reduced POM can supply the charges to the anode. The oxygen in the cathode then captures these electron.

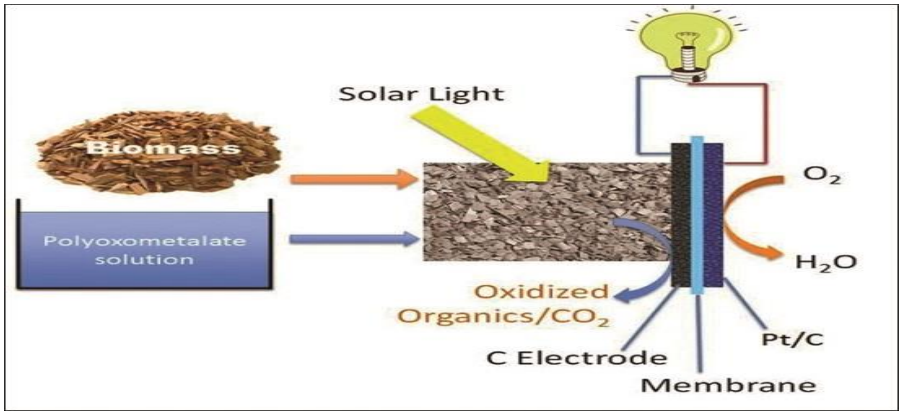


Figure 3: Solar-induced direct biomass-to-electricity hybrid fuel cell (Adapted from Dave and Kumar, 2019)

4. Completely renewable hybrid power plant

Solar, wind, biomass, and hydrogen Hybrid Power Plant (solar, wind, biomass, and hydrogen) A hybrid power plant made up of these four renewable energy sources can be put into operation by careful management of these resources (Sinha and Chandel, 2014).

5. Hydro-wind

Wind energy is one of the most rapidly developing renewable energy sources. Due to low wind speeds and significant unpredictability concerns, stand-alone wind energy systems may not be able to meet the demands of certain loads. A wind-hybrid energy system combines wind energy with one or more other renewable energy technologies, as well as an appropriate backup system, such as a battery bank or diesel generator. The reliability of combined operation in the form of a hybrid system is improved, and it compensates for the shortcomings of stand-alone systems (Bajpai *et al.*, 2012)

Hybrid renewable energy systems utilization

The usage of renewable energy is critical for the world since global energy consumption is rising, and traditional energy sources are no longer sufficient to supply the demand, resulting in energy crises. Climate and weather-related variations in solar radiation and wind speed, on the other hand, limit the stable operation of renewable energy sources, causing output to fluctuate. A hybrid renewable energy (HRE) system is a viable solution to the above issue because it can be very efficient by combining multiple renewable energy sources.

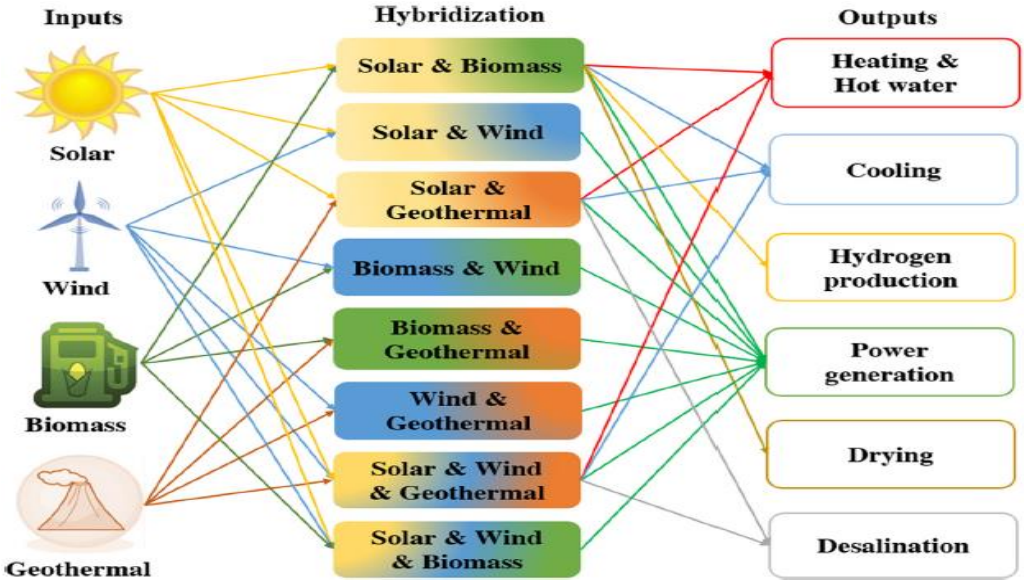


Figure 4: Hybrid renewable energy systems utilization road map

(Adapted from Guoa *et al.*, 2018)

Hybrid renewable energy systems is utilized for the following categories including space heating, cooling, hot water usage, power generation, hydrogen production, drying and multi-generation.

1. Space heating, cooling and hot water usage

Alternative energy resources, in addition to conventional fossil fuels, have been seriously studied to reduce energy consumption and greenhouse gas emissions in order to fulfil the

objective of keeping global average temperature increases below 2 degrees Celsius. Solar energy, as the most readily available renewable energy source, is simple to collect and use for space heating, cooling and hot water.

2. Cooling

The HRE system can be used for cooling as well as space heating and hot water, thanks to the integration of an absorption refrigeration cycle. A solar-biomass hybrid cooling system is made up of three parts: a solar water heater, a biomass gasifier-boiler, and an absorption chiller. A solar water heater collects solar energy for heating water and stores it in a storage tank. When solar radiation is scarce or unavailable, the biomass gasifier-boiler is used as a backup or primary heat source. The absorption cooling cycle is usually carried out with a binary solution, such as a lithium bromide-water solution, in which the former acts as an absorbent and the latter acts as a refrigerant.

3. Power generation

Solar, wind, biomass, and geothermal energy can all be combined to generate electricity. This section is organised around the various combinations of renewable energy sources in order to clearly characterise the power generating processes in various HRE systems.

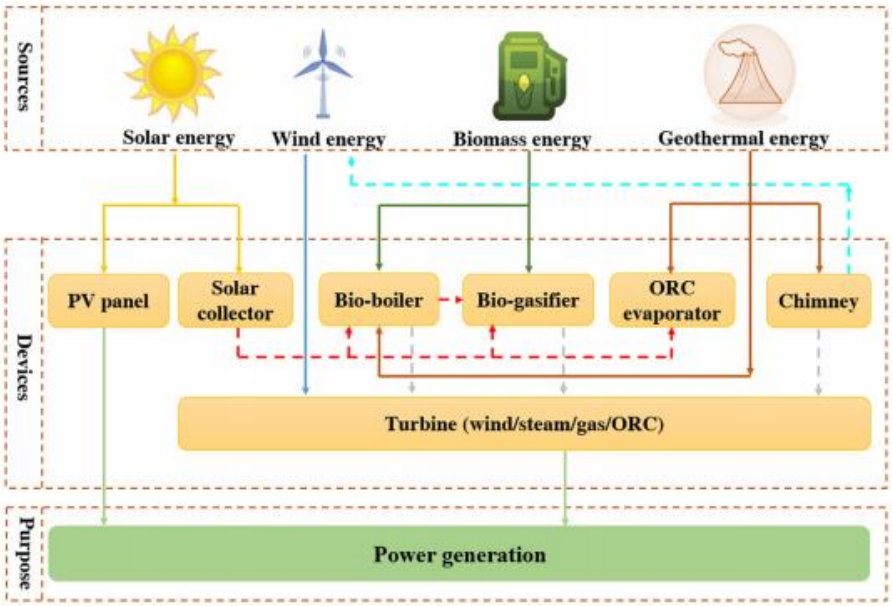


Figure 5: Power generation (Adapted from Guoa *et al*., 2018)

4. Hydrogen production

The production of hydrogen via HRE system is mainly based on thermochemical or biological processes including

- Pyrolysis,
- Gasification,

- Supercritical water extraction,
- Biophotolysis, and
- Photo fermentation

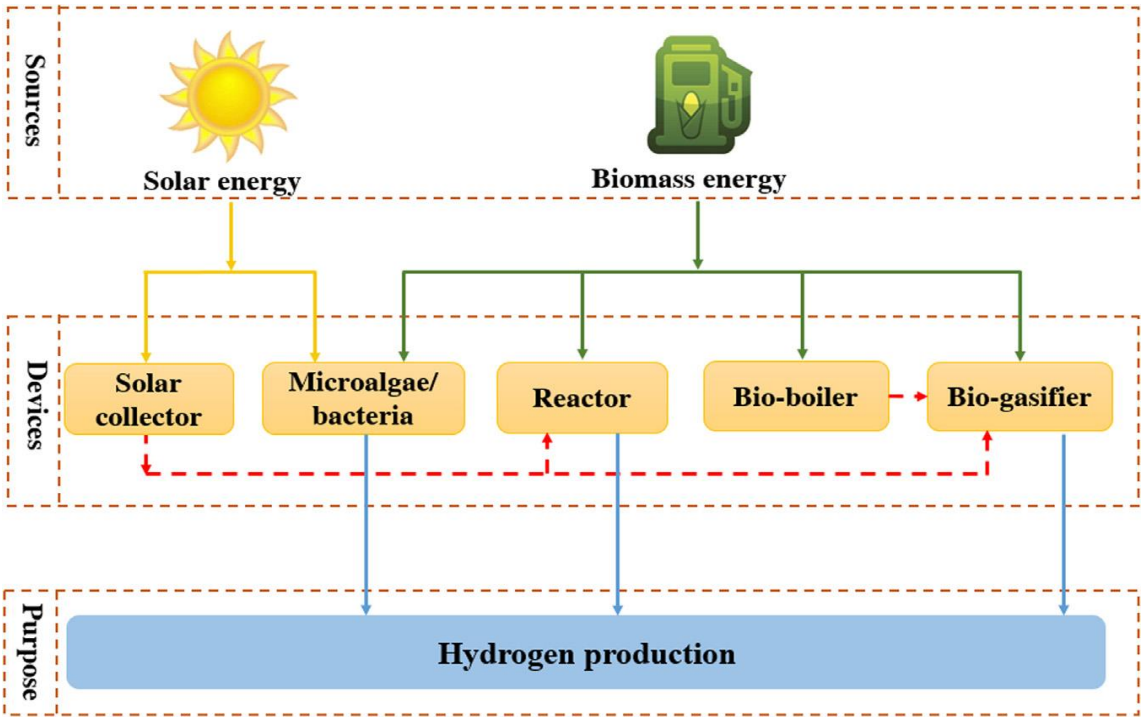


Figure 6: Road map for hydrogen production (Adapted from Guoa *et al*., 2018)

5. Drying

Drying crop by-products including paddy rice, cassava, chile, and rubber sheets is an important agricultural activity, particularly in tropical and subtropical nations. A sun drying device was proposed to alleviate the drawbacks of standard drying processes. However, there are substantial biomass resources produced during agricultural production that can be used to provide drying energy. For drying, a combination of solar and biomass energy can be used.

6. Multi-generation

The multi-generation system is intended to meet the energy demands for heating, cooling, electricity, and desalination at the same time. The combined heat and power (CHP) system is powered by solar and geothermal energy to deliver both thermal and electrical energy for heating and power generation. The absorption refrigeration cycle can be supported by the heat gathered from solar radiation and recovered from turbine exhaust gas (Olatomiwa *et al*., 2016). Desalination by reverse osmosis and distillation can be powered by solar collector/geothermal heat exchanger heat and generated power, respectively.

Steps involved hybrid system based power generation

It is essential to have a well-defined and standardized frame work/steps taken for hybrid system based power generation for rural electrification. These steps are as follows:

a) Demand Assessment: The load demand can be calculated using reliable load forecasting of remote settlements. Interviewing gramme pradhans, school teachers, local people, and workers, for example, can help with load assessment.

b) Resource Assessment: Using existing meteorological data, resource evaluation can be done by assessing potential available in wind, MHP, solar, Biomass, Biogas, and other renewable energy resources.

c) Barriers/Constraints: Annual electricity demand, reliability, net present cost, environmental considerations, and employment are all impediments to these activities.

d) Demand is fulfilled by Hybrid renewable energy system. Combining one or more renewable energy sources with traditional energy sources can accomplish this. Some Hybrid renewable system configurations are as follows:

- PV/Wind/diesel generator HRES,
- PV/wind/fuel cell HRES
- Wind/battery HRES
- Biomass/wind/diesel generator HRES
- PV/Wind/Biomass/fuel cell HRES

Criteria for hybrid system optimizations

In order to select an optimum combination for hybrid system to meet the load demand, evaluation must be carried out on the basis of power reliability and system life-cycle cost (Gupta *et al.*, 2016).

1. Power reliability analysis: A variety of methods used to calculate the reliability of the hybrid system includes Loss of power supply probability (LPSP), Loss of Load Probability (LOLP), System Performance Level (SPL) [4], and Loss of Load Hours (LOLH).
    - LPSP: The probability that an insufficient power supply results when the hybrid system is unable to satisfy the load demand.
    - LOLP: This measure of the probability that the system demand will exceed the system's power supply capacity in a given time period.
    - SPL: It is defined as the probability that the load cannot be satisfied.
    - LOLR: To decide a proportion for solar and wind energy in a hybrid system.

2. System cost analysis: There are several economic criteria for the system cost analysis, such as Net present Cost, Levelised Cost of Energy and life-cycle cost (Sharma *et al.*, 2021).

Storage

For assuring a constant supply of electricity to the load, storage technology is vital. Compressed air energy storage (CAES), pumped hydro storage (PHS), hydrogen fuel cells, flywheels, supercapacitors, superconducting magnetic energy storage (SMES), and batteries are just a few examples of energy storage that can be employed in a hybrid renewable energy system.

Table 2: Key characteristics of each of the energy storage

Attributes	Efficiency	Maturity of technology	Cost	Energy density	Power density
CAES	70%	Mature	High	High	High
PHS	75-85%	Mature	High initial cost	Depends on size of the reservoir	Depends on the height distance between the reservoir
Hydrogen fuel cells	50-60%	Early stages of maturity	High	Depends on the hydrogen resorvoir	Depends on speed on reaction
Flywheels	80-90%	Mature	Low	Low	High
Super capacitors	80-95%	Immature	High	Low	High
SMES	90 - 95%	Immature	High	Low	High
Battery	75- 85%	Mature	Low	High	High

Future trends and limitations:

The renewable technologies have come a long way in terms of research and development. However there are still certain constraints in terms of their optimal use and efficiency. The challenges faced due to this technologies includes

- Renewable energy sources, such as solar PV and fuel cells, require novel technologies to extract more useful electricity. Solar's inefficiency is a major roadblock to its widespread adoption.

- Because the high capital cost leads to a longer payback time, the production cost of renewable energy sources must be significantly reduced.
- It should be assured that the power electrical equipment lose as little power as possible.
- Innovative technologies must be used to extend the life of storage technology
- These stand-alone systems are less flexible to variations in load. Large variations in load could cause the entire system to fail.

Conclusion:

In India, the state of renewable energy sources such as solar and wind systems are adequate, but they require further attention for greater development. Although cost reductions and technological advancements in renewable energy systems have been encouraging in recent years, they remain a costly source of energy. Further R&D developments in solar PV and wind technologies that can reduce the cost of renewable systems are needed to allow widespread adoption of this developing technology. India will achieve "Grid Parity" in solar energy in 2017 and wind energy in 2022, according to the above debate. For further development, it is required to concentrate on a certain technological system, which necessitates greater work and better policy measurement.

References:

A. K. (2016). PV-wind hybrid system: A review with case study. *Cogent Engineering*, *3*(1), 1189305

Bajpai, P., and Dash, V. (2012). Hybrid renewable energy systems for power generation in stand-alone applications: A review. *Renewable and Sustainable Energy Reviews*, *16* (5), 2926-2939

Dave V and Kumar S. 2019. Hybrid Renewable Energy Technologies for Rural India. *Electrical India.*

Deshmukh, M. K., and Deshmukh, S. S. (2015). Modeling of hybrid renewable energy systems. *Renewable and sustainable energy reviews*, *12*(1): 235-249

Guoa, S., Liu, Q., Sun, J., and Jin, H. (2018). A review on the utilization of hybrid renewable energy. *Renewable and Sustainable Energy Reviews*, *91*, 1121-1147

Gupta, A., Saini, R. P., and Sharma, M. P. (2016, December). Optimised application of hybrid renewable energy system in rural electrification. In *2016 India international conference on power electronics* (pp. 337-340). IEEE

Khare, V., Nema, S., and Baredar, P. (2013). Status of solar wind renewable energy in India. *Renewable and Sustainable Energy Reviews*, *27*, 1-10

Krishna, K. S., and Kumar, K. S. (2015). A review on hybrid renewable energy systems. *Renewable and Sustainable Energy Reviews*, *52*, 907-916

Kumar, S., Paswan, M. K., and Behera, S. (2018). Micro Study of Hybrid Power System for Rural Electrification-A Case Study. *International Journal of Applied Engineering Research*, *13*(7), 4888-4896

Negi, S., and Mathew, L. (2014). Hybrid renewable energy system: a review. *International Journal of Electronic and Electrical Engineering*, *7*(5), 535-542

Neves, D., Silva, C. A., and Connors, S. (2014). Design and implementation of hybrid renewable energy systems on micro-communities: A review on case studies. *Renewable and Sustainable Energy Reviews*, *31*, 935-946

Olatomiwa, L., Mekhilef, S., Ismail, M. S., and Moghavvemi, M. (2016). Energy management strategies in hybrid renewable energy systems: A review. *Renewable and Sustainable Energy Reviews*, *62*, 821-835

Sharma, K. K., Gupta, A., Kumar, R., Chohan, J. S., Sharma, S., Singh, J. and Dwivedi, S. P. (2021). Economic evaluation of a hybrid renewable energy system (HRES) using hybrid optimization model for electric renewable (HOMER) software—a case study of rural India. *International Journal of Low-Carbon Technologies.* Oxford University Press, vol. 16(3), pages 814-821.

Sinha, S., and Chandel, S. S. (2014). Review of software tools for hybrid renewable energy systems. *Renewable and Sustainable Energy Reviews*, *32*, 192-205.

ABATING ETHNO MEDICINAL KNOWLEDGE

Manohara Acharya*[1, 2], Ravindra B. Malabadi[1, 3] and Rajukrishna Chalannavar[1*]

[1]Department of Applied Botany,

Mangalore University, Mangalagangotri, Mangaluru-574 199, Karnataka, India

[2]Department of Botany,

Canara College, Mangaluru-575 003, Karnataka, India

[3]Miller Blvd, NW, Edmonton, Alberta, Canada

*Corresponding author E-mail: acharya.manohara@gmail.com

Abstract:

The Dakshina Kannada district is well recognised for its traditional, folk, or Ayurvedic medicine preparation and practises. Traditional healers, pandits, and Ayurvedic physicians can all be found in the district. Traditional Healer is a person in a primitive civilization who treats people suffering from various illnesses using long-established methods passed down from one healer to the next. Because of poverty and lack of access to modern medicine, tribal and rural communities in various sections of Dakshina Kannada rely more on medicinal plant therapy to meet their health care needs. New generations are hesitant to continue the family's indigenous medicine techniques due to modernisation and a loss of faith in TK. Meanwhile, most traditional healers are unwilling to share their knowledge for a variety of reasons, including a false belief in knowledge sharing, educated cheaters, illiteracy about knowledge conservation, and a lack of integration among traditional healers, which is why folk medicine knowledge is deteriorating from generation to generation.

Introduction:

Many traditional practitioners and folk healers reside in the Dakshina Kannada district, which has a diverse ethnic and cultural population. Traditional medicine (also known as indigenous or folk medicine) refers to therapeutic knowledge passed down through generations in diverse civilizations prior to the advent of modern medicine (Bhandary and Chandrashekar, 2003). Traditional medicine is defined by the World Health Organization (WHO) as "the sum total of knowledge, skills, and practices based on theories, beliefs, and experiences indigenous to various cultures, whether explainable or not, used in the maintenance of health as well as the prevention, diagnosis, improvement, or treatment of physical and mental illness, whether explainable or not" (Poornima *et al.*, 2010).

Different ethnic groups such as Koraga, Malekudiya, Naika, and other communities such as Brahmin, Bunts, Vishwakarmas, Billavas, Musilms, and others live in the district. Herpes,

Jaundice, Skin Diseases, Male and Female Sterility, Sexual Problems, Indigestion, Blood Pressure (High & Low BP), Diabetes, Menstrual problems (White discharge, Bleeding, etc), Piles, Constipation, Kidney Stones, Mental Disorders, and Child Problems are all treated by these traditional healers using locally available herbs (Shivaprasad and Chandrashekar, 2003).

The unique trait of folk medicine is that it is always linked to divinity. Traditional healers give medications for various physical and mental disorders in the name of Deities such as Naga, Bairava, Dhanvanthari, Mantraguliga, Mantradevate, Satyadevate, and others. Villagers rely on plants for their primary healthcare due to poverty and a lack of access to modern treatment. Fewer side effects, higher patient tolerance, lower cost, and acceptability owing to long history of use are all advantages of folk medicine (Kshirsagar, 2001).

Traditional herbal medicinal knowledge has been passed down orally from generation to generation, thereby eroding this important and priceless knowledge. The study discovered that the majority of Dakshina Kannada tribes' or communities' folklore medicine has remained unpublished or unknown until now (Kingston *et al.,* 2009; Gupta *et al.,* 2010; Rajakumar and Shivanna, 2010).

Study area:

The coastal district of Dakshina Kannada is located in the state of Karnataka (Fig 1). On the east, it is protected by the Western Ghats, while on the west, it is encircled by the Arabian Sea. During the monsoon, Dakshina Kannada receives a lot of rain. The district is bordered on the north by Udupi District, on the northeast by Chikkamagaluru District, on the east by Hassan District, on the southeast by Kodagu, and on the south by Kasaragod District in Kerala (Dakshina Kannada District Profile, 2015). Mangaluru, Putturu, Belthengady, Bantwala, and Sullia are the five Taluks of Dakshina Kannada District (Fig 2).

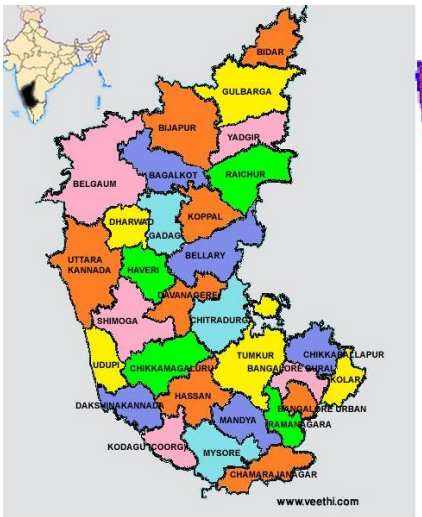


Figure 1: Map of Karnataka state and Figure 2: Map of Dakshina Kannada district

Reasons for folk medicinal knowledge deterioration

1. Modernization: Dakshina Kannada district has a high literacy rate compared to other districts in Karnataka, and many educational institutions related to medical, engineering, paramedical, law, preuniversity, and undergraduate colleges are springing up year after year, earning the district the title of Shikshana Kashi (Educational Centre) of Karnataka. As a result, students from all across Karnataka, as well as neighboring states such as Kerala, Tamilnadu, Assam, Manipur, Telangana, Andra, and the northern states, are flocking to the area. Because of the aforementioned reason, the district is rapidly expanding in all areas, which is causing the decline of vegetation to the point where medicinal plants are no longer readily available to traditional healers. Because of contemporary education, the current generation is hesitant to adopt folk medicine, rejecting it as an old-fashioned superstition. As a result, traditional healers have lost their professional lives in this sector, forcing them to seek alternative employment in order to support their families.

2. Disbelief in Folk Knowledge: In comparison to Allopathic medicine, the restorative period is longer since they prepare medicine as needed, which takes time, but it is impractical to accept in emergency situations in the computer age. Another reason is that phoney traditional healers are springing up every day, deceiving people in the name of deities, causing people to lose faith in folk medicinal expertise.

3. Illiteracy about the Conservation of Knowledge: The majority of traditional healers are illiterate and rely solely on memory to pass on their knowledge of medicine. These people will not disclose their knowledge until they find a trustworthy person; in the meantime, if the person dies or becomes ill (Paralysis), the entire knowledge will be buried in the earth. Another myth (superstition) held by traditional healers is that if they share their knowledge, medicine will lose its healing properties. As a result, without adequate documentation, folk medicinal knowledge is fading from generation to generation.

4. Educated Cheaters: Ethno botany is a relatively new branch of study in the world of biological sciences. Pharmaceutical companies, in particular, profit from these ethnic groups by using them without regard for their loyalty. On the other side, life science researchers collect data from traditional healers or the tribal community and publish it as their own without disclosing their names or obtaining their permission; such well-educated persons purposefully breach the Intellectual Property Rights (IPR) rules.

5. Lack of Integration among Traditional Healers: Because there were no adequate norms and regulations for medication in ancient times, as well as a lack of medical accebility, village people relied heavily on traditional healers for various ailments, and they also received the best care at a

low or free cost. However, the government has tightened medication restrictions in recent years, requiring traditional healers to register their names with the Forest Department, failing which department officers will refuse to enable them to pick medicinal plants from the forest. . Due to a lack of cooperation among them, they were unable to stand up to the government's norms and regulations, as well as modern medicine, and they were unable to defend folk medical knowledge in relation to literature.

Discussion:

Traditional healers in Dakshina Kannada come from a variety of backgrounds, including astrologers, priests, black magicians, spiritual healers, agriculturists, and others, and they provide promising folk medicine for a variety of ailments, including fatal diseases such as Dengue, Cancer, and jaundice. There were numerous examples of traditional healers treating failed allopathic cases based on the results of the field survey. This knowledge was derived from their ancestors' wisdom or from their Teacher (Guru) through living practical experiences at the treatment site, rather than from a book or laboratory knowledge. As a result, due to the aforementioned rationale, traditional healers have not documented this information in the form of literature. Because most folk medicines are unknown, there is a chance that promising medicines for lethal diseases such as Dengue fever, jaundice, cancer, HIV, and other infectious diseases will emerge.

Conclusion:

Traditional Medicinal Knowledge is a wealth of the district, state, or country; thus, we must preserve it by raising awareness among traditional healers about proper documentation, Intellectual Property Rights (IPR), marketing, and valid publication. Traditional healer's organizations will play a vital role in the preservation of folk medicinal knowledge as well as legal backing from the government.

Reference:

Bhandary, M. J. and Chandrashekar, K. R. (2003). Sacred groves of Dakshina Kannada and Udupi districts of Karnataka. Current science. Vol.85 (12):1655-1656.

Bhandary, M. J. and Chandrashekar, K. R. (2011).Herbal therapy for herpes in the ethnomedicine of Coastal Karnataka.Indian Journal of Traditional Knowledge.,Vol.10(3):258-532.

Dakshina Kannada District Profile (PDF). Government of Karnataka. Retrieved 22 February 2015.

Gupta, R., Vairale, M.G., Deshmukh, R.R., Chaudhary,P.R., Wate, S.R. (2010). Ethnomedicinal uses of some plants used by *Gond* tribe of Bhandara district,Maharashtra. *Indian Journal of Traditional knowledge* 9(4):713-717.

Kingston, C., Jeeva, S., Jeeva, G.M., Kiruba, S.,Mishra, B.P., Kannan, D. (2009). Indigenous knowledge of using medicinal plants in treating skin diseases in Kanyakumari district, Southern India.Indian Journal of Traditional Knowledge 8(2): 196-200.

Kshirsagar, R.D.(2001). Some Less Known Ethnomedicinal uses from Mysore and coorg districts, Karnataka, Southern India. Ancient Science of Life.,Vol. 20:20-25.

Poornima, G., Manasa, M., Rudrappa, D. and Prashith Kekuda, T.R.(2010). Medicinal Plants Used by Herbal Healers in Narasipura and Manchale Villages of Sagara Taluk, Karnataka, India. Science, Technology and Arts Research Journals. Vol. 1(2):12-17.

Shivanna, M.B., Rajakumar, N. (2010). Ethno-medicobotanical knowledge of rural folks in Bhadravathi taluk of Shimoga district, Karnataka. Indian Journal of Traditional Knowledge 9(1): 158-162.

HEALTH MANAGEMENT PRACTICES FOR SOME IMPORTANT VIRAL POULTRY DISEASES

Devendra Singh Porte* and Pushpraj Singh

Department of Rural Technology and Social Development,

Guru Ghasidas Vishwavidyalaya (A Central University),

Bilaspur-495009, Chhattisgarh, India

*Corresponding Author E-mail: portedevendra26@gmail.com

Abstract:

In the last half century, significant increases in the productivity of modern poultry stocks have been achieved for both the meat and the egg production sectors of the global poultry industry. Improvements in poultry management and housing, nutrition, ration formulation, the use of poultry genetics in commercial breeding programmes, and improved diagnosis and control of avian diseases have all led to synergies. The health and disease of poultry can be the least predictable of all these essential components.

Although poultry diseases from nutritional and metabolic causes, the focus of this information note is on minimizing diseases brought on by infectious agents, which can have detrimental and sometimes immediate negative effects on the effectiveness of commercial processes. Although poultry diseases from nutritional and metabolic causes can be have concern. The development of an intensive poultry industry in many of the countries discussed here depends on the growth in number and size of small and medium-sized commercial poultry operations. Therefore, the main focus of this review is on elevating poultry health for operations of this size. However, the effects on poultry health for and from such flocks are also included due to the significance of small-scale, village-based production units in many developing countries.

Keywords: Viral diseases, immunization, disease symptoms disease control

Introduction:

Agriculture is the backbone of the rural economy. It also has a significant impact on the national economy, contributing 13–16 percent of GDP. The number of people who keep chickens in their homes is not known with precision. The estimated agricultural households are all thought to be involved in small-scale domestic poultry production using native chickens in various parts of the nation depending on climatic conditions. Natural selection primarily favours regional or native breeds when it comes to the chickens raised in free-range and backyard

systems. Because of this, chickens in rural areas continue to perform poorly, as shown by their extreme broodiness, slow growth rates, small body size, and low production of eggs and meat. In the majority of the country, separate poultry houses are uncommon, and chickens typically coexist in family homes with people without any or very little feed supplementation. Additionally, raising them has been thought of as a supplemental farming action. Almost every household raises chickens for social and economic reasons in rural India, which is one of the potential locations for household-based poultry production systems. The rural poultry production and management system was not well described in relation to the leading agro-ecological set up of the area, in spite of its great importance in the household economy and food security.

After creating a clean and hygienic environment through good poultry Production activities, routine precautionary process represent the next line of defence against disease. Among the preventive steps are:

- Immunization
- Parasite control
- Identifying and treating sick birds
- Separating multi-age flocks
- Putting routine bio-safety activity into practise between staff members who work with flocks.

Immunization

Numerous poultry diseases can be stopped by immunization. Use a suitable vaccination schedule, or only purchase properly immunised stock. When buying chicks or pullets, you can ask your supplier for vaccination records. Marek's disease, infectious bronchitis, infectious coryza, infectious bursal disease, infectious laryngotracheitis and Newcastle disease are among the diseases against which poultry vaccinations are available.

For breeders of poultry, when vaccinating:

- Always abide by the label's instructions, including those regarding storage.
- Make use of disposable needles and syringes.
- Properly dispose of all vaccines, used syringes and needles.
- Keep things organized, but keep cleaning products and disinfectants away from vaccination equipment.
- Disinfecting the skin before receiving Marek's HVT or the fowl pox vaccine will destroy the vaccine virus.
- Confirm the accessibility of vaccines with your vaccine supplier or veterinarian

Control of parasite

Internal and external parasite exposure will be greater in birds kept on the floor with access to pastures and outdoor spaces. It's crucial to implement a prevention programme and administer treatments as necessary for birds kept in these circumstances. By doing so, physical strain is abridged and poultry birds are kept fit and disease-resistant. Eliminate parasites by:

- Frequently checking poultry birds for parasites on the outside
- Spraying or dusting the poultry birds with a standard insecticide thoroughly if you notice mites or lice; make sure the insecticide gets into crevices in the shed, nest and perches.
- Rotating ranges and cleaning out sheds to avoid worms.
- Constantly looking for any worms in the faeces.
- Always check the label for withholding periods on worming treatments because some are not suitable for production poultry birds.
- Consulting a veterinarian. Speaking with a veterinarian.

Eliminate ill poultry birds

Keep an eye out for any symptoms of sickness or issues with the flock, such as feather picking, in your poultry birds. Eliminate sick poultry birds and other animals from the main flock and get a professional diagnosis. Usually, sick birds don't look like healthy birds. Once the disease or issue has been identified, the proper treatment can be administered. Until they are fully recovered, keep sick birds isolated from the flock. If medication is administered, it's crucial to follow any withholding limits.

Management of multi-age flocks

There is a higher risk of disease communication from an older flock of birds to a flock of younger birds when younger birds are introduced. Older birds frequently develop resistance to ailments that younger birds have not yet encountered. When introducing new birds to a flock, there may also be an increased risk of feather plucking and social problems. Running single-aged flocks is best in terms of disease prevention. However, if this is not possible and you are managing flocks with multiple ages, such as Keep age groups apart; implement an all-in, all-out system for each age group to enable thorough cleaning and equipment disinfection in between batches. Always begin with younger and work your way up to the oldest poultry birds.

Some important viral disease of poultry birds

1. Newcastle disease (ND)

Newcastle disease (ND) affects domestic poultry, birds kept in cages and aviaries, and wild birds. It is a highly contagious viral disease. It typically manifests as a rapidly fatal, high-mortality condition in poultry birds and is characterised by respiratory, gastrointestinal, and

nervous signs. The disease caused by virulent ND viruses can be clinically undetectable or quickly fatal in other bird species. ND viruses can cause clinical disease in poultry birds in pathological conditions with several virus strains displaying high levels of virulent while other strains produce no disease and are categorised as avirulent. The disease caused by viruses with high and moderate virulent as shown in tests on domestic birds or based on their genetic makeup is known as neurodegenerative disease (ND), according to the World Organisation for Animal Health (OIE). ND virus is infective for almost all avian species, both domestic and wild. Natural infection has been reported in humans and rodents, and a variety of laboratory animals have been infected experimentally. Infections in non-avian species could spread the disease but the significance of this is not known. However, these animals pose a significant risk because they can act as mechanical vectors of ND.

- **Chickens:** Highly prone to ND virus infection, including APMV-1's pigeon variant. Among poultry birds, is thought to be the most vulnerable.
- **Turkeys:** Susceptible to ND. Outbreaks can occur in turkey flocks but they are usually less severe than those in chickens. Effects on egg production are similar to those in chickens. Some outbreaks have resulted in high mortalities, others in leg paralysis.
- **Ducks and geese:** Ducks are reported to be readily infected with ND virus and to be competent of dispersal the virus. Nervous signs and diarrhoea are the most notable clinical features of the pigeon variant of APMV-1, which can result in up to 80% morbidity. There are few reports of clinical ND virus in ducks, Turkeys can also be infected with the virus, but are apparently not very susceptible to disease**.**
- **Pigeons:** Susceptible to ND. APMV-1 the pigeon variant can create up to 80% morbidity, with diarrhoea and nervous symptom being the nearly all distinguished medical characters.
- **Wild waterfowl:** Another reservoir of avirulent ND viruses usually associated with intestinal infection. However, wild waterfowl have been strongly implicated in the spread of outbreaks across Europe. Infections have occurred in cormorants in Filename: ND3.1-16FINAL (29Nov06) 10 AUSVETPLAN Edition 3 the United States and Canada over a number of years without infecting domestic poultry**.**
- **Peafowl, pheasants, quail and guinea fowl:** All are susceptible to natural ND virus infection. Although mortalities have been recorded, infection usually produces only mild disease unless it occurs in quail, which are very susceptible.

Disease Symptoms

The clinical signs of ND virus infection are very variable, influenced greatly by the virulence and tissue tropism of the virus; the species, age, immune status and condition of the bird; the route of exposure; the magnitude of the infecting dose; and external factors, such as type of housing and environmental and social stress. An outbreak of ND in poultry birds may be so severe that approximately all birds of a pretentious flock die within 72 hours without noticeable signs, often causing a suspicion of poisoning. In adult layers, a marked drop in production may be the first sign, followed in 24–48 hours by mortality, which can reach 100%. Clinical signs and symptoms noted may be:

- A sudden drop in egg production often accompanied by production of abnormal eggs (misshapen, soft or missing shells with loss of normal pigment);
- Loss of appetite, fever, weakness;
- Swelling and cyanosis of the comb and wattles;
- Watery, bile-stained, distinctive bright green or bloody diarrhoea;
- Respiratory signs, which may include increased respiratory rate, respiratory distress, coughing and a high-pitched sneeze (‘snick’); and Filename.
- Nervous signs, which can include loss of balance, circling, backward progression and convulsive somersaulting, rhythmic spasms, stiff and wry neck, head tremors, and wing and leg paralysis.

Disease Control

Innate and passive immunity: Different strains of chickens vary in their response to ND infection. Younger birds develop clinical signs more quickly and are more severely affected, although chicks from immune hens may be protected by antibody derived from the yolk.

Active immunity: It is likely that the bird’s full range of immune mechanisms is involved in the immune response. Cell-mediated immunity can be demonstrated two days after infection. All ND virus strains cause an antibody response in chickens and other avian species. However, titres in cage and aviary birds following natural infection with lentogenic strains are not known. Serum antibody can be detected in chickens 6–10 days after infection. Titres peak after 3–4 weeks and decline to undetectable levels in 8–12 months. Neutralising antibody protects chickens, chicken embryos and cell cultures from infection. Birds resistant to infection have high levels of circulating antibody. Low levels of antibody may not prevent infection but can protect chickens from severe disease and mortality. It has been demonstrated that vaccinated birds without detectable antibody may survive challenge with virulent virus. This may be due to low levels of

humoral antibody, interference between vaccine and challenge virus competing for cell attachment sites, cell-mediated immunity, and/or local immunity.

Newcastle disease (ND) vaccination

Immunity brought on by vaccinations is thought to last between 10 and 12 weeks. Repeated vaccinations are required to maintain a sufficient level of protection. Parental immunity also interferes with vaccine effectiveness. Vaccination programs are therefore often delayed until chicks are 1–2 weeks old. Both naturally occurring ('live') and inactivated ('killed') vaccines have been developed overseas and experiments conducted locally and overseas to determine the vaccine effectiveness of the lentogenic V4 strain (Australian virus isolated in 1966).

Virulence

i. **Lentogenic virus:** Lentogenic virus vaccines are generally administered by eye drop, in drinking water, by aerosol or intranasally. A vaccine using a heat-tolerant V4 strain has been developed for feeding to village chickens in countries where these constitute a significant proportion of poultry production.

Vaccine type: V4 strain, Ulster 2C, Hitchner B1, Asplin F strain, La Sota.

ii. **Mesogenic strains:** Mesogenic strains are not considered for use in Australia because the vaccine virus is capable of causing significant disease in fully susceptible poultry. Vaccines based on lentogenic strains of virus, such as B1, La Sota, F and V4, which have proven efficacy against ND, have been successful in controlling ND outbreaks in many parts of the world.

Vaccine type: Komarov K strain, Roakin strain, n Mukteswar strain.

2. Fowl Pox

The majority of bird species, including all types of commercial poultry, are susceptible to the relatively slow-moving viral infection known as fowl pox. Both a wet and dry form is present. Plaques in the mouth and upper respiratory tract define the wet form. The wart-like skin lesions that develop into thick scabs are the defining feature of the dry form. The disease may occur in any age of bird, at any time. Unless the respiratory involvement is severe, mortality is typically not significant. Depression, decreased appetite, and poor growth or egg production can all be symptoms of fowl pox. The disease progresses over three to five weeks in each individual bird.

An avian DNA pox virus is what causes fowl pox. Although there is some cross-infection, there are five or six closely related viruses that primarily affect various species of birds. Skin bites or abrasions lead to infection, as do respiratory infections and possibly eating

infected scabs. Birds, mosquitoes, or termites can all spread it (inanimate objects such as equipment). In dried scabs, the virus is incredibly resilient and can, in some circumstances, last for months. After feeding on sick birds, mosquitoes can carry infectious viruses for a month or longer before spreading them to other birds. Recovered birds do not remain carriers. A flock may be affected for several months as fowl pox spreads slowly.

Disease Symptoms

Fowl pox is a common disease in backyard chickens that have not been vaccinated. Although very young or sick birds may not survive the infections, the majority of birds do. On the comb, wattles, and other skin areas, the lesions initially resemble a whitish blister. Lesions can occasionally be found on the legs, body, and even the softer parts of the beak. The blisters take about three weeks to heal and fall off after developing into a dark scab. Fowl pox lesions in the mouth and throat of an infected bird can make breathing difficult or even fatal. Exhibition poultry breeders prefer to vaccinate their birds to prevent this disease because scarring could result. Management of the mosquito population can help reduce outbreaks of fowl pox.

Disease Control

Fowl pox has no known cure, so the only way to prevent it is to vaccinate any replacement birds. When using preventative vaccination, all replacement chickens are immunised between six and ten weeks of age, and one dose of the fowl pox vaccine provides lifetime immunity. Broilers typically do not need to be immunised unless there is a high mosquito population or a history of infections. Chicks as young as one day old can receive vaccinations. Unaffected flocks and individuals may receive vaccinations during outbreaks to aid in containing the spread. Broad-spectrum antibiotics may help lower morbidity and mortality if there is evidence of a secondary bacterial infection. As mosquitoes are known reservoirs, mosquito control procedures may be of some benefit in limiting spread in poultry confined in houses.

Fowl Pox vaccination

Vaccines are available for fowl pox (ATC vet code: QI01AD12 (WHO)). Chicken are usually vaccinated with *pigeon pox virus*. The wing web method of injection is typically used to administer this vaccine to chickens between the ages of 8 and 14 weeks. A bird should be in perfect health prior to vaccination because it will only expose it to a mild form of the active virus. Also regularly immunised are turkeys. There are no cures for bird flu once it has been contracted; the only options are vaccination and mosquito control.

3. Avian influenza

The term "bird flu" (also known as "avian flu" or "avian influenza") describes influenza strains that primarily infect wild and domesticated birds. Depending on the proteins found on the virus surface, bird flu is given the names H or N. Avian influenza, also known as bird flu, is a condition brought on by influenza virus strains that primarily affects birds. A new strain of bird flu emerged in the late 1990s that was notable for its capacity to kill domesticated birds like ducks, chickens, and turkeys and cause severe illness and disease. As a result, this strain was called highly pathogenic (meaning very severe and contagious) avian influenza and termed H5N1.Avian influenza viruses are type A orthomyxoviruses (*Influenzavirus A*) characterized by antigenically homologous nucleoprotein and matrix internal proteins, which are identified by serology in agar gel immune diffusion (AGID) tests. AI viruses are further divided into 16 hemagglutinin (H1-16) and 9 neuraminidase (N1-9) subtypes.

A new strain of bird flu was identified in China in 2013. H7N9 is the name of the influenza A virus (H7N9 Chinese bird flu). The virus (H7N9) was identified on March 31, 2013, and is antigenically distinct from the H5N1 bird flu virus. Sadly, the H7N9 bird flu strain appears to have unstable genetics. At least 48 different subtypes of H7N9 have been identified since its discovery. Researchers are worried that some H7N9 strains will continue to swap genes with other flu viruses and may cause a new pandemic because the viruses are persistent in some chicken flocks in China.

Infected birds shed the virus in their saliva, nasal secretions, and droppings, which is how it spreads. When healthy birds come into contact with contaminated faeces or secretions from sick birds, they become infected. The virus may also spread from bird to bird through contact with contaminated surfaces, such as cages. A bird's symptoms can range from a slight decrease in egg production to the failure of several major organs and eventual death.

Influenza virus strains that have evolved to be specifically adapted to enter avian cells are what cause bird flu. The three main influenza subtypes are A, B, and C. The influenza A type virus that causes bird flu has eight RNA strands in its genome. By examining two proteins on the surface of the virus, influenza viruses are further categorised. Hemagglutinin (H) and neuraminidase are the names of the proteins (N). Hemagglutinin and neuraminidase proteins come in a wide variety of forms. Type 5 hemagglutinin and type 1 neuraminidase, for instance, are present in the most recent pathogenic bird flu virus. The influenza A virus is therefore known as "H5N1" (also termed HPAI or highly pathogenic avian influenza). The

2013 virus is known as H7N9 because it has two different surface proteins on its surface. H7N7, H5N8, H5N2, and H9N2 are additional strains of bird flu.

Disease Symptoms

Bird flu symptoms include fever, cough, sore throat, and nausea. Symptoms often progress to severe breathing problems, pneumonia, and acute respiratory distress syndrome (ARDS).

Disease Control

Prevention Techniques

Exclusion bio-security strategies are the best protection against the introduction of AI into poultry. Suspected outbreaks should be reported to the relevant regulatory authorities. In addition to preventing clinical symptoms and death, vaccines that are antigenically matched and given correctly can significantly reduce viral replication and shedding from the respiratory and gastrointestinal tracts. Specific defense is offered by autologous virus vaccines or vaccines created from AI viruses with the same hemagglutinin subtype. Antibodies to homologous viral neuraminidase antigens may partially protect against them. Only the inactivated whole AI virus, DNA of H5 hemagglutinin, defective eastern equine encephalitis virus, recombinant fowlpox-AI-H5, and recombinant herpesvirus-turkey-AI-H5 (rHVT-AI-H5) vaccines are currently approved for use in the USA. The state veterinarian must give their consent before using any approval AI vaccine against the H1-4, H6, and H8-16 hemagglutinin subtypes. Additionally, a state of emergency must be declared and the Secretary of Agriculture must approve the use of H5 and H7 AI vaccines in the USA.

Providing Support

Morbidity and mortality may be decreased by treating flocks affected by the LPAI with broad-spectrum antibiotics to control secondary pathogens and raising house temperatures. Antiviral drug therapy is neither approved nor advised.

Vaccination

In the US, efforts are made to lessen the prevalence of HPAI in poultry through routine surveillance of poultry flocks in commercial poultry operations. The flock might need to be immediately destroyed if an HPAI virus is discovered. Less pathogenic viruses can be controlled through vaccination, which is mainly used in flocks of turkeys (ATC vet codes: QI01AA 23 (WHO) for the inactivated fowl vaccine and QI01CL01 (WHO) for the inactivated turkey combination vaccine).

4. Polyneuritis

Marek's disease is a highly contagious lympho-prolifative disease in chickens. Birds like Quail and Turkeys can be affected naturally/ artificially but chickens are more prone to this disease, as they are most important natural host for Marek's disease virus (MDV). The chickens become carriers for life, once infected with MDV. In the environment where chickens are raised, the virus is present everywhere. Human beings are not harmed by this virus. Renowned veterinarian Dr. Joseph Marek of the Royal Hungarian Veterinary School in Budapest published the first research on Marek's disease in 1907. The Marek's disease virus (MDV) is a member of the Herpes viridae family's Alpha-herpesvirinae subfamily and is a member of the Mardivirus genus.

Additionally, MDV type 1 (MDV1), also known as Gallid herpesvirus 2 (GaHV-2), and MDV-2 are two distinct MDV species found in the herpesvirus of turkeys (HVT), which is a member of the Mardivirus genus (GaHV-3). The pathogenic strains for MDV-1 and MDV-2 were originally isolated from chickens that appeared to be normal, while MDV-1 contains all the pathogenic strains and some vaccine strains. According to D. J. McGeoch's personal communication, the Mardivirus lineage split off from the mammalian Herpesvirus about 131 million years ago. Feather dander—a white coating that surrounds growing feathers—is the main method of MDV transmission, but it can also spread through blood, saliva, poultry house dust, and other bodily fluids. The condition was initially referred to as "Polyneuritis." the sacral plexus and spinal routes becoming thicker, allowing mononuclear cells to invade. Innate and adaptive immune responses work together to produce immunity against MDV. However, because the virus is cell-associated, vaccination results in a cell-mediated immune response. The activation of T helper (TH-cells) 1 cells and the release of pro-inflammatory cytokines are necessary for protection. Additionally released are the interferons (IFNs), interleukin (IL)-1, IFN-, and inducible nitric oxide synthase (iNOS).

Etiology: Gallid Herpesvirus 2, also known as Alpha-herpesvirus, is the etiological agent. There are three known serotypes, including the avirulent serotype 3 and the less virulent serotype 1 of the turkey herpesvirus. Serotype 1 (MDV-1) has attenuated strains and is more virulent. Serotype 3 (MDV-3) is the herpesvirus of turkeys (HVT), which is used as a vaccine against MDV. Serotype 2 (MDV-2) is an avirulent virus isolated from chickens. MDV consists of a 256-capsomere capsid, an outer envelope, and an inner core. Serotype 1 MDVs are the most virulent of these three serotypes, and they can be further divided into mild (m), virulent (v), very virulent (vv), and very virulent plus (vv+) strains based on their ability to cause cancer.

Disease Symptoms

MD-related clinical symptoms include T-cell lymphoma formation in visceral and ectoderm derived tissues, immunosuppression, and polyneuritis. Neurolymphomatosis causes paralysis in the legs and wings. Ocular lymphomatosis causes the iris of one or both chicken eyes to turn grey. Round, nodular lesions up to 1 cm in diameter, particularly on the feather follicles, are a symptom of cutaneous disease. Organs like the kidney, muscles, gonads, lungs, and spleen can also develop nodular and lymphoid tumours. Meningoencephalomyelitis without suppuration and lymphoma.

Depending on the age of the affected birds, the symptoms change. Sneezing, gasping, and frequently droopiness are the first symptoms that are typically seen in young birds. The symptoms in this stage of the illness resemble bronchitis-related infections quite closely. A flock experiences deaths in quick succession and in growing numbers each day within a short period of the onset of respiratory symptoms.

In some cases, sudden deaths happen to adult or growing birds, and many birds exhibit respiratory symptoms afterward. The affected birds' feathers are ruffled, and they are stuffed and depressed. Diarrhea, which is characterized by passing watery, pungent-smelling stools, occurs along with these symptoms. There is significant salivation. The production of gurgling sounds is caused by saliva that frequently builds up in the mouth and obstructs breathing. Disused birds may also have soft-shelled and malformed birds. The disease in turkeys progresses very slowly. Except for a slight sense of dullness, a loss of appetite, and other minor symptoms, it may go unnoticed in adults in particular.

Control

There is no worthwhile treatment available right now. In an effort to reduce the duration and severity of the infection, proper housing and general good care are advised. Early disease detection and the implementation of sanitary measures are extremely important for disease control. Segregating infected birds into groups of 10 to 15 each, removing all infectious materials like droppings and poultry cleanliness residues, and providing individual attendants for each group of birds are some crucial steps in its prevention.

The location of the poultry farm should be far from a busy intersection. Prior to bringing any newly acquired birds into the farm, they should be kept in isolation for at least 10 days. The chicken runs should occasionally be ploughed, and lime should be applied there as a general disinfectant. By creating a barrier of wire netting, it should be as difficult as possible for free-flying birds to access the pens and runs.

Conclusion:

Viral infections are the main causes of losses in poultry production. Ethnoveterinary medicine (EVM) plays an important role in health management of family poultry as shown by a high percentage of rearers that use it compared to vaccines and chemical dusts. The majority of respondents used EVM as broad spectrum medicines. The low use of vaccines and chemical dusts by poultry rearers indicate inadequacy of extension service. Therefore, it is important that the government extension service train family poultry rearers in health management. It is necessary to assess the effectiveness of traditional treatments that are frequently used by farmers to control parasites and diseases in their flocks. So, indigenous chicken ecotypes and a very small number of exotic breeds are the foundation of the poultry production system (RIR). Agro-ecology affects how the households manage their resources and their production systems. While traditional medical practises were used to heal and protect chickens from disease, poultry vaccination was extremely inadequate. The system's main bottlenecks are diseases, inadequate veterinary care, subpar housing, subpar nutrition, and the neglect of local chickens in extension programmes, but the farmers' desire to encourage poultry production and their innate understanding of culling and selection practises may present an opportunity to advance the industry.

References:

http://www.agrilearner.com/diseases-poultry/

https://www.researchgate.net/publication/320711795_Etiology_Epidemiology_Pathogenesis_and_Diagnosis_of_Marek's_Disease_in_Chickens_A_Mini_Review/

https://www.pashudhanpraharee.com/ranikhet-disease-in-poultry/

https://www.academia.edu/5842754/Rural_poultry_production_and_health_management_practices_in_central_zone_of_tigray_Ethiopia_A_R_T_I_C_L_E_I_N_F_O

https://www.medicinenet.com/avian_influenza_bird_flu/article.htm

https://www.msdvetmanual.com/poultry/avian-influenza/avian-influenza

https://en.wikipedia.org/wiki/Fowlpox

https://www.poultryhub.org/all-about-poultry/health-management/disease/fowl-pox

https://www.researchgate.net/publication/340598889_Bird_Flu_Avian_Flu_H5N1

https://animal-diseases1.blogspot.com/2020/02/article-3-disease-strategy-newcastle.htm

https://www.fao.org/3/al729e/al729e00.pd

https://en.wikipedia.org/wiki/Avian_influenza

https://www.backyardchickens.com/articles/avian-pox-in-chickens-warning graphic-pictures-underconstruction.47704/?page=4

https://academicjournals.org/journal/AJAR/article-full-text/CACD40148874

https://schoolsonlinemw.com/agri-business-news/diseases-in-poultry-and-their organic-treatment-or-naturalremedies

http://www.kvsmt.com/chicken

https://www.semanticscholar.org/paper/Poultry-health-and-disease-control-in-developingBagust/a8765d788ef58d5f079be008fa7a268cd7ef202b

https://www.standardmedia.co.ke/farmkenya/article/2001380464/what-you-need-to-know-about-fowlpo

https://www.dpi.nsw.gov.au/__data/assets/pdf_file/0007/596689/Recognising-exotic-diseases-of-birds.pdf

https://link.springer.com/article/10.1007/s11250-009-9442-4

https://www.iiste.org/Journals/index.php/JBAH/article/viewFile/23957/24528

https://www.academia.edu/es/21101472/Ethnoveterinary_Practices_in_Rural_Poultry_Production_in_Atiba_Local_Government_Area_of_Oyo_State_Nigeria

https://www.fao.org/3/i3531e/i3531e08.pdf

https://www.fao.org/3/i3531e/i3531e08.pdf

https://www.researchgate.net/publication/329337970_Review_on_Newcastle_Disease_of_Poultry_and_its_Public_Health_Importance

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8404124/

https://link.springer.com/article/10.1007/s00705-010-0866-/

CHILD HEALTH AND NUTRITION IN INDIA

Shashi A. Mishra

R. J. College of Arts, Science and Commerce (Autonomous)

Ghatkopar (West), Mumbai 400086

Corresponding author E-mail: sash2mash@yahoo.co.in

Abstarct:

In human beings life childhood is the period related with physical, mental and social wellbeing.With increasing age there is physical and psychological maturation, which influences activity, body composition, feeding skills and food choices. Perfect diet with nutritional food is essential for overall development in infants and children .Thus Proper intake of nutrition among children is necessary for the physical and mental growth of child. Poor nutrition increases the risk of health illness and is responsible directly or indirectly for children deaths among less than five years of age. Unhealthy food habits and lack of proper nutritional diets leads to obesity among children. While adequate nutrition is important throughout childhood, it is crucial during the first five years of a child's health when rapid growth occurs in the physical structure of child and this is the period where family members, grandparents, caregiver to infants and mother play a very important role to fulfil the nutrition level of the child. Nutrition has major effects on health of every individual and crucial effect on child's health.

Keywords: Childhood, food choices, poor nutrition, physical structure

Introduction:

Mental, emotional and physical growth and development depends on the food and nutrition of Child diet. Poor nutrition increases the risk of health illness and is responsible directly or indirectly for children deaths among less than five years of age. Malnutrition refers to any imbalance in satisfying nutritional requirements. Malnutrition among children is often caused by the collective effects of inadequate or improper food intake, repeated episodes of parasitic or other childhood diseases such as diarrhoea. Malnutrition is an important factor for high morbidity and mortality among children. It can also affect growth potential and risk of morbidity and mortality in later years of life. Proper intake of nutrition among children is necessary for the physical and mental growth of children. Infants who are by birth underweight are more vulnerable and are at higher risk towards morbidity.Focusing on the young child's health and their improved nutritional level is directly linked with socio-economic development of nation.

Factors affecting child nutrition

A number of factors affect the health and child nutrition. The most common factors are food availability and dietary intake, breastfeeding, prevalence of infectious and parasitic diseases, access to health care, immunization against childhood diseases, vitamin A supplementation, maternal care during pregnancy, water supply and sanitation, socioeconomic status, and health-seeking behaviour.In India due to illiteracy blind belief ,early marriage of girls child bearing years for women increases and due to lack of awareness of proper family planning programe birth interval in two children among rural women is very low .It affects the availability of nutrition level among children which in turn affects the health of child and mother .Inadequate or improper food intake and repeated episodes of infectious diseases adversely affect children's nutritional status and health. If disease like diarrhoea, among children is tackled carefully it will have positive impact on survival rate of children's mental health and personality growth .Sanitation, washroom facilities , supply of clean water is equally important for the positive health of mother and child. Discrimination against girls in feeding and health care are reasons for poorer nutrition and higher mortality among girls than boys in many developing countries.

Impact of Malnutrition on Children's Health

Allover the world 792 people are affected by malnutrition as it is reported by the world health organization. At least one third of them are children.In childhood malnourishment among children's health have very negative effects on their health in terms of **Stunting**. Malnutrition can hinder a child's ability to grow normally, leaving both his height and his weight well under normal when he's compared with children of the same age. Severe malnourishment can lead to permanent damage among child. ***Marasmus*** is characterized by a lack of nearly all nutrients, particularly protein and calories, severe weight loss, thin and papery skin, hair loss and leads to severe health damage among children. In India protein deficiency among children,known as ***Kwashiorkor*** is found large in number.Symptoms of kwashiorkor include discoloured, brittle hair that has a copper sheen, rashes, water retention, a distended belly caused by bloating, an enlarged liver. Malnutrition can involve not only insufficient macronutrients such as protein, carbohydrates and fat, but also insufficient micronutrients such as vitamins and minerals and also water.

Malnutrition among Children's Health in India

According to the 2010 Global Hunger Index, India has more hungry people and the highest burden of child malnutrition than any country in the world. Though India is having higher GDP compared to many less developed countries but child nutritional deficiency is very high in India .Close to 1.3 million children die every year in India because of malnutrition,

according to the World Health Organization (WHO).Death among children due to malaria in rainy season, diarrhoea related to unhealthy and unhygienic food, prevalence of pneumonia is very high.Worldwide, over 100 million children are underweight. In India large number of young children are underweight, going through the disease like stunting and child nutritional level ailments.

In India deficiency of vitamins and protein in the body number of anaemic children is high. Although poverty is an important factor, nutritional deficiencies are widespread even in households that are economically well off. Inadequate feeding practices for children make it difficult to achieve the needed improvements in children's nutritional status, and nutrition programmes have been unable to make much impact with these serious nutritional problems. India is in the position of having very high levels of malnutrition despite large stocks of food grains resulting from increased agricultural productivity. Major two situations responsible for child malnutrition are -firstly, a significant proportion of the population remains unable to buy enough food; secondly, the whole population is vulnerable to becoming malnourished due to exposure to diseases like diarrhoea and parasitic infections resulting from poor sanitation and living conditions.

Malnutrition among children: Health in Maharashtra

According to National Family Healthy Survey 2005-2006 the nutrition situation in Maharashtra is slightly better than the national average with improvements from 1998-99. The feeding practice for children aged 6-9 months shows an alarming pattern with only 48% of children receiving solid or semisolid food and breast milk.56% less in terms of national average chart requirement. The prevalence drops to 40% for rural areas compared with the national average of 54% and as low as 23.3% for non-educated mothers compared with the national average of 49%.Rural areas of Maharashtra malnutrition is very high .Almost 38% of children under age three are stunted (India - 38.4%) and almost 40% are underweight (India 45.9%). Wasting affects 14.6% of children under age (India 19%). Compared with urban areas, under-nutrition is higher in rural areas and also in Mumbai. In Maharashtra, there is a strong correlation between child malnutrition and the level of maternal education showing a two-fold difference between non-educated and well-educated mothers. Almost 72% of children under age three are anaemic (India 79.2). There is a significant urban-rural divide with Mumbai having the lowest prevalence with 59.5% compared with 76.8% in rural areas. The non-educated versus educated mothers difference is not as strong with 75% and 71% relatively. This may be linked to a more general poor quality of nutrition and hygiene conditions and limited access to iron supplementation.53% of children are fed only breast milk for the first 6 months (India 46%).

Breast feeding among illiterate women in rural areas as well as in urban area is high. 51.8% of children under three years are breastfed within one hour of birth (India 23.4%) with no significant difference between urban and rural areas and between well-educated and non-educated mothers. Only 47.8% of children aged 6-9 months receive solid or semisolid food and breast milk. 32% of children age 12-35 months received vitamin A supplements in the six months (India 23%) with the highest prevalence in urban areas (34.2%) followed by rural areas (29.9%) and in Mumbai (27%).

Factors Affecting India's Nutritional Health Challenges

Population growth, unemployment, livelihood issues, fragmentation of land holding, contractual jobs has reduced purchasing power and led to poverty in Indian society. India's rapid urbanization and overcrowding makes households vulnerable to malnutrition by reducing access to support services, healthcare, clean water, and sanitation. Gender equity is considered as a particularly strong factor in the high rates of maternal and child malnutrition seen in South Asia; women are undervalued in society and eat least and last. National rates of child anaemia, calorie deficiency, and child illness also point to non-optimal feeding practices, which in turn reflects poor maternal nutritional status, economic limitations, sociocultural settings, high fertility rates, limited access to education, and mothers' young ages. India's notably low public health expenditures compound issues of access.These wide factors show that undernutrition follows lines of high and rising levels of inequity in the country. Compare to urban area in rural areas malnourishment and undernourishment is very high.Main reason is poverty which is widely prevalent in rural areas.Children from scheduled tribes have the poorest nutritional status on nearly every measure, and the highest prevalence of wasting among under-fives.

Government's Initiatives in India towards Child's Health and Malnutrition

To check malnutrition, is one of the top agendas in the priority list of the government. Indian Government has implemented various developmental schemes and approach to curb the issues of malnutrition by adopting various sectoral approach. Integrated Child Development Services (ICDS) specially programmed for lactating and pregnant mothers and children below the age of 3, National Rural Health Mission (NRHM), Mid Day Meal Scheme (MDM), Rajiv Gandhi Scheme for Employment of Adolescent Girls(RGSEAG),Indira Gandhi Matriyo Sahayog Yojana (IGMSY). However, in spite of the sincere efforts of the government, gaps exist between the government envisaged nutrition programs and their actual implementations due to various factors like lack of voluntary manpower in the different states. Madhya Pradesh has the highest malnutrition rate (55%) and Kerala the lowest (27%). Even today one in three children in India are stunted, therefore the Niti Aayog has come out with a national strategy to fight maternal and

child malnutrition and anaemia. The focus is on 100 poor performing districts in terms of stunting zero. The national nutrition strategy aims to bring nutrition to the centre stage of the National Development Agenda and has outlined a vision of Khuposhan Mukt Bharat (Malnutrition Free India), reducing all forms of malnutrition by 2030.

Way Forward

Each of the factors affecting malnutrition are extremely complex and emphasizes that there are significant social dimensions to it among children. We must think about such growth among children, where rising incomes and government revenues target improvements in health, nutrition, infrastructure, and education of all. It is also important to emphasize that India is an enormous and diverse country, and much of health and nutrition programming is directed at the state level. By keeping regional issues and their problems in society steps can be taken to address the problems of malnutrition. It is an important thought process that how India is going to address its nutrition challenges, and what opportunities exist for improvement. In India among infants due to lack of awareness programmes for parents especially to young mothers certain communicable disease like measles and diarrhoea which are highly prevalent in India are responsible for leading into malnutrition ad undernutrition among children and infants. Mothers should made informed about importance of exclusive breastfeeding for six months and continuing to breast feed up to two years or beyond. Other important knowledge which should be conveyed are damage caused by irrational beliefs and cultural practices of feeding, Proper work and implementation by all stakeholders at various levels should be addressed efficiently.

References:

Basavanthappa, B (2015). Child health nursing.Jaypee Brothers Medical Publishers

David A. S. (2022). Global meternal and child health.Series Editor

Jonathan, B. K. (2012).Maternal and child health: programes, problems and policy in public health.Jones and Bartlett Publishers.

Klaus V. G. *et al.* (2010) Global Hunger Index: The challenge of hunger, Focus on the crisis of child undernutrition, IFPRI, Concern worldwide

Michaelsen K. *et al.* (2003), Feeding and Nutriton of ifants and young children, World Health Organisation, Regional Office for Europe Copenhagen

Ministry of Health and Family Welfare Government of India. (2007), National Family Health Survey (NFHS-3) 2005-2006

THE POTENTIAL APPLICATION OF THIN CELL LAYER (TCL) CULTURE IN PROPAGATION OF ORCHIDS

Girish K[1] and Ashok N. Pyati*[2]

[1]Department of Microbiology, Government College for Women, Kolar, Karnataka

[2]Plant Tissue Culture Laboratory, Department of PG Botany,

Maharani's Science College for Women, Mysore-570 005, Karnataka, India

* Corresponding author E-mail: drashoknpyati@gmail.com

Absrtact:

Orchids are among the most diverse flowering plant families, prized for their beautiful long lasting flowers exhibiting an incredible range of diversity in size shape and color. Large scale multiplication of exquisite and rare orchids using tissue culture techniques has helped orchids occupy a position as one of the top ten cut flowers. As orchids are out breeders, their propagation using seeds leads to the production of heterozygous plants. Hence protocols providing regeneration from various vegetative parts of the plant are needed. Though orchid micropropagation has shown spectacular development in the recent years. Now Plant thin cell layers (TCLs) have proven to be an effective tool for the in vitro culture of many plants including orchids. TCLs consists of few layers of cells, typically 0.5-1.0 mm thick. The limited amount of cells in a TCL is a paramount importance because marker molecules/genes of differentiation can be easily located in situ in the target cells. Since then TCLs have been successfully used in the micropropagation of many plant species whose previous in vitro regeneration was not successful using conventional methods. This book chapter examines fundamentals behind TCLs, and their applications in orchid micropropagation.

Introduction:

Tran Thanh Van (1973) developed Thin Cell Layer (TCL) culture technique, first described in tobacco pedicel epidermal cell cultures. Based on the use of very small explants derived from a limited cell number of uniform tissue. It is useful for reducing the time period, producing a high frequency of shoot regeneration, direct PLB formation and callus induction in many plants including orchids, simply by manipulating the auxin to cytokinin ratio. TCL serve as a simple, yet effective way to control the developmental pathway and organogenic out come and provide, where tested an effective way of mass producing clones of specific organs (Teixeira da Silva *et al.,* 2015). Since the remarkable discovery of the TCL technique various cellular, biochemical and In vitro micropropagation aspects of several monocotyledons and

dicotyledonous plants, have been examined with explants from roots, shoots and somatic embryogenesis/organogenesis of isolated plant tissues (Teixeira da Silva, 2013; Tung *et al.,* 2021). This culture method was developed for programming different patterns of morphogenesis Tran Thanh Van (1981) and has been applied to short regeneration and somatic embryogenesis in dicot plants. From the last decade this culture method was also used in orchids and other monocotyledonous species. This culture system was proved to be more efficient than other conventional In vitro culture methods with regard to the total output of plantlets (Lakshmanan *et al.,* 1995). So, in order to obtain rapid plant regeneration with a high frequency the TCL culture method was exploited for mass propagation of many orchids. Effects of various PGRs were also evaluated in TCL culture.

The TCL system consists of an explant of a small size excised from different plant organs namely stems, leaves, inflorescences, floral organs, apical meristems, pseudobulbs and protocorms either longitudinal (lTCL) or transverse (tTCL) sections (Vyas *et al.,* 2010). TCL contains only one tissue type, such as monolayer of epidermal cells, whereas tTCL includes small number of cells from different tissue types; epidermal, cortical, cambium, perivascular, medullar, and parenchyma cells. TCL systems allow for the isolation of specific cell or tissue layers which depending on the genetic state and epigenetic requirements and in conjugation with strictly controlled growth conditions namely light, temperature, pH, PGRs, media additives and others, may lead to the In vitro induction of specific morphogenic programs (Teixeira da Silva, 2003). Within the TCL system the morphogenic and developmental pathways of specific organs, derived from other specific or nonspecific cells, tissues or organs may be clearly directed and controlled. Moreover, it allows for the study of cytological, physiological, biochemical and molecular changes occurring in a particular morphogenic program. This strict regulation of the morphogenic pathways will allow for the controlled production of somatic embryos and their subsequent use as synthetic seeds or as mass propagation units. It would also enhance the production capacity of secondary metabolites and pharmaceuticals through transgenic organ cultures. This culture system is promising and efficient with regard to the total output of plantlets obtained, higher than in other conventional In vitro methods for rapid regeneration (Hossain *et al.,* 2013). This technique is also for mass propagation of ornamental plants and conventional cash crops as well as difficult to propagate species, for research and commercial purpose. This protocol requires special attention to selecting appropriate explants, technical skills and careful handling of explants (Teixeira da Silva, 2013). The application of TCL technology is very efficient in terms of using a small number of plants as an explant source, but resulting in the great number of plantlets in a more controlled manner than a conventional explant (Teixeira da

Silva and Dobranszki, 2014). This technique is low cost effective for tissue culture industries and commercial production of plantlets and also generates employment for rural people. This technique is also useful method, would the conservation of RET orchids and can be used for the large-scale production to meet the growing demand of native orchids. Therefore, TCL technique has made micropropagation easier and more efficient for many angiosperms species including orchids (Bose *et al.*, 2017).

In order to have successful results in genetic engineering, high rates of regeneration and low rates of escape are needed. Using explants with large size in gene transformation studies increase the chance of regeneration for both transformed and untransformed cells which leads chimerism (Teixeira da Silva, 2003). Regarding the advantages of thin cell layer method, this method could significantly reduce the possibility of chimerism. This TCL system consists of small sized explants of different plant organs longitudinally (lTCL) or transversely ((tTCL) which are different in tissue types. Therefore, they could be used as suitable system for selecting a specific cell or tissue layer, in order to induce a specific morphogenic programs which lead to an efficient and successful regeneration of transformed tissue. The regeneration of many plant species through TCL method has been reported so far and this method has also been successful as an efficient method for enhanced production of secondary metabolites and pharmaceuticals through transgenic organ cultures such as those produce by *Agrobacterium rhizogenes* (Teixeira da Silva, 2003).

TCL and Orchid In vitro Culture

Orchids were once considered to be particularly difficult-to-propagate plants In vitro, but TCL technology has helped to develop methods for their tissue culture, making mass propagation easier and more reproducible (Teixeira da Silva, 2013). The TCL system employs various small sized explants from different plant organs excised either longitudinally (lTCL) or transversely (tTCL). TCL culture systems are promising and efficient with regard to the total output of orchid plantlets compared to other conventional In vitro methods for rapid regeneration systems. TCLs have been successively used for callus or direct PLBs induction from different explants in many orchid species. The advantage of the TCL system is to produce high frequency organ regeneration and to reduce the time interval required to generate plantlets. TCLs have been used extensively to study the differentiation and with the successful manipulation of all morphogenic programs in orchids. Plant tissues such as shoot tips, root tips, floral stalks, stem nodes, apical buds, protocorm like bodies, leaf segments, rhizomes and mature seeds have been used as explants in, In vitro propagation of orchids (Zhao *et al.*, 2008).

Since decades TCL technique have been used to culture several orchids In vitro; *Brasilidium forbesii* (Gomes *et al.,* 2015), *Dendrobium aqueum* (Parthibhan *et al.,* 2018), *Phalaenopsis* hybrid (Lo *et al.,* 2022), *Dendrobium candidum* (Zhao *et al.,* 2007), *Paphiopedilum callosum* (Wattanapan *et al.,* 2018), *Hadrolaelia grandis* (Vudala *et al.,* 2019), *Dendrobium* hybrid Sonia (Mandal *et al.,* 2020), *Dendrobium malones* Victory (Anjum *et al.,* 2006), *Cymbidium* sleeping Nymph (Vyas *et al.,* 2010), *Dendrobium gratiosissimum* (Jaiphet and Rgasayatorn, 2010), *Dendrobium aphyllum* (Bhattacharyya *et al.,* 2018), *Vanilla planifolia* (Jose and Nirmal Babu, 2018) and *Cattleya forbesii* (Ekmekcigil *et al.,* 2019). Therefore, TCL technique has made micropropagation easier and more efficient for many species, including orchids (Bose *et al.,* 2017). Micropropagation by using TCL technique can provide a rapid large scale production on a commercial scale of valuable plant species, which may be difficult to produce through traditional methods, in a limited space under the controlled environment without any seasonal constraints all over the year. Tissue culture and biotechnology of *Dendrobium*, an important medicinal orchid and ornamental plant are evolving rapidly with many advances being made annually. Novel techniques that offer assistance and solutions to orchid researchers who may encounter difficulties or limitations in tissue culture are always welcomed. Advancing both basic tissue culture and conservation biology, Parthibhan *et al.* (2018) induced somatic embryos from stem tTCLs of *Dendrobium aqueum* in MS medium supplemented with 2.3 µM Zeatin or in the presence of 8.3 µM N^6 (2-isopentenyl) adenine. In this as many as 43 globular somatic embryos per tTCL were formed. Bose *et al.* (2017), induced adventitious shoots in *Malaxis wallichii* a threatened medicinal orchid from stem tTCL in 89% explants developed 22.5 shoots/TCL when inoculated on to MS medium with 4.1 µM meta-topolin and 2.7 µM NAA. Shoots were then rooted in ½ strength MS medium with 5.7 µM IAA. In a study by Bhattacharyya *et al.* (2018) induced the shoots on nodal tTCL of *Dendrobium aphyllum*, cultured on MS medium supplemented with 15 µM mta-topolin, 10 µM TDZ and 10 µM $AgNO_3$, forming 39.4 shoots per explant. These shoots were rooted in medium containing 15 µM IBA and plantlets were successfully acclimatized in a mixture of vermiculite and Saw dust in the ratio of 1:1.

Table 1: Thin Cell Layer (TCL) culture in orchids from different explants

Sl No	Species	TCL derived from	Nutrient media with Additives	Results	Ref
1	*Hadrolaelia grandis*	PLBs	Woody plant medium + BA (2.2 µM) and BAP (8.8 µM)	High percentage of PLBs from tTCL and 83.3% of PLBs from lTCL	39
2	*Bletilla striata*	Pseudobulbs	MS + BA (2.0 mg/l) + TDZ (1.0 mg/l)	Shoot induction (93.3%)	12
3	*Paphiopedilum callosum var. sublaeve*	PLBs	MVW + Ascorbic acid (0.1 mg/l) + AC (2 g/l)	Shoots (29.17% and PLBs (50.89%) induction	41
4	*Eria dalzelli*	Shoot tips	Mitra et al. + TDZ (9.08 µM)	Induction of PLBs (96.0%)	16
5	*Cattleya forbesii*	stem nodes	KC + BAP (1.2 mg/l) + NAA 1.2 mg/l)	RITA bioreactor culture produced PLBs and Shoot regeneration	05
6	*Vanilla planifolia*	axillary buds	MS + BA (1.0 mg/l) + IBA (0.5 mg/l) + Tryptone (2 g/l)	Induction and proliferation of PLBs	09
7	*Doritaenopsis Hybrid*	Leaves	MS + TDZ (9.0 µM)	Highest percentage of PLBs formation (72.3%)	25
8	*Cymbidium Hybrid cv. Twilight Mon 'Daylight'*	PLBs	VW + NAA (0.1 mg/l) + Kin (0.1 mg/l) + Tryptone (2 g/l)	High frequency PLBs regeneration	30
9	*Aerides maculosum*	Shoot tips	Mitra et al. + TDZ (13.62 µM)	High percentage of PLBs (81%) ultimately produced healthy shoots	18
10	*Cymbidium bicolor*	Shoot tips	Mitra et al. + 24-epiBL (13.62 µM)	Induction of PLBs (86%)	17
11	*Liparis elliptica*	Shoot tips	Mitra et al. + TDZ (4 µM)	Induction of PLBs (93%)	19
12	*Dendrobium draconis*	Stem	MS + BA (2.0 mg/l) + NAA (1.0 mg/l)	Development of Maximum PLBs (68%) formation	27

13	*Dendrobium gratiosissimum*	Protocorms	MS + Kin (2.0 mg/l)	Highest percentage of PLBs (83%) formation	08
14	*Cymbidium Hybrid cv. Sleeping Nymph*	PLBs	KC + CW (5%)	Highest percentage of PLBs (83%) formation	40
15	*Coelogyne cristata*	PLBs	MS + BA (2.0 mg/l)	Highest shoot multiplication	22
16	*Paphiopedilum deperle*	Floral buds	MS + BA (44.39 µM) + NAA (26.85 µM)	Highest shoot production	13
17	*Xenikophyton smeeanum*	Shoot tips	Mitra et al. + TDZ (11.35 µM)	Highest PLB induction	21
18	*Renanthera Tom Thumb 'Qilin'*	leaves	VW + TDZ (1.0 mg/l) + Peptone (1 g/l) + CW (10%)	High frequency PLBs formation	43
19	*Dendrobium candidum* Wall ex Lindl.	PLBs	MS + NAA (1.2 mg/l) + BA (1.2 mg/l)	High frequency shoot regeneration	44
20	*Dendrobium nobile* Lindl.	PLBs	MS + BA (11.0 µM)	Induction of 34 PLBs/TCL	23
21	*Phalaenopsis amabilis* (L.) Bl. cv. Cool Breez	Inflorescence axis	MS + BAP (2.0 mg/l) + NAA (1.0 mg/l) + CW (10%) + Peptone (2 g/l) + AC (1 g/l)	Induction of PLBs	28
22	*Cymbidium aloifolium* (L.)	PLBs	MS + Zea (14.0 µM)	Induction of 28.2 PLBs/TCL.	23

Success of TCL technology

Over the past few decades, methods based on TCL culture as explants were developed and successfully applied to numerous plant species for in vitro mass propagation, genetic transformation, and production of artificial seed, cryopreservation and in vitro selection. For plant biotechnology, the use of TCL technology might be highly beneficial in many ways, Teixeira da Silva and Dobranszki (2019) were listed below, seven parameters contributing to its success.

A. When TCL explants are used, the surface area of the explant in contact with the medium is relatively greater in a conventional explant and the transport of medium components is more efficient because they can reach relatively more receptive cells of the explants, that is which would enable an organogenic or embryogenic response, relative to conventional explants. As

a result of these properties, when used as explants TCLs can be more receptive to environmental, chemical, or physical stimuli. TCLs may thus be beneficial when used in cause-and-effect style studies.

B. The sensitivity of TCLs to different inputs from the in vitro environment is high. For example, light at the beginning of culture blocked shoot regeneration from leaf tTCLs of apple 'Freedom' completely (Dobranszki and Teixeira da Silva (2013). Due to their higher sensitivity to environmental factors, TCLs have been successfully applied for in vitro selection (Kozgar and Kahn,2012).

C. The stress response caused by wounding can induce callus formation and differentiation. In TCLs the wounded surface of the explant is relatively higher than in a conventional explant. This may be one reason why TCLs may be a simple but practical solution for breaking recalcitrance to in vitro growth and morphogenesis, as was observed in *Dendrobium aphyllum* (Bhattacharyya et al., 2018). However, there is no guaranty that the increased callus production will increase organogenesis or somatic embryogenesis. Some organs, if used as conventional explants, may display a low morphogenic or organogenic potential, but can be involved in regeneration or even in mass propagation if TCLs are prepared from them, as was demonstrated in apple in vitro stem segment (tTCLs) for shoot regeneration (Teixeira da Silva and Dobranszki, 2015).

D. In genetic transformation, it is advantageous to employ explants that have only a single tissue layer such as lTCLs or few layers such as the epidermis, especially where regeneration occurs from epidermal or sub-epidermal tissues (Nhut et al. 2003). The use of TCL based regeneration systems would allow a greater surface area to be infected, although this can pose challenges in removing the *Agrobacterium* or avoiding excessive bacterial growth and explant contamination. However, *Agrobacterium* overgrowth can be successfully suppressed during transformation by using the *Agrobacterium* strain harboring the SacB-SacR gene cassette (Liu et al., 2016). In this construction the SacB-SacR gene cassette is used as a negative selection marker, which is able to hinder the overgrowth of Agrobacterium. Inserting SacB-SacR gene at the recA locus of Agrobacterium tumefaciens strain GV2260 made the Agrobacterium sensitive to the quantity of sucrose in the medium that is, its growth was inhibited when the sucrose content in the medium was increased. By contrast, the use of TCLs would effectively match the use of particle bombardment to introduce plasmid or other DNA constructs of interest, since the DNA coated microprojectiles would enter the few layers of tissues in a TCL. This should ensure that the greater proportion of tissue in a subsequent regeneration is genetically transformed. These

issues have previously been discussed in the context of orchid genetic transformation and biotechnology.

E. If studies are conducted on morphoanatomical changes and morphogenesis, the use of TCLs is more beneficial because preparations of samples for light or electron microscopy is much easier from TLC explants than from thicker conventional explants, which would facilitate histological observations and confirmation of the origin of an organ.

F. If the availability of plant material is limited during in vitro culture establishment or subcultures, it is beneficial to use TCLs as explants for increasing the number of explants and, thereby, the efficiency of the subculture and culture establishment.

G. TCLs might actually be more productive than a conventional explant, especially when assessing the number of regenerating organs per source organ, even if the actual productivity per TCL is less than that from the conventional explant. This was demonstrated in Cymbidium hybrid, Chrysanthemum and Apple, when two new concepts the plant growth correction factor were applied. Across species, when these two factors were applied, the relative productivity of TCL explants was between 10-fold and 13-fold higher than that of conventional explants.

Conclusions:

The TCL system is a simplified system that requires only a small amount of plant material and medium volume and provides a good system for the study of fundamental and applied aspects of regeneration and transformation. The TCL system has been effectively utilized to study organogenesis and embryogenesis in ornamental and floricultural plant species and promises to be extended to the micropropagation of others. One of the strongest positive aspects of TCLs is the inherent capacity to strictly control an organogenic programme more than a conventional explants, which has multiply advantages and applications in plant tissue culture.

Acknowledgements

The authors gratefully acknowledge University Grants Commission (UGC), New Delhi, India for the financial assistance in the form of Major Research Project F. No. 42-927/2103 (SR).

References

Anjum S, Zia M and Chaudhary MF (2006). Investigations of different strategies for high frequency regeneration of *Dendrobium malones* Victory. Afr. J. Biotechnol. 5(19): 1738-1743.

Bhattacharyya P, Paul P, Kumaria S and Tandon P (2018). Transverse thin cell layers (tTCL) mediated improvised micropropagation protocol for endangered medicinal orchid

Dendrobium aphyllum Roxb. an integrated phyto-molecular approach. Acta Physiol. Plantarum 40:137. DOI: 10.1007/s11738-018-2703-y

Bose B, Kumaria S, Choudhary H and Tandon P (2017). Insight into nuclear DNA content hydrogen peroxide and antioxidative enzyme activities during transverse thin cell layer organogenesis and ex-vitro acclimatization of *Malaxis wallichii* a threatened medicinal orchid. Phsiol. Mol. Biol plants 23 (4): 955-968. DOI: 10.1007/s12298-017-0474-3

Dobranszki J and Teixeira da Silva JA (2013). In vitro shoot regeneration from transverse thin cell layers of apple leaves in response to various factors. J. Horticult. Sci. Biotechnol. 88: 60-66.

Ekmekcigil M, Bayraktar M, Akkus O and Gurel A (2019). High frequency protocorm like bodies and shoot regeneration through a combination of thin cell layer and RITA temporary immersion bioreactor in *Cattleya forbesii* Lindl. Plant Cell Tiss. Org. Cult. 136: 451-464 DOI: 10.1007/s11240-018-1526-2

Gomes LRP, Franceschi CRB and Ribas LLF (2015). Micropropagation of *Brasilidium forbesii* (orchidaceae) through transverse and longitudinal thin cell layer culture. Acta Scientiarum Biol.Sci. 37(2): 143-149.

Hossain MM, Kant R, Thanh Van P, Winarto B, Zeng S and Teixeira da Silva JA (2013). The application of biotechnology to orchids Crtical Rev.in Plant Sci. 32(2): 69-139.

Jaiphet C and Rangsayatorn N (2010). Micropropagation of a rare orchid *Dendrobium gratiosissimum* using thin cell layers. Acta Horticult. 878: 185-189.

Jose V and Nirmal Babu K (2018). Efficient plant regeneration through protocorm like bodies derived from shoot tip cultures of *Vanilla planifolia* Andr. Plant Tiss. Cult. Biotechnol. 28(1): 91-98.

Kozgar MI and Khan S (2012). Induced mutagenesis in crop plants. Biorem. Biodiv. Bioavail. 6(1): 118

Lakshmanan P, Loh CS and Goh CJ (1995). An In vitro method for rapid regeneration of a monopodial orchid hybrid *Aranda Deborah* using thin cell layer culture. Plant Cell Rep. 14: 510-514.

Li F, Ren-Rui W, Ma YU, Jin-Huan L, Da-Bin H and Zhi-Kang T (2020). Shoot regeneration from transverse thin cell layers of pseudobulbs and micropropagation of *Bletilla striata*. Bulletin Bot. Res. 40(3): 368-375 DOI: 10.7525/j.issn.1673-5102.2020.03.007

Liao YJ, Tsai YC, Sun YW, Lin RS and Wu FS (2011). In vitro shoot induction from flower buds in *Paphiopedilum* orchids. In Vitro Cell Dev. Biol. Plant 47: 702-709.

Liu Y, Miao J, Traore S, Kong D, Liu Y, Zhang X, Nimchuk ZL, Liu Z and Zhao B (2016). SacB-SacR gene cassette as the negative selection marker to suppress *Agrobacterium* overgrowth in *Agrobacterium* mediated plant transformation. Front. Mol. Biosci. 3: 70.

Lo KC, Gansau JA, Shih CH and Kao CY (2022). Shoot development through modified transverse thin cell layer (tTCL) culture of *Phalaenopsis* hybrid protocorms. Horticulturae 8, 206, DOI: 10.3390/ horticulturae8030206

Malabadi RB, Teixeira da Silva JA and Mulgund GS (2008). Micropropagation of *Eria dalzelli* (Dalz.) Lindl. through TCL in vitro culture. Floricult. Ornamen. Biotechnol. 2(2): 77-80.

Malabadi RB, Teixeira da Silva JA, Nataraj K and Mulgund GS (2008). Shoot tip transverse thin cell layers and 24-epibrassinolide in the micropropagation of *Cymbidium bicolor* Lindl. Floricult.Ornmen. Biotechnol. 2: 44-48.

Malabadi RB, Teixeira da Silva JA and Mulgund GS (2009). TDZ induced in vitro shoot regeneration of *Aerides maculosum* Lindl. from shoot tip thin cell layers. Floricult. Ornamental Biotechnol. 3: 35-39.

Malabadi RB, Teixeira da Silva JA and Mulgund GS (2009). In vitro shoot regeneration by culture of *Liparis elliptica* (Rees) Lindl.shoot tip derived transverse thin cell layers induced by 24-epibrassinolide. Int. J. Plant Dev. Biol. 3: 47-51.

Mandal S, Pal, Mondal T and Banerjee N (2020). Comparative efficiency analysis of different explants and contribution of polyamines on In vitro propagation of *Dendrobium* hybrid Sonia. Plant Tissue Cult. Biotechnol. 30(1): 77-86.

Mulgund GS, Nataraj K, Malabadi RB and Kumar SV (2011). TDZ induced in vitro propagation of an epiphytic orchid *Xenikophyton smeeanum* (Reichb.f.). Res. Plant Biol. 1:7-15.

Naing AH, Chung JD, Park IS and Lim KB (2011). Efficient plant regeneration of the endangered medicinal orchid *Coelogyne cristata* using protocorm like bodies. Acta Physiol. Plant. 33: 659-666.

Nayak NR, Sahoo S, Patnaik S and Rath SP (2002). Establishment of thin cross section (TCS) culture method for rapid micropropagation of *Cymbidium aloifolium* (L.) Sw. and *Dendrobium nobile* Lindl. (Orchidaceae). Sci. Horticult. 94: 107-116.

Nhut DT, Murthy HN and Teixeira da Silva JA (2003). Usefulness of thin cell layers in plant transformation. Prop. Ornamental Plants 2(2): 30-38.

Park SY, Yeung EC, Chakrabarty D and Paek KY (2002). An efficient direct induction of protocorm like bodies from leaf subepidermal cells of *Doritaenopsis* hybrid using thin section culture. Plant Cell Rep. 21: 46-51.

Parthibhan S, Rao MV, Teixeira da Silva JA and SenthilkumarT(2018). Somatic embryogenesis from stem thin cell layers of *Dendrobium aqueum*. Biol. Plantarum 62(3): 439-450.

Rangsayatorn N (2009). Micropropagation of *Dendrobium draconis* Rchb.f. from thin cross section culture. Sci. Horticult. 122: 662-665.

Sinha P, Hakim MK and Alam MF (2007). Efficient micropropagation of *Phalaenopsis amabilis* (L.) Bl. cv. "Cool Breeze" using inflorescence axis thin sections as explants. Propagation Ornamental Plants 7: 9-15.

Teixeira da Silva JA (2003). Thin cell layer technology in ornamental plant micropropagation and biotechnology. Afri. J. Biotechnol. 2(12): 683-691.

Teixeira da Silva JA, Singh N and Tanaka M (2006). Priming biotic factor for optimal protocorm like body and callus induction in hybrid *Cymbidium* (Orchidaceae) and assessment of cytogenetic stability in regenerated plants. Plant Cell Tiss. Org. Cult. 84: 135-144.

Teixeira da Silva JA (2013). The role of thin cell layers in regeneration and transformation in Orchids. Plant Cell Tiss. Org. Cult. 113: 149-161.

Teixeira da Silva JA and Dobranszki J (2014). Dissecting the concept of thin cell layers: theoretical basis and practical application of the plant growth correction factor to *apple, Cymbidium* and *Chrysanthemum*. J. Plant Growth Reg. 33: 881-895.

Teixeira da Silva JA and Dobranszki J (2015). Plant thin cell layers; update and perspectives. Folia Horticult. 27: 183-190.

Teixeira da Silva JA and Dobranszki J (2019). Recent advances and novelties in the thin cell layer based plant biotechnology- a mini review. Biotechnologia 100(1): 89-96.

Teixeira da Silva JA, Altamura MM and Dobranszki J (2015). The untapped potential of plant thin cell layers J. Horticult. Res. 23(2): 127-131.

Tran Thanh Van K 1981. Control of morphogenesis. Ann. Rev. Plant Physiol. 32: 291-311.

Tran Thanh Van M (1973). In vitro control of de novo flowers, bud, root and callus differentiation from excised epidermal tissues. Nature 246: 44-45. DOI: 10.1038/246044a0

Tung HT, Van HT, Bao HG, Bein LH, Khai HD, Luan VQ, Cuong DM, Phong TH and Nhut DT (2021). Silver nano particles enhanced efficiency of explant surface disinfection and somatic embryogenesis in *Begonia tuberous* via thin cell layer culture. Vietnam J. Biotecnol. 19: 337-347.

Vudala SM, Padial AA, and Ribas LLF (2019). Micropropagation of *Hadrolaelia grandis* through transverse and longitudinal thin cell layer culture. South Afr. J. Bot. 121: 76-82.

Vyas S, Guha S, Kapoor P and Rao IU (2010). Micropropagation of *Cymbidium* Sleeping Nymph through protocorm like bodies production by thin cell layer culture. Sci. Horticult. 123(4): 551-557. DOI: 10.1016/j.scienta.2009.11.020

Wattanapan N, Nualsri C and Meesawat U (2018). In vitro propagation through transverse thin cell layer (tTCL) culture system of lady's slipper orchid *Phaphiopedilum callosum* var. sublaeve. Songklanakarin J. Sci. Technol. 40(2): 306-313.

Wattanapan N, Srichuay W, Klinnawee L and Meesawat U (2018). Effect of anti-browning agents on protocorm like body induction using thin cell layer of endangered Lady's slipper orchid *Paphiopedilum callosum* var. sublaeve. Princess of Naradhiwas Univ. J. 11: 107-116.

Wu KL, Zeng SJ, Teixeira da Silva JA, Chen ZL, Zhang JX, Yang YS and Duan J (2012). Efficient regeneration of *Renanthera* Tom Thumb 'Qilin' from leaf explants. Sci. Horticul. 135: 194-201

Zhao P, Wang W, Feng FS, Wu F, Yang ZQ and Wang WJ (2007). High frequency shoot regeneration through transverse thin cell layer culture in *Dendrobium candidum* Wall ex. Lindl. Plant Cell Tiss. Org. Cult. 90: 131-139.

Zhao P, Wu F, Feng FS and Wang WJ (2008). Protocorm like body (PLB) formation and plant regeneration from the callus culture of *Dendrobium candidum* Wall ex Lindl. In vitro Cell Dev. Biol. Plant 44: 178-185 DOI: 10.1007/s11627-007-9101-2.

CHARECTERIZAION OF CHITINASES AND ANTIMICROBIAL ACTIVITY

Aboli Kshirsagar
Department of Botany,
Arts and Science College, Pulgaon
(Affiliated to RTM Nagpur University, Nagpur)
Corresponding author E-mail: kshirsagaraboli@gmail.com

Abstract:

Chitin is present in the exoskeletons of insects, fungus, yeast, and algae, as well as the internal structures of various vertebrates, and is the second most prevalent polysaccharide in nature after cellulose. Chitinases are enzymes that break down the chitin molecule. Chitinases have a role in the production of carbon and nitrogen in the environment. Chitin and chitinolytic enzymes are gaining popularity for their biotechnological applications, particularly chitinases, which are used to control infections in agriculture fields. Chitinases are used in human health care, particularly in the treatment of disorders such as asthma. Chitinases are used for a variety of things, including the production of pharmaceutically important chitooligosaccharides and N-acetyl D glucosamine, the isolation of protoplasts from fungi and yeast, the control of pathogenic fungi, the treatment of chitinous waste, mosquito control and morphogenesis, and so on.

Keywords: Chitinases, Chitinolytic Enzymes, Endochitinase, Exochitinases

Introduction:

Chitin is the second most abundant biopolymer on the planet, consisting of a linear polymer of -1, 4-N-acetylglucosamine (GlcNAC). [1] Chitin can be found in the outer skeletons of insects, fungi, yeasts, algae, crabs, shrimps, and lobsters, as well as other invertebrates' internal structures. [2] Chitin accounts for 20-58 percent of the dry weight of discarded shellfish (e.g. crab, krill, and shrimp) [3]. Chitin is used to increase the formation of extracellular chitinase among a variety of applications. Chitin and its derivatives are used in a variety of applications, including drug delivery, wound healing, dietary fibre, and waste water treatment. [4] Chitin is a white, hard, inelastic polysaccharide that contributes significantly to pollution in coastal areas [5]. Chitin has a high nitrogen content (6.89%), making it an effective chelating agent. [6] -chitin and -chitin are two allomorphic forms of chitin. The packing and polarities of adjacent chains in subsequent sheets differ between these two types of chitin. [7,8] Chitinase can break down chitin. Chitin is catabolized in two steps, with chitinases cleaving the polymer into chitin oligosaccharides and chitobiases cleaving the oligosaccharides into N-acetylglucosamine and monosaccharides [9].

Chitinases (EC 3.2.2.14) are glycosyl hydrolases that range in size from 20 to 90 kDa. [2] Bacteria, fungi, yeasts, plants, actinomycetes, arthropods, and humans are among the creatures

that contain them. Chitinases may degrade chitin directly into low-molecular-weight chitooligomers, which can be used for a variety of industrial, agricultural, and medical purposes, including elicitor action and anti-tumor activity. [10] For the treatment of osteoarthritis, N-acetylglucosamine (GlcNAc) has gotten a lot of interest. [11] Because of their role in the biocontrol of fungal phytopathogens[12] and hazardous insects, chitinases have gotten a lot of attention. [13] Chitinases have recently gotten a lot of interest because they're thought to be important in mosquito control and plant defence systems against pests.

Endochitinases (EC 3.2.1.14) and exo-chitinases are the two main categories of chitinases. Endochitinases split chitin at random internal locations, creating the dimer di-cetylchitobiose and soluble low molecular mass GlcNAc multimers such chitotriose and chitotetraose. [19] Exo-chitinases are further split into two subcategories: chitobiosidases (E.C. 3.2.1.29), which catalyse the gradual release of di-acetylchitobiose from the non-reducing end of the chitin microfibril, and 1-4-glucosaminidases (E.C. 3.2.1.30), which cleave the oligomeric products of endochit [19]. The identification of different chitinases in *Serratia plymuthica* strains IC1270, IC14, and HRO-C48 has been carried out using this nomenclature. It has been reported that the strains IC14 and HRO-C48 create an endochitinase and a 100 kDa N-acetyl-1,4-D-hexosaminidase or chitobiase, whilst the strain IC1270 generates N-acetyl—D-glucosaminidases of 89 and 67 kDa, a 50 kDa chitobios [21,22] When it comes to the amino acid similarity of chitinases from different organisms, five classes have been presented, and they've been divided into two families, which include glycosyl hydrolase families 18 and 19. [23] Plants, bacteria, fungi (classes III and V), mammals, and viruses are among the creatures that include family 18 chitinases. Chitinases are additionally classified according to their N-terminal sequence, isoelectric pH, enzyme location, signal peptide, and inducers. Plants possess class I chitinases, but fungus, bacteria, and plants all contain class II chitinases. Class III chitinases have no sequence resemblance to class I or II chitinases. Class I chitinases share many of the same qualities as class IV chitinases, such as immunological properties, although they are much smaller. [24]

Chitinases are a large and diversified family of enzymes with distinct molecular structures, substrate specificities, and catalytic mechanisms. [25] It's crucial to research chitinases' substrate specificity since it not only reveals the link between substrate specificity and physiological roles, but it also enables the degradation of chitin into innovative compounds with industrial applications. [26] All chitinase classes have diverse substrate specificities and reaction processes, according to research. Tobacco class III chitinases, for example, have a high level of lysozyme as well as chitinase activity, whereas class VI chitinases solely have chitinase activity. [27] To hydrolyze the -glycosidic bond, class I and class II chitinases require an inverting mechanism, whereas class III chitinases do not this through a retaining mechanism.[28,29] It has

been established by Sasaki *et al*. (2006) [30] that class III chitinases do not act against GlcNAc oligomer or polymer, but could be active on an endogenous complex carbohydrate containing a GlcNAc residue, whereas a GlcNAc sequence was the most likely substrate of the class I enzyme.

The two groups of chitinases have completely different 3-dimensional (3D) structures and molecular processes, with no amino acid sequence commonality. As a result, they are likely to have descended from separate forebears. A number of conserved amino acid repeats make up Family 18. It is made up of an enzyme core with 8 strands of parallel sheets forming a barrel placed down helices, which then creates a ring on the outside. [31] The transglycosylation processes can be catalysed by GH family 18 chitinases. Transglycosylation products have previously been described for *T. harzianum* Chit42 and Chit33, as well as Aspergillus fumigatus ChiB1. [32] One of the structural hallmarks of chitinases seen in diverse mammals and microorganisms is a multidomain structure with catalytic domains and both a cysteine-rich chitin-binding domain (different from the catalytic domain) and a serine/threonine-rich glycosylated domain. [33] The similarity between bacterial and fungal chitinases suggests that their catalytic domains are the same. [34]

The 8 cysteins inside the chitin-binding domain are highly conserved, according to a large analysis of chitin-binding domains in plant proteins. Plants also have chitin-binding proteins (CBPs) without chitinase activity that have a cystein-rich chitin-binding domain. [35] According to Poole *et al*., 1993 [36], the chitin-binding domain in bacteria differs from that in plants, which has eight conserved cystein residues. On the other hand, several amino acids, particularly tryptophan, are among the residues that are conserved in bacteria's chitin-binding domain, and their role in cellulase-cellulose binding has been discovered. Chitinases have also been linked to the binding of chitin by a non-catalytic chitin-binding protein. [37] Watanabe *et al.*, 1997[38], discovered that just four amino acids in the catalytic domain are conserved across bacterial and plant class III chitinases. The chitin-binding domain of fungal chitinases is hypothesised to assist them connect to their substrate or cell wall. CTS1 and K1Cts1p have a 6-cystein conserved area in their chitin-binding domains that is likely involved in protein-protein interaction or tertiary structure through disulphide bond formation. The C-terminal chitin-binding domain in insect chitinases binds to the substrate and has a 6-cystein motif similar to nematode chitinases, according to Villagomez *et al*., 1996[39].

Bacterial chitinases

The chitin-binding domain of bacterial chitinases can be found in either the amino terminal or carboxyl terminal domains of the enzyme. [40] The majority of the bacterial chitinases that have been identified and sequenced so far belong to glycosyl hydrolase family 18, with the exception of a chitinase (C-1) isolated from *S. griseus* IIUT 6037 that belongs to

glycosyl hydrolase family 19. [41] Unlike bacterial chitinases, this enzyme can only hydrolyze GlcNAc-GlcNAc and GlcNAc-glucosamine links; chitinase C-1 from *S. griseus* HUT 6037 can hydrolyze glucosamine-GlcNAc and GlcNAc-GlcNAc connections. As a result, chitinase C-1's catalytic site differs from that of other microbial chitinases. The non-catalytic domains of other bacterial lytic enzymes, such as chitinases, proteases, and cellulases, have sequence similarities with the amino terminal region of chitinase C-1, and it is thought that this domain is involved in chitin binding. [41] *Serratia* is another bacterial species that produces significant quantities of chitinolytic enzymes. [38,42] Bacterial chitinases have a molecular weight of 20-60 kDa, which is less than insect chitinases (40-85 kDa) but similar to plant chitinases (40-85 kDa). [2] Depending on the bacteria from which they were isolated, bacterial chitinases are active over a wide range of pH and temperatures. For example, *Streptomyces violaceusniger endochitinase* [43] and *Streptomyces thermoviolaceus* OPC-520 thermostable chitinase [16] have optimal temperatures of 28°C and 80°C, respectively. Furthermore, the final enzyme has a pH optimal range of 8.0 to 10.075, whereas *Stenotrophomonas maltophilia* C3 chitinase has a pH optimal range of 4.5 to 5.0. Bacterial chitinases have a wide range of isoelectric values as well (pI 4.5-8.5) [38]

Bacteria are thought to produce chitinases in order to provide nitrogen and carbon as a source of nutrients. [44] Chitinases are produced by bacteria primarily for the purpose of degrading chitin and using it as an energy source. Some chitinases of chitinolytic bacteria, such as the chiA gene products from *Serratia marcescens* and *S. plymuthica*, are potential agents for the biological control of plant diseases caused by various phytopathogenic fungi, according to Chernin, 1997[45] and Downing, 2000[46]. The latter enzymes break down the chitin in the fungal cell wall, preventing it from growing. Anti-fungal proteins like chitinases have a lot of biotech potential because they can be used as food and seed preservatives, as well as for engineering plants to be resistant to phytopathogenic pathogens. [47] Ordentlich (1988) [48] *Sclerotium marcescens* chitinolytic culture filtrate was found to be effective as a biocontrol agent against *Sclerotium rolfsii*. Family 18 contains the vast majority of known bacterial chitinases. [49] Since group A chitinases have been identified in the majority of bacterial chitinases studied thus far, it has been hypothesised that group A chitinase genes are more abundant in nature than enzymes from groups B or C. [50] Bacterial chitinases have multiple functional domains linked to the catalytic domain, such as the chitin-binding domain (CBD) and Wibronectin type III-like domain (Fn3 domains). Some bacterial chitinase has demonstrated the importance of CBD in the degradation of insoluble chitin. [51] Family 19 chitinases, on the other hand, have been discovered to be present only in a few bacterial strains and plants. [52] Several microorganisms, including *Aeromonas sp*. No. 10S-24, [53] Pseudomonas aeruginosa K-187 [3] *Bacillus circulans* WL-12, have been found to produce multiple chitinase-producing enzymes. [54]

Suzuki *et al*. (2002) [42] found that Chi A, Chi B, and Chi C1 of *S. marcescens* 2170 have a synergistic effect on chitin degradation. Despite having similar catalytic domains, Chi A and Chi B were thought to digest chitin chains in opposite directions, with Chi A beginning at the reducing end and Chi B beginning at the non-reducing end.

Fungal chitinases

Fungal chitinases, like bacterial chitinases, have a variety of functions because they are involved in nutrition, morphogenesis, and fungal development. Chitin is a major component of fungi's cell walls. [19] Class III plant chitinases have a high amino acid homology with fungal chitinases. [55] They are mostly members of the glycosyl hydrolase superfamily's family 18 [56].

- The catalytic domain,
- N-terminal signal peptide region,
- chitin-binding domain,
- serine/threonine rich-region, and
- C-terminal extension region

Make up the basic structure of family 18 fungal chitinases. However, most fungal chitinases lack the serine/threonine rich-region, chitin-binding domain, and C-terminal extension region, which appear to be unnecessary for chitinase activity because naturally-occurring chitinases lacking these regions are still enzymatically active. Fungal chitinases are not as well classified as bacterial and plant chitinases, and are identified by their resemblance to bacterial or plant family 18 chitinases. [57] As a result, fungal chitinases have been classified as fungal/plant chitinases, which are class III chitinases that are related to class V chitinases found in plants, fungi, and bacteria. [57] Group C fungal chitinases are a novel group of fungal chitinases that have yet to be identified. They are predicted to be between 140 and 170 kDa in size, with two LysM domains and a chitin-binding domain. They've been compared to yeast killer toxins. Chitinases serve a variety of physiological and biological functions, including morphogenetic, autolytic, nutritional, and parasitic functions. In the yeast *Saccharomyces cerevisiae*, for example, disruption of the chitinase gene (CTS1) causes cell clumping and failure to separate after division, while functional expression of chitosanase and chitinase has been shown to influence morphogenesis (*Schizosaccharomyces pombe*). [58][59] (Lorito *et al.* (1994) [60] Ulhoa and Peberdy, 1991). Purification and molecular weights of three N-acetylglucosaminidaes (GlcNAcases) from various *Trichoderma* isolates were determined using sodium dodecyl sulphate-polyacrylamide gel electrophoresis, or SDS-PAGE. The cloning of three GlcNAcase genes from *Trichoderma sp*., exc1, exc2, and nag1, was reported by Draborg *et al*. (1995) [61] and Peterbauer *et al*. (1996) [62]. Draborg *et al*. (1995) [61] and Peterbauer *et al*. (1996) [62] reported the cloning of 3 genes of GlcNAcases from *Trichoderma sp.: exc1, exc2,* and *nag1*. The genes *exc1, exc2* were isolated from T25-1, thereby resulting in the conformation

that *Trichoderma* has 2 different GlcNcases. Lorito *et al*., 1998**, [**63] reported the isolation of about 42 kDa endochitinase from *Trichoderma*. As reported by Yamanaka *et al*., 1994 [64] Sandor *et al*., 1998 [65] Since the chitinase inhibitors demethylallosamidin or allosamidin inhibited the fragmentation of hyphae into arthroconidia, fungal cell wall chitinases have been linked to their role in filamentous fungal sporulation. Among the various chitinolytic fungi and bacteria, *Trichoderma sp*. have received the most attention as biocontrol agents in the case of soil-borne fungal pathogens. [66–68] Purification and characterization of chitinases and 1,3-glucanases from *Talaromyces flavus* and *Trichoderma sp*. have been reported, as well as their role in mycoparasitism of soilborne pathogens such as *Rhizoctonia solani, S. rolfsii*, and *Fusarium sp*. [20,69,70] According to Harman (2000)[71] and Yedidia *et al*. (2000)[72], the beneficial effect of *Trichoderma* on fungi is due to its direct mycoparasitism, which causes induced resistance and increased development in fungi.

According to Yaun and Crawford (1995) [73], the antifungal biocontrol agent *Streptomyces lydicus* WYEC108 has the ability to damage the fungal cell wall hyphae as well as destroy *Phytium ultimum* germinating oospores. Chitinase genes from *Trichoderma harzianum* (such as ech42, chi33, nag1, chi18-13) have previously been shown to play an important role in mycoparasitism. [74,75] Disruption of the ech42 gene has been shown to affect mycoparasitism in *T. harzianum*. [76] *T. harzianum* isolate TM yielded a novel endochitinase called CHIT36, which was isolated. Viterbo *et al*., 2001.[77] Other important applications of fungal chitinases include the ability to improve plant resistance through genetic manipulation. *T. harzianum's* chi42 gene encodes a potent endochitinase with much stronger anti-fungal activity against a variety of phytopathogenic fungi and is expressed constitutively in apple, tobacco, and potato. As a result, these transgenic plants have a high level of resistance to phytopathogenic fungi. [63] Fungal chitinases are also used to control insects**.**

Plant chitinases

Chitinases are found naturally in plants, stems, seeds, flowers, and tubers. They are both developmentally and tissue-specific. Plant chitinases have been classified into 5 or 6 classes based on amino acid sequences. Globular domains are found in the key structure of enzymes of classes I, II, and IV. Class III and V plant chitinases have 8 -helices and 8 -strands. The former uses an inverting mechanism to hydrolyze the -1, 4-glycosidic linkage, while the latter uses a retaining mechanism. [78] Plant chitinases are pathogenesis-related proteins that are produced in plant self-defense in response to phytopathogen attack or by contact with elicitors such as chitooligosaccharides or growth regulators such as ethylene. [79] There are some chitinases, which are expressed in response to environmental stresses, (i.e., high salt concentration, cold, and drought). There are also reports of some chitinases, which take part in vital physiological processes of plants, like embryogenesis and ethylene synthesis.[78] Chitinase, which is a

polypeptide and a major pathogenesis-related protein, accumulates in the infected plant tissue extracellularly. Garg and Gupta, 2010 [80,81] reported the isolation and purification of chitinase from moth beans against the fungal pathogen *Macrophomina Phaseolina* strain 2165. The chitinases of plants can be detected during their development in the early stages of growth. The chitinases of plants are generally endochitinases of smaller molecular weight as compared to the chitinases of insects.

Insect chitinases

Chitinases found in insects such as *Manduca sexta* and *Bombyx mori* have been described. During ecdysis, when endochitinases randomly break the cuticle into chitooligosaccharides, which are then hydrolyzed by exoenzymes to N-acetyl-glucosamine, these enzymes play a critical role as degradative enzymes. The monomer is recycled to make new cuticles. Insect chitinases also play a defensive role against their own parasites, and the production of the enzyme is controlled by hormones during the larvae's transformation. Insect chitinases are inhibited by allosaminidin. [82] Crustaceans such as shrimp, krill, and prawns also contain chitinases.

Mammalian chitinases

Chitinases in mammals are members of the glycosyl hydrolase family 18 (GH18), which includes chitinase-like proteins with no enzymatic activity and enzymatically active true chitinases. [7] The first mammalian chitinase to be discovered was chitotriosidase. [83] The N-terminal catalytic domain of GH18 family members contains a triose-phosphate isomerase fold, which is characterised by the (/)8-barrel structure, with the 4 strand containing a conserved sequence motif (DXXDXDXDXE, where D = aspartic acid, E = glutamic acid, and X = any amino acid) forming the enzyme's active site. The key residue donating a proton required for hydrolyzing the (1-4) glycosidic bond in chitin is glutamic acid.[84] The lack of chitinolytic activity in chitinase-like proteins is due to the substitution of this essential glutamic acid for glutamine, leucine, and isoleucine. The conserved chitin-binding aromatic residues on the triose-phosphate isomerase barrel, on the other hand, are unaffected and can still bind to chitin with high affinity. [85]

Methods of production of chitinase

For the production of microbial chitinases, several methods have been used, including "fed-batch fermentation, continuous fermentation, and liquid batch fermentation." According to Khan *et al*., 2010 [86], in the presence of chitin, $MgSO_4 7H_2O$, and KH_2PO_4, yeast extract has a positive effect on chitinase production, whereas chitin has a negative effect on yeast extract. It was also reported that an increase in maltose and chitin concentrations increased chitinase secretion. At lower concentration levels, components such as $MgSO_4 7H_2O$, KH_2PO_4, and yeast extract demonstrated the highest chitinase secretion. According to Bhushan, 1998 [87] and

Dahiya, 2005 [88] Extracellular chitinase production is influenced by media constituents for nitrogen and carbon sources, as well as agricultural remains (e.g., wheat bran, rice bran, etc.). He also reported that when glucose was used in the production medium alongside chitin, it increased chitinase production. However, Miyashita *et al*., 1991, discovered that glucose inhibits the production of chitinase. [89]

According to Bhushan (1998) [87], physical factors such as pH, aeration, and incubation temperature influence chitinase production. He also reported that increasing the concentration of amino acids and their analogues, such as tryptophan, tyrosine, glutamine, and arginine, in the growth medium stimulated chitinase production in Bacillus sp. BG-11 (0.1 mM). In order to boost chitinase production, several methods, such as biphasic cell systems, cell immobilization, solid-state fermentations, etc., have been used.[87,90] There have been reports of natural and synthetic enzyme inhibitors, as well as oxidizing/reducing agents and organic compounds. Allosomadin, a competitive inhibitor produced by *Streptomyces sp*., has been identified as a specific inhibitor of yeast, insects, fungi, and human serum chitinases. Allosamidin inhibits the enzyme by acting as a non-hydrolysable analogue of the oxazolinium ion intermediate. [91,92] Psammaplin A is a brominated tryrosine-derived compound that has gained recognition as a non-competitive inhibitor of chitinase B from *S. marcescens*, which belongs to the family 18 chitinase. A disordered Psammaplin A molecule binds near the active site, according to crystallographic studies. [93] Another chitinase inhibitor is argadin, which was isolated from *Clonostachys sp*. FO-7314.[94] Several attempts have been made with *S. plymuthica* [95] *B. circulans* WL-12, to carry out the cloning and expression of genes from various organisms into *E. coli*. [54] ChiA, a chitinase gene from family 18, was cloned and expressed in *E. coli* from the thermophilic species *Rhodothermus marinus*. The *R. marinus* chitinase is the most thermostable chitinase isolated from bacteria, according to Hobel *et al*., 2005[96]. *Bacillus* chitinase genes ChiCW and ChiCH have been cloned into the pGEX-6P-1 vector and expressed in *E. coli* as soluble glutathione S-transferase chitinase fusion proteins. [97] Many Streptomyces and non-Streptomyces bacteria are known for producing chitin and are antagonistic to *Sclerotinia minor*, the pathogen that causes lettuce's basal drop. *Streptomyces viridodiasticus* and *Micromonospora carbonacea* were identified as high-level chitinase producers, which significantly reduced the growth of S. minor in vitro and under controlled greenhouse conditions, resulting in a reduction in disease occurrence. [98]

Uses:

Chitinases can be used to depolymerize chitin-containing biomass and turn it into useful components. Chitinases can be used to control plant pathogens such as fungi and insects. [99,100] Fungal protoplasts have been used as a powerful tool for studying cell wall synthesis, enzyme synthesis and secretion, and strain improvement in biotechnological applications. [101]

Chitinase activity also serves as a fungi activity indicator in the soil. There is a strong link between chitinase activity and the fungal population in the soil, according to research. As a result, chitinases activity appears to be a good indicator of actively growing fungi in the soil.. Miller *et al*., (1998) [102] by making use of specific methylumbelliferyl substrates reported the correlation of chitinase activity with the content of fungus-specific indicator molecules 18:2ωb phospholipid fatty acid and ergosterol.

Medicinal functions

Chitooligosaccharides have a lot of potential in the pharmaceutical industry. They play a role in root nodule formation signalling, act as elicitors of plant defence, and have the potential to be used in human medicine (e.g., anti-tumor activity is shown by chitohexaose and chitoheptaose). Murao *et al.,* 1999 [103] reported that chitotriose was produced from colloidal chitin using a chitinase from Vibrio alginolyticus. By combining GlcNAc and a sugar oxazoline derivative, Kobayashi *et al*., 1997 [104] reported the use of Bacillus chitinase for the production of chitobiose. GlcNAc is an anti-inflammatory drug that is synthesised from glucose in the human body and then incorporated into glycoproteins and glycosaminoglycans.. GlcNAc has been shown to be an effective anti-inflammatory drug in the treatment of ulcerative colitis and other gastrointestinal inflammation disorders when administered orally, intravenously (IV), or intramuscularly (IM). [105] Horsch *et al*. (1997) [106] suggested that N-acetylhexosaminidase be investigated as a target for developing low-molecular-weight antifungals. Chitin and chitin binding proteins, according to Laine and Lo (1996) [107], can be used to detect fungal infections in humans.

Chitinases play an important role in human health care. Chitinases have also been suggested as a way to boost the effectiveness of antifungal drugs in the treatment of fungal diseases**.** [108] They could be used in anti-fungal creams and lotions because of their topical applications. Chitin derivatives have been used to make a variety of artificial medical items, including contact lenses, artificial skin, and surgical stitches. Because many of these chitin derivatives are non-toxic, non-allergic, biocompatible, and biodegradable, they have a wide range of medical applications. [109] Chitinases have a variety of other medical applications. Because mammals do not use chitin as an energy source or produce any chitinous structure, the first discovery of the involvement of acidic mammalian chitinase (AMCase) in the pathogenesis of asthma was novel and unexpected.[110] The importance of chitinases as a host defence effector in the mammalian immune system has been demonstrated by several lines of evidence. Humans lacking chitotriosidase, for example, have a higher rate of microfilarial infection due to reduced chitinolytic activity, allowing the parasite to thrive within the host.

In vitro, recombinant human chitotriosidase inhibits *Candida albicans* hyphae formation, demonstrating antifungal activity and lowering mortality in neutropenic candidiasis and

aspergillosis mouse models. Zhu and co-workers, 1984 [110] Exaggerated quantities of AMCase were detected in the epithelial cells and macrophages of lung biopsies taken from asthma patients, which was the first clinically significant finding related to the role of chitinase in asthma. In the lungs of an ovalbumin-induced mouse asthma model, BAL fluid chitinase activity and AMCase levels were also reported to be induced. Furthermore, AMCase, serum, and lung tissue levels of a chitinase-like protein, YKL-40, have recently been discovered to be elevated in asthma patients. Furthermore, circulating YKL-40 levels in asthmatic subjects were found to be associated with lung sub-epithelial basement membrane thickening, the use of rescue inhalers, asthma severity, and deterioration in pulmonary function. [111].

Despite the fact that mammals are unable to synthesise chitin, some chitinolytic enzymes or true chitinases (-e.g., acidic mammalian chitinase AMCase and chitotriosidases, or chitinases like proteins (CLPs)) or CBPs (e.g., breast regression protein 39 (BRP-39, chondrocyte protein-Chitinase activity is absent in mammalian chitinases, but it is present in AMCase and chitotriosidase. [112] Mammalian chitinases have enzymatic activity due to their chitin-binding domain, which consists of 6 cystein residues with chitin binding properties. [113] CLPs, on the other hand, lack a typical chitin-binding domain, but they still have a high chitin-binding affinity. [114] Because an essential glutamic acid residue has been replaced with leucine, CHI3L1 lacks chitinase activity, but it has a high chitinase affinity for chitooligosaccharides and chitin, which is due to conserved substrate binding cleft.[116] It's also been suggested that YKL-40/BRP-39 plays a key role in the development of intestinal bowel disease as an active pathogenic mediator in acute colitis (IBD). Even though it lacks chitinolytic activity, YKL-40 is thought to play a role in tissue remodelling and inflammation. It can bind to chitin, type I collagen, hyaluronan, and heparin. [117] Various chitinase family proteins have been found to express constitutively in macrophages, the digestive tract, and pulmonary epithelial cells, indicating that they are the body's first line of defence against external agents, which include chitin-containing pathogens. [110,117] It has been reported that the development of Th2 inflammation in the human asthmatic airway, as well as in allergic animal models, is accompanied by an increase in the expression of AMCases in the lungs. [110]

AMCases have also been shown to play a critical role in the activation of the IL-13 effector pathway and the pathogenesis of Th2 inflammation. Studies of cancer, arthritis, and liver fibrosis have suggested that chitinase 3-like protein 1 (CHI3L1) plays a role in tissue remodelling and inflammation. [115,118] CaCO3, chitin, and protein are the main components of solid waste from shellfish processing. Their group used *S. marcescens* chitinase to hydrolyze chitinous material and *yeast, Pichia kudriavzevii*, to produce SCP that could be used in aquaculture. *Hensenula polymorpha, Candida tropicalis, S. cerevisiae*, and *M. verrucaria* have been commonly used for the production of SCP. The chitinase from *M. verrucaria* and *S.*

cerevisiae have been used to produce SCP from chitinous waste by Wang and Hwang, 2001.[119] *M. verrucaria* chitinase preparation for chitin hydrolysis and *S. cerevisiae* chitinase preparation for SCP have also been reported by these authors. Chitinases are used in a variety of fields, including agriculture and mosquito control. Chitinases can also be used as additives to supplement commonly used insecticides and fungicides, making them more potent while lowering the concentration of chemically synthesised active agents in the ingredients, which are otherwise harmful to human health and the environment. [120,121] Chitinases can also be used in the bioconversion of chitin waste into fertiliser. [122] The use of microorganisms as biological control agents or the secretions of microorganisms to prevent plant pathogens and insect pests gives us a compelling option to control plant diseases. As a result, biological control strategies have become a critical step toward achieving sustainable agriculture. [123] Chitinase-producing organisms could also be used as biocontrol agents, either directly or indirectly, through the use of purified proteins or through gene manipulation. [124]

Future prospects:

It may be possible to develop chitinases with new functions in the future. Chitinases have the potential to be used as food preservatives, extending the shelf life of foods. A thorough understanding of the biological functions of various chitinases would aid in the development of novel therapeutic approaches for a variety of diseases, including asthma and chronic rhinosinusitis. Since chitohexaose and chitoheptaose have shown anti-tumor activity, chitinases could be used as anti-tumor drugs. These enzymes can be used to boost the immune system of humans. This research could be focused on identifying the active sites of chitinases as well as the novel functions they have. Protein engineering can be used to create chitinases that have unique functions.

Acknowledgement:

I would like to thank Professor A.D. Choudhary Ex-Head PGTD Botany RTMNU Nagpur for critical reading of the manuscript and helpful discussions on the activity of chitinase and analysis. This work was supported and fund grant by BRNS from BARC Mumbai as a research project.

References:

1. Shahidi F, Abozaytoun R. Chitin, chitosan and co-products: Chemistry, production, applications and health effects. *Adv Food Nutr Res.* 2005;49:93–135.
2. Bhattachrya D, Nagpure A, Gupta RK. Bacterial chitinase: Properties and potential. *Crit Rev Biotechnol.* 2007;27:21–8.
3. Wang SL, Chang WT. Purification and characterization of two bifunctional chitinases/lysozymes extracellularly produced by *Pseudomonas aeruginosa* K-187 in a shrimp and crab shell powder medium. *Appl Environ Microbiol.* 1997;63:380–6.

4. Muzzarelli RA. Clinical and biochemical evaluation of chitosan for hypercholesterolemia and overweight control. *EXS.* 1999;87:293–304.
5. Zikakis JP, editor. *Chitin, Chitosan, and Related Enzymes.* Orlando, New York: Academic Press Inc; 1984. p. XVII.
6. Muzzarelli RA. *Natural Chelating Polymers, per gives it the ability to bond chemically with negatively charged lipids, fats and bile acids.* New York: The gamon Press; 1997. p. 83. [
7. Bussink AP, Speijer D, Aerts JM, Boot RG. Evolution of mammalian chitinase (-like) members of family 18 glycosyl hydrolases. *Genetics.* 2007;177:959–70.
8. Chen JK, Shen CR, Liu CL. N-acetylglucosamine: Production and applications. *Mar Drugs.* 2010;8:2493–516.
9. Suginta W, Robertson PA, Austin B, Fry SC, Fothergill-Gillmore LA. Chitinases from vibrio: Activity screening and purification of chiA from V.carchariae. *J Appl Microbiol.* 2000;89:76–84.
10. Yuli PE, Suhartono MT, Rukayadi Y, Hwang JK, Pyunb YR. Characteristics of thermostable chitinase enzymes from the Indonesian *Bacillus sp*.13.26. *Enzyme Microb Technol.* 2004;35:147–53.
11. Shiro M, Ueda M, Kawaguchi T, Arai M. Cloning of a cluster of chitinase genes from Aeromonas sp. no. 10S-24. *Biochim Biophys Acta.* 1996;1305:44–8.
12. Mathivanan N, Kabilan V, Murugesan K. Purification, characterization and antifungal activity of chitinase from *Fusarium chlamydosporum*, a mycoparasite to groundnut rust, Puccinia arachidis. *Can J Microbiol.* 1998;44:646–51.
13. Mendonsa ES, Vartak PH, Rao JU, Deshpande MV. An enzyme from *Myrothecium verrucaria* that degrades insect cuticles for biocontrol of Aedes aegypti mosquito. *Biotechnol Lett.* 1996;18:373–6.
14. Shahabudin M, Vinetz JM. Chitinases of human parasites and their implications as antiparasitic targets. In: Jolles P, Muzzarelli RA, editors. *Chitin and Chitinases.* Switzerland: Birkhauser Verlag Basel; 1999. pp. 223–8.
15. Blaak H, Schrempf H. Binding and substrate specificities of a Streptomyces olivaceovirldis chitinase in comparison with its proteolytically processed form. *Eur J Biochem.* 1995;229:132–9.
16. Tsujibo II, Orikoshi II, Tanno H, Fujimoto K, MIyamoto IL, Imada C, et al. Cloning, sequence and expression of a chitinase gene from a marine bacterium, *Alteromonas sp*. strain O-7. *J Bacteriol.* 1993;175:176–81.
17. West PA, Colwell RR. Identification and classification of Vibrionaceae: An overiew. In: Colweii RR, editor. *Vibrios in the environment.* New York: John Wiley and Sons, Inc; 1984. pp. 285–363.

18. Sitrit Y, Vorgias CE, Chet I, Oppenheim AB. Cloning and primary structure of Chia gene from Aeromonas caviae. *J Bacteriol.* 1995;177:4187–9.
19. Sahai AS, Manocha MS. Chitinases of fungi and plants: Their involvement in morphogenesis and host-parasite interaction. *FEMS Microbiol Rev.* 1993;11:317–38.
20. Harman GE, Hayes CK, Lorito M, Broadway RM, Di Pietro A, Peterbauer C, et al. Chitinolytic enzymes of *Trichoderma harzianum*: Purification of chitobiosidase and endochitinase. *Phytopathol.* 1993;83:313–8.
21. Frankowski J, Berg G, Bahl H. Purification and properties of two chitinolytic enzymes of the biocontrol agent *Serratia plymuthica* HRO C48. *Arch Microbiol.* 2001;176:421–6.
22. Kamensky M, Ovadis M, Chet I, Chernin L. Soil-borne strain IC14 of *Serratia plymuthica* with multiple mechanisms of antifungal activity provides biocontrol of *Botrytis cinerea* and *Sclerotinia sclerotiorum* diseases. *Soil Biol Biochem.* 2003;35:323–31.
23. Henrissat B, Bairoch A. New families in the classification of glycosyl hydrolases based on amino acid sequence similarities. *Biochem J.* 1993;293:781–8.
24. Patil RS, Ghormade V, Deshpande MV. Chitinolytic enzymes: An exploration. *Enzyme Microb Technol.* 2000;26:473–83.
25. Kasprzewska A. Plant chitinases-regulation and function-a review. *Cell Mol Bio Lett.* 2003;8:809–24.
26. Matsumiya M, Kasasuda S, Miyauchi K, Mochizuki A. Substrate specificity and partial amino acid sequence of a Chitinase from the stomach of coelacanth Latimeria chalumnae. *Fish Sci.* 2008;74:1360–2.
27. Brunner F, Stintzi A, Fritig B, Legrand M. Substrate specificities of tobacco chitinases. *Plant J.* 1998;14:225–34.
28. Iseli B, Armand S, Boller T, Neuhaus JM, Henrissat B. Plant chitinases use two different hydrolytic mechanisms. *FEBS Lett.* 1996;382:186–8.
29. Sasaki C, Itoh Y, Takehara H, Kuhara S, Fukamizo T. Family 19 chitinase from rice (Oryza sativa L.): Substrate-binding subsites demonstrated by kinetic and molecular modeling studies. *Plant Mol Biol.* 2003;52:43–52.
30. Sasaki C, Vårum KM, Itoh Y, Tamoi M, Fukamizo T. Rice chitinases: Sugar recognition specificities of the individual subsites. *Glycobiology.* 2006;16:1242–50.
31. Gooday GW. Aggressive and defensive roles of chitinases. *EXS.* 1999;87:157–69.
32. Andres E, Boer H, Koivula A, Samain E, Driguez H, Armand S, Cottaz S. Engineering chitinases for the synthesis of chitin oligosaccharides: catalytic amino acid mutations convert the GHfamily 18 glycoside hydrolases into transglycosylases. *J Mol Cat B: Enzym.* 2012;74:89–96.
33. Tellam RL. Protein motifs in filarial chitinases: An alternative view. *Parasitol Today.* 1996;12:291–2.

34. Alam MM, Nikaidou N, Tanaka H, Watanabe T. Cloning and sequencing of chiC gene of *Bacillus circulans* WL-12 and relationship of its product to some other chitinases and chitinase-like proteins. *J Ferment Bioeng.* 1995;80:454–61.
35. Graham LS, Sticklen MS. Plant chitinases. *Can J Bot.* 1994:721057–83.
36. Poole DM, Hazelwood GP, Huskisson NS, Virden R, Gilbert H. The role of conserved tryptophan residues in the interaction of a bacterial cellulose binding domain with its ligand. *FEMS Microbiol Lett.* 1993;106:77–84.
37. Schnellmann J, Zeltins A, Blaak H, Schrempf H. The novel lectinlike protein CHBl is encoded by a chitin-inducible *Streptomyces olivaceoviridis* gene and binds specifically to crystalline a-chitin of fungi and other organisms. *Mol Microbiol.* 1994;13:807–19.
38. Watanabe T, Kimura K, Sumiya T, Nikaidou N, Suzuki K, Suzuki M, et al. Genetic analysis of the chitinase system of *Serratia marcescens* 2170. *J Bactriol.* 1997;179:7111–7.
39. Venegas A, Goldstein JC, Beauregard K, Oles A, Abdulhayoglu N, Fuhrman JA. Expression of recombinant microfilarial chitinase and analysis of domain function. *Mol Biochem Parasitol.* 1996;78:149–59.
40. Watanabe T, Ito Y, Yamada T, Hashimoto M, Sekine S, Tanaka H. The roles of the C-terminal domain and type III domains of chitinase A1 from *Bacillus circulans* WL-12 in chitin degradation. *J Bacteriol.* 1994;176:4465–72.
41. Ohno T, Armand S, Hata T, Nikaidou N, Henrissat B, Mitsutomi M, et al. A modular family 19 chitinase found in the prokaryotic organism *Streptomyces griseus* HUT 603Z. *J Bacteriol.* 1996;178:5065–70.
42. Suzuki K, Sugawara N, Suzuki M, Uchiyama T, Katouno F, Nikaidou N, et al. Chitinases A, B, and C1 of *Serratia marcescens* 2170 produced by recombinant *Escherichia coli*: Enzymatic properties and synergism on chitin degradation. *Biosci Biotechnol Biochem.* 2002;66:1075–83.
43. Shekhar N, Bhattacharya D, Kumar D, Gupta RK. Biocontrol of wood- rotting fungi using *Streptomyces violaceusniger* XL-2. *Can J Microbiol.* 2006;52:805–8.
44. Cohen-Kupiec R, Chet I. The molecular biology of chitin digestion. *Curr Opin Biotechnol.* 1998;9:270–7.
45. Chernin LS, De la Fuente L, Sobolev V. Molecular cloning structural analysis and expression in Escherichia coli of a chitinase gene from *Enterobacter agglomerans*. *Appl Environ Microbiol.* 1997;63:834–9.
46. Downing KJ, Thomson JA. Introduction of the *Serratia marcescens* chiA gene into an endophytic Pseudomonas fluorescens for the biocontrol of phytopathogenic fungi. *Can J Microbiol.* 2000;46:363–9.

47. Dempsey DA, Silva H, Klessig DF. Engineering disease and pest resistance in plants. *Trends Microbiol.* 1998;6:54–61.
48. Ordentlich A, Elad Y, Chet I. The role of chitinase of *Serratia marcescens* in biological control of *Sclerotium rolfsii*. *Phytopathol.* 1988;78:84–8.
49. Svitil AL, Kirchman DL. A chitin-binding domain in a marine bacterial chitinase and other microbial chitinases: Implications for the ecology and evolution of 1,4-beta-glycanases. *Microbiology.* 1998;144:1299–308.
50. Metcalfe AC, Krsek M, Gooday GW, Prosser JI, Wellington EM. Molecular analysis of a bacterial chitinolytic community in an upland pasture. *Appl Environ Microbiol.* 2002;68:5042–50.
51. Morimoto K.S, Karita T, Kimura T, Sakka K, Ohmiya K. Cloning, sequencing, and expression of the gene encoding *Clostridium paraprtrificum* chitinase chiB and analysis of the functions of novel cadherin-like domains and a chitin-binding domain. *J. Bacteriol.* 1997;179(23):7306–7314.
52. Watanabe T, Kanai R, Kawase T, Tanabe T, Mitsutomi M, Sakuda M, et al. Family 19 chitinases of *Streptomyces species*: Characterization and distribution. *Microbiology.* 1999; 145:3353–63.
53. Ueda M, Fujiwara A, Kawaguchi T, Arai M. Purification and some properties of six chitinases from Aeromonas sp. No. 10S-24. *Biosci Biotechnol Biochem.* 1995;59:2162–4.
54. Mitsutomi O, Isono M, Uchiyama A, Nikaidov N, Ikegami T, Watanabe T. Chitosanase activity of the enzyme previously reported as β-1,3-1,4-glucanase from Bacillus circulans WL 12. *Biosci Biotechnol Biochem.* 1998;62:2107–14.
55. Hayes CK, Klemsdal S, Lorito M, Di PA, Peterbauer C, Nakas JP, et al. Isolation and sequence of an endochitinase-encoding gene from a cDNA libray of *Trichoderma harzianum*. *Gene.* 1994;138:143–8.
56. Henrissat B. Classification of chitinase modules. In: Jolles P, Muzzarelli RA, editors. *Chitin and Chitinases.* Switzerland: Burkhauser Basel; 1999. pp. 137–56.
57. Takaya N, Yamazaki D, Horiuchi H, Ohta A, Takagi M. Cloning and characterization of a chitinase-encoding gene (ChiA) from *Aspergillus nidulans*, disruption of that decreases germination frequency and hyphal growth. *Biosci Biotechnol Biochem.* 1998a;62:60–5.
58. Shimono K, Matsuda H, Kawamukai M. Functional expression of chitinase and chitosanase, and their effects on morphologies in the yeast Schizosaccharomyces pombe. *Biosci Biotechnol Biochem.* 2002;66:1143–7.
59. Lorito M, Hayes CK, Di Pietro A, Woo SL, Harman GE. Purification, characterization and synergistic activity of a glucan 1,3-β-glucosidase and an N-acetylglucosaminidase from *T. harzianum*. *Phytopathology.* 1994;84:398–405.

60. Ulhoa CJ, Peberdy JF. Purification and characterization of an extracellular chitobiase from *T. harzianum*. *Curr Microbiol.* 1991;23:285–9.
61. Draborg H, Kauppinen S, Dalboge H, Christgau S. Molecular cloning and expression in *S. cerevisiae* of two exochitinases from *T. harzianum*. *Biochem Molec Biol Int.* 1995;36:781–91.
62. Peterbauer CK, Lorito M, Hayes CK, Harman GE, Kubicek CP. Molecular cloning and expression of the nag1 gene (N-acetylbeta-D-glucosaminidase-encod ing gene) from *Trichoderma harzianum* P1. *Curr Genet.* 1996;30:325–31.
63. Lorito M, Woo SL, Fernandez IG, Colucci G, Harman GE, Pintor-Toro JA, et al. Genes from mycoparisitic fungi as a source for improving resistance to fungal pathogens P. *Natl Acad Sci USA.* 1998;95:7860–5.
64. Yamanaka S, Tsuyoshi N, Kikuchi R, Takayama S, Sakuda S, Yamada Y. Effect of demethylallosamidin, a chitinase inhibitor, on morphology of fungus *Geotrichum candidum*. *J Gen Appl Microbiol.* 1994;40:171–4.
65. Sandor E, Pusztahelyi T, Karaffa L, Karanyi Z, Pocsi I, Biro S, et al. Allosamidin inhibits the fragmentation of *Acremonium chrysogenum* but does not influence the cephalosporin-C production of the fungus. *FEMS Microbiol Lett.* 1998;164:231–6.
66. Chérif M, Benhamou N. Cytochemical aspects of chitin breakdown during the parasitic action of a *Trichoderma sp*. on *Fusarium oxysporum* f. sp. radicis-lycopersici. *Phytopathol.* 1990;80:1406–14.
67. Chet I. Trichoderma- Application, mode of action, and potential as a biocontrol agent of soilborne plant pathogenic fungi. In: Chet I, editor. *Innovative Approaches to Plant Disease Control.* New York: John Wiley and Sons; 1987. pp. 137–60.
68. Lorito M, Harman GE, Hayes CK, Broadway RM, Tronsmo A, Woo SL, et al. Chitinolytic enzymes produced by *Trichoderma harzianum*: Antifungal activity of purified endochitinase and chitobiosidase. *Phytopathology.* 1993;83:302–7.
69. Haran S, Schickler H, Oppenheim A, Chet I. Differential expression of *Trichoderma harzianum* chitinases during mycoparasitism. *Phytopathology.* 1996;86:980–5
70. Madi L, Katan T, Katan J, Henis Y. Biological control of *Sclerotium rolfsii* and Verticillium dahliae by Talaromyces flavus is mediated by different mechanisms. *Phytopathology.* 1997;87:1054–60.
71. Harman GE. Myths and dogmas of biocontrol. *Plant Dis.* 2000;84:377–91.
72. Yedidia I, Benhamou N, Kapulnik Y, Chet I. Induction and accumulation of PR proteins activity during early stages of root colonization by the mycoparasite *T. harzianum* strain T-203. *Plant Physiol Biochem.* 2000;38:863–73.

73. Yuan WM, Crawford DL. Characterization of *Streptomyces lydicus* WYEC108 as a potential biocontrol agent against fungal root and seed rots. *Appl Environ Microbiol.* 1995;61:3119–28.
74. Seidl V, Huemer B, Seiboth B, Kubicek CP. A complete survey of *Trichoderma chitinases* reveals three distinct subgroups of family 18 chitinases. *FEBS J.* 2005;272:5923–39.
75. Zeilinger S, Galhaup C, Payer K, Woo SL, Mach RL, Fekete C, et al. Chitinase gene expression during mycoparasitic interaction of *Trichoderma harzianum* with its host. *Fungal Genet Biol.* 1999;26:131–40.
76. Woo SL, Donzelli B, Scala F, Mach RL, Harman GE, Kubicek CP, et al. Disruption of ech42 (endochitinase-encoding) gene affects biocontrol activity in *Trichoderma harzianum* strain P1. *Mol Plant Microbe Interact.* 1998;12:419–29.
77. Viterbo A, Haran S, Friesem D, Ramot O, Chet I. Antifungal activity of a novel endochitinase gene (chit36) from *T. harzianum* Rifai TM. *FEMS Microbiol Lett.* 2001;200:169–74.
78. Fukamizo T, Sakai C, Tamoi M. Plant Chitinases: Structure-function relationships and their physiology. *Foods Food Ingredients J Jpn.* 2003;208:631–2.
79. Gooday GW. Aggresive and Defensive Roles for Chitinases. In: Muzzarelli RA, editor. *Chitin Enzymology.* Italia: Atec Edizioni; 1996. pp. 125–133.
80. Garg N, Gupta H. Isolation and purification of fungal pathogen *(Macrophomina Phaseolina)* induced chitinase from moth beans *(Phaseolus aconitifolius) J Pharm Bioall Sci.* 2010;2:38–43.
81. Gupta R, Saxena R, Chaturvedi P, Virdi J. Chitinase production by Streptomyces viridificans: Its potential in fungal cell wall lysis. *J Appl Bacteriol.* 1995;78:378–83.
82. Koga D, Sasaki Y, Uchiumi Y, Hirai N, Arakane Y, Nagamatsu Y. Purification and Characterization of Bombyx mori Chitinases. *Insect Biochem Mol Biol.* 1997;27:757–67.
83. Bussink AP, van Eijk M, Renkema GH, Aerts JM, Boot RG. The biology of the Gaucher cell: The cradle of human chitinases. *Int Rev Cytol.* 2006;252:71–128.
84. Chou YT, Yao S, Czerwinski R, Fleming M, Krykbaev R, Xuan D, et al. Kinetic characterization of recombinant human acidic mammalian chitinase. *Biochemistry.* 2006;45:4444–54.
85. Webb DC, McKenzie AN, Foster PS. Expression of the Ym2 lectinbinding protein is dependent on interleukin (IL)-4 and IL-13 signal transduction: Identification of a novel allergy-associated protein. *J Biol Chem.* 2001;276:41969–76
86. Khan MA, Hamid R, Ahmad M, Abdin MZ, Javed S. Optimization of culture media for enhanced chitinase production from a novel strain of *Stenotrophomonas maltophilia* using response surface methodology. *J Microbiol Biotechnol.* 2010;20:597–1602.

87. Bhushan B. *Ph.D. thesis, Department of Microbiology*. Chandigarh: Punjab University; 1998. Isolation, purification, characterization and scale up production of a thermostable chitinase from an alkalophilic microorganism.
88. Dahiya N, Tewari R, Tiwari RP, Hoondal GS. Chitinase production in solid state fermentation by Enterobacter sp. NRG4 using statistical experimental design. *Curr Microbiol.* 2005b;51:222–8.
89. Miyashita K, Fujii T, Sawada Y. Molecular cloning and characterization of chitinase gene from *Streptomyces lividans* 66. *J Microbiol.* 1991;137:2065–72.
90. Chen JP, Lee MS. Enhanced production of *Serratia marcescens* chitinase in PEG/dextran aqueous two-phase system. *Enzyme Microb Technol.* 1995;17:1021–7.
91. Sakuda S. Studies on the chitinase inhibitors, allosamidins. In: Muzzarelli RA, editor. *Chitin Enzymology*. Grottammare: Eur. Chitin Soc; 1996. pp. 203–12.
92. Tews I, Scheltinga AC, Terwisscha V, Perrakis A, Wilson KS, Dijkstra BW. Substrate assisted catalysis unifies two families of chitinolytic enzymes. *J Am Chem Soc.* 1997;119:7954–9.
93. Tabudravu JN, Eijsink VA, Gooday GW, Jaspar M, Komander D, Legg M, et al. Psammaplin A, a chitinase inhibitor isolated from the *Fijian marine* sponge *Aplysinella rhax*. *Bioorg Med Chem.* 2002;10:1123–8.
94. Arai N, Shiomi K, Yamaguchi Y, Masuma R, Iwai Y, Turberg A, et al. Argadin, a new chitinase inhibitor, produced by Clonostachy sp. FO. 7314. *Chem Pharm Bull.* 2000;48:1442–6.
95. Ovadis M, Liu XG, Gavriel S, Ismailov Z, Chet I, Chernin L. The global regulator genes from biocontrol strain *Serratia plymuthica* IC1270: Cloning, sequencing, and functional studies. *J Bacteriol.* 2004;186:4986–93.
96. Hobel CF, Hreggvidsson GO, Marteinsson VT, Bahrani-Mougeot F, Einarsson JM, Kristjansson JK. Cloning, expression and characterization of a highly thermostable family 18 chitinase from Rhodothermus marinus. *Extremophiles.* 2005;9:53–64.
97. Huang CJ, Chen CY. High level expression and characterization of two chitinases ChiCH and ChiCW of Bacillus cereus 28-9 in Escherichia coli. *Biochem Biophys Res Commun.* 2005;327:8–17.
98. El-Tarabily KA, Soliman M, Nassar A, Al-Hassani H, Sivasithamparam K, McKenna F, et al. Biological control of sclerotinia minor using a chitinolytic bacterium and actinomycetes. *Plant Pathol.* 2000;49:573–83.
99. Melchers LS, Stuiver MH. Novel genes for disease-resistance breeding. *Curr Opin Plant Biol.* 2000;3:147–52.
100. Roberts WK, Selitrennikoff CP. Plant and bacterial chitinases differ in antifungal activity. *J Gen Microbiol.* 1988;134:169–76.

101. Dahiya N, Tewari R, Tiwari RP, Hoondal GS. Production of an antifungal chitinase from Enterobacter sp. NRG4 and its application in protoplast production. *World J Microbiol Biotechnol.* 2005a;21:1611–6.
102. Miller M, Palojarvi A, Rangger A, Reeslev M, Kjoller A. The use of fluorogeic substrates to measure fungal presence and activity in soil. *Appl Environ Microbiol.* 1998;64:613–7.
103. Murao S, Kawada T, Itoh H, Oyama H, Shin T. Purification and characterization of a novel type of chitinase from *Vibrio alginolyticus*. *Biosci Biotech Biochem.* 1992;56:368–9
104. Kobayashi S, Kiyosada T, Shoda SI. A novel method for synthesis of chitobiose via enzymatic glycosylation using a sugar oxazoline as glycosyl donor. *Tetrahedron Lett.* 1997;38:2111–2.
105. Aloise PA, Lumme M, Haynes CA. N-Acetyl-D-glucosamine production from chitin-waste using chitinases from *Serratia marcescens*. In: Muzzarelli RAA, editor. *Chitin Enzymology.* Grottammare: Eur. Chitin Soc; 1996. pp. 581–94.
106. Horsch M, Mayer C, Sennhauser U, Rast DM. β-N- acetylhexosaminidase: A target for the design of antifungal agents. *Pharmacol Ther.* 1997;76:187–218.
107. Laine LA, Lo CJ. Diagnosis of fungal infections with a chitinase. 1996 US patent 5587292.
108. Orunsi NA, Trinci AP. Growth of bacteria on chitin, fungal cell walls and fungal biomass, and the effect of extracellular enzymes produced by these cultures on the antifungal activity of amphotericin B. *Microbios.* 1985;43:17–30.
109. Muzzarelli RA. Human enzymatic activities related to the therapeutical administration of chitin derivatives. *Cell Mol Life Sci.* 1997;53:131–40.
110. Zhu Z, Zheng T, Homer RJ, Kim YK, Chen NY, Cohn L, et al. Acidic mammalian chitinase in asthmatic Th2 inflammation and IL-13 pathway activation. *Science.* 2004;304:1678–82.
111. Chupp GL, Lee CG, Jarjour N, Shim YM, Holm CT, He S, et al. A chitinase-like protein in the lung and circulation of patients with severe asthma. *N Engl J Med.* 2007;357:2016–27. Chang NC, Hung SI, Hwa KY, Kato I, Chen JE, Liu CH, et al. A macrophage protein, Ym1, transiently expressed during inflammation is a novel mammalian lectin. J Biol Chem 2001;276:17497-506.
112. Chang NC, Hung SI, Hwa KY, Kato I, Chen JE, Liu CH, Chang AC. A macrophage protein, Ym1, transiently expressed during in-flammation is a novel mammalian lectin. *J Biol Chem.* 2001;276:17497–506.
113. Tjoelker LW, Gosting L, Frey S, Hunter CL, Trong HL, Steiner B, et al. Structural and functional definition of the human chitinase chitin- binding domain. *J Biol Chem.* 2000;275:514–20.

114. Kzhyshkowska J, Mamidi S, Gratchev A, Kremmer E, Schmuttermaier C, Krusell L, et al. Novel stabilin-1 interacting chitinase-like protein (SICLP) is up-regulated in alternatively activated macrophages and secreted via lysosomal pathway. *Blood.* 2006;107:3221–8.
115. Hakala BE, White C, Recklies AD. Human cartilage gp-39, a major secretory product of articular chondrocytes and synovial cells, is a mammalian member of a chitinase protein family. *J Biol Chem.* 1993;268:25803–10.
116. Renkema GH, Boot RG, Au FL, Donker-Koopman WE, Strijland A, Muijsers AO, et al. Chitotriosidase, a chitinase, and the 39-kDa human cartilage glycoprotein, a chitin-binding lectin, are homologues of family 18 glycosyl hydrolases secreted by human macrophages. *Eur J Biochem.* 1998;251:504–9.
117. Kawada M, Hachiya Y, Arihiro A, Mizoguchi E. Role of mammalian chitinases in inflammatory conditions. *Keio J Med.* 2007;56:21–7.
118. Volck B, Price PA, Johansen JS, Sorensen O, Benfield TL, Nielsen HJ, et al. YKL-40, a mammalian member of the chitinase family, is a matrix protein of specific granules in human neutrophils. *Proc Assoc Am Physicians.* 1998;110:351–60.
119. Wang S, Hwang J. Microbial reclamation of shellfish wastes for the production of chitinases. *Enzyme Microb Technol.* 2001;28:376–82.
120. Giambattista RD, Federici F, Petruccioli M, Fence M. The chitinolytic activity of *Penicillium janthinellum* P9: Purification, partial characterization and potential application. *J Appl Microbiol.* 2001;91:498–505.
121. Govindsamy V, Gunaratna KR, Balasubramanian R. Properties of extracellular chitinase from *Myrothecium verrucaria*, an antagonist to the groundnut rust Puccinia arachidis. *Can J Plant Pathol.* 1998;20:62–8.
122. Sakai K, Yakota A, Kurokawa H, Wakayama M, Moriguchi M. Purification and characterization of three thermostable endochitinases of Bacillus noble strain MH-1 isolated from chitin containing compost. *Appl Environ Microbiol.* 1998;64:3340–97.
123. Wang SL, Yen YH, Tsiao WJ, Chang WT, Wang CL. Production of antimicrobial compounds by *Monascus purpureus* CCRC31499 using shrimp and crab shell powder as a carbon source. *Enzyme Microb Technol.* 2002;31:337–44.
124. Singh P, Shin Y, Park C, Chung Y. Biological control of Fusarium wilt of cucumber by chitinolytic bacteria. *Phytopathology.* 1999;89:92–9.

LYMPHATIC FILARIASIS AND MASS DRUG ADMINISTRATION: A REVIEW

Dilip Haribhau Dudhmal

Department of Zoology,

MVP Samaj's Arts, Commerce and Science College, Dindori (Nashik), Maharashtra

Corresponding author E-mail: dilipdudhmal@gmail.com

Introduction:

World Health Organization (WHO) has counted Lymphatic Filariasis (LF) as Neglected Tropical Disease (NTD) which is a parasitic disease transmitted through mosquitoes to humans. It is commonly called as 'Elephantiasis' or Filariasis. It stands second in the list of diseases which leads to long term physical disability in the tropical and subtropical countries of the world [(1)]. It is caused when the female mosquito sucks up blood along with round worms i.e. nematodes in the form of microfilariae from a carrier human being and bites to a healthy human being. The neamtodes which are attributed as the pathogens of this disability are *Wuchereria bancrofti, Brugia malayi and Brugia timori* [(2,3,4,5)]. These parasites are sucked up and transmitted to healthy humans as vectors by mosquito genera like *Aedes, Culex* and *Anopheles*[(3,6,7, 8)]**.** These parasites, after entering a healthy person's body, migrate to stay in the lymphatic vessels thereby causing impairment in lymphatic functions [(7)]. Broadly, the symptoms can be divided into two types a. Lymphoedema which refers to abnormal swellings in legs, arms and breasts, b. Hydrocoele is an accumulation of fluid in the scrotal sac [(9)]. It is recorded that 25 million males were diagnosed with filarial hydrocoele whereas lymphoedema was observed in 15 million people [(9)] with 1.4 billion people living in the endemic countries in 73 countries with over 120 million people currently affected [(10)]. India has 650 millions of people living in 257 filaria endemic districts from 21 states and Union Territories of the country [(11)] that accounts for 40% of the world's burden of LF [(12)]. In the year 2000, World Health Organization (WHO) introduced Global Program to Eliminate Lymphatic Filariasis (GPELF) by 2020 but currently, the target is changed as 2030[(13)]. The endemic population is supplied with Ivermectin, Diethylcarbamazine citrate and Albendazole though Mass Drug Administration (MDA)[(7,8,9,10,13)]. Since the GPELF was launched, 74% reduction was recorded in new cases from the endemic countries[(14)] Researchers across the world have been reporting that the compliance of the drugs through MDA could not meet the expected success because of the low degree of knowledge about LF in the endemic population. For the complete eradication of this neglected tropical disease, people's awareness

may play a crucial role. Improper knowledge of the disease may lead to carelessness in preventive and controlling practices [15, 16].

The Pathogen:

Wuchereria bancrofti is a nematode i.e. a roundworm that causes lymphatic filariasis along with three other parasitic worms, *Brugia malayi* and *B. timori*, infecting the lymphatic system. *W. bancrofti* is found in most of the cases than the other two pathogens.

Phylum: Nematoda

Class: Chromadorea

Order: Rhabditida

Family: Onchoceridae

Genus: *Wuchereria*

Species: *bancrofti*

Vector:

The filarial worms i.e. nematodes are sucked up by female mosquitoes when they bite a carrier person and are injected into a healthy human being thereby causing the transmission of this disease. Worldover, *Culex quinquefasciatus* is attributed for the transmission but there are a wide range of mosquitoes which can transmit the parasite. This differs as the geographical area changes. e.g. in Africa, it is transmitted by Anopheles *while in* America, by Aedes and *Mansonia* is credited for the transmission in some parts of Asia.

Classification:

Phylum: Arthropoda

Class: Insecta

Order Diptera

Family: Culicidae

Genus: *Culex*

Species: *quinquesfasciatus*

Life Cycle:

During a blood meal, an infected mosquito introduces third-stage filarial larvae onto the skin of the human host, where they penetrate into the bite wound. They develop in adults that commonly reside in the lymphatics. The female worms measure 80 to 100 mm in length and 0.24 to 0.30 mm in diameter, while the males measure about 40 mm by .1 mm. Adults produce microfilariae measuring 244 to 296 μm by 7.5 to 10 μm, which are sheathed and have nocturnal periodicity, except the South Pacific microfilariae which have the absence of marked periodicity. The microfilariae migrate into lymph and blood channels moving actively through lymph and

blood. A mosquito ingests the microfilariae during a blood meal. After ingestion, the microfilariae lose their sheaths and some of them work their way through the wall of the proventriculus and cardiac portion of the mosquito's midgut and reach the thoracic muscles. There the microfilariae develop into first-stage larvae and subsequently into third-stage infective larvae. The third-stage infective larvae migrate through the hemocoel to the mosquito's prosbocis and can infect another human when the mosquito takes a blood meal [(18)]

Symptoms:

Lymphatic filariasis infection involves asymptomatic, acute, and chronic conditions. The majority of infections are asymptomatic, showing no external signs of infection while contributing to transmission of the parasite. These asymptomatic infections still cause damage to the lymphatic system and the kidneys and alter the body's immune system.

When lymphatic filariasis develops into chronic conditions it leads to lymphoedema (tissue swelling) or elephantiasis (skin/tissue thickening) of limbs and hydrocele (scrotal swelling). Involvement of breasts and genital organs is common. Such body deformities often lead to social stigma and sub-optimal mental health, loss of income-earning opportunities and increased medical expenses for patients and their caretakers. The socioeconomic burdens of isolation and poverty are immense.

Acute episodes of local inflammation involving skin, lymph nodes and lymphatic vessels often accompany chronic lymphoedema or elephantiasis. Some of these episodes are caused by the body's immune response to the parasite. Most are the result of secondary bacterial skin infection where normal defenses have been partially lost due to underlying lymphatic damage. These acute attacks are debilitating, may last for weeks and are the primary cause of lost wages among people suffering with lymphatic filariasis.

Treatment:

Large scale treatment through preventive chemotherapy (it can also be called as Anti-parasitic treatment) is possible in eliminating LF from the world. Mass Drug Administration (MDA) is the strategy adopted by World Health Organization (WHO) wherein at risk population is administered with anti-filarial drugs. The medicines used have a limited effect on adult parasites but effectively reduce the density of microfilariae in the bloodstream and prevent the spread of parasites to mosquitoes. The drugs supplied through MDA are as follows [(19)]:

1. **Diethylcarbamazine citrate (DEC):**

   It is a drug generally used as an anti-parasitic drug, killing certain adult parasites as well as microfilariae in the body. Earlier, it was used to be supplemented with Albendazole

but according to the recent recommendations of WHO, it should be given with Ivermection (200 mcg/kg) and Albendazole (400mg).

2. **Ivermectin:**

   This medication is used to treat certain parasitic roundworm infections[20]. Ivermectin is effective against the microfilariae of *W. bancrofti*, but has no effect on the adult parasite[18].

3. **Albendazole:**

   Albendazole, also known as albendazolum, is a medication used for the treatment of a variety of parasitic worm infections. It is useful for giardiasis, trichuriasis, filariasis, neurocysticerosis etc [21].

References:

1. WHO (2007) Global programme to eliminate lymphatic filariasis. Wkly Epidemiol Rec 82: 361–380. (second leading cause of long term disability)
2. Ottesen EA, Duke BO, Karam M, Behbehani K. Strategies and tools for the control/elimination of lymphatic filariasis. Bull World Health Organ 1997; 75: 491–503. (infection caused by filarial nematodes B. malayi, B. timori and W. bancrofti) and
3. Centers for Disease Control and Prevention (CDC), 2019.Parasites–Lymphatic filariasis/Epidemiology & Risk Factors.Available at: https://www.cdc.gov/parasites/lymphaticfilariasis/ accessed May 24, 2021.
4. K.D. Ramaiah and Eric A. Ottesen Progress and Impact of 13 Years of the Global Programme to Eliminate Lymphatic Filariasis on Reducing the Burden of Filarial Disease 2014.
5. WHO Global Programe to eliminate Lymphatic Filariasis Progress Report 2015.
6. WHO. Lymphatic filariasis: a handbook of practical entomology for national lymphatic filariasis elimination programmes. World Health Organization, 2013. https://apps.who.int/iris/handle/10665/87989 (accessed Dec 19, 2019) (Transmitted through mosquito vectors of *Aedes, Culex* and *Anopheles* genera)
7. WHO Global Programme to eliminate Lymphatic Filariasis Progress Report 2020.
8. WHO 2013 Lymphatic Filariasis: Practical Handbook.
9. WHO, 2021.Lymphatic filariasis https://www.who.int/news-room/fact-sheets/detail/lymphatic-filariasis Accessed on 24th May 2021.
10. WHO Lympahtic Filariasis Fact Sheet, 2014: https://applications.emro.who.int/docs/Fact_Sheet_WHD_2014_EN_1631.pdf accessed on 30th May, 2021.

11. National Vector Borne Disease Control Programme, Filaria Endemic districts https://nvbdcp.gov.in/index4.php?lang=1&level=0&linkid=453&lid=3733 accessed 30th May 2021.
12. Ramaiah KD. Lymphatic filariasis elimination programme in India: progress and challenges. Trends in parasitology. 2009; 25(1):7–8. doi: 10.1016/j.pt.2008.10.001 PMID: 19008150. (40% burden India).
13. WHO Guidelines Alternative Mass Drug Administration Regimens to Eliminate Lymphatic Filariasis 2017.
14. The Lancet 2020. The global distribution of lymphatic filariasis, 2000–18: a geospatial analysis (GPELF success) the lancet Glob Health. (74% reduction in cases - WHO https://www.who.int/news/item/29-10-2020-lymphatic-filariasis-reporting-continued-progress-towards-elimination-as-a-public-health-problem)
15. K. D. Ramaiah, K. N. Vijay Kumar and K. Ramu, 1996 Knowledge and beliefs about transmission, preventionand control of lymphatic filariasis in rural areas of South India Tropical Medicine and International Health, VOLUME I NO. 4 **PI'** 433-438**.**
16. Rath, K., Nath, N., Mishra Shaloumy, Swain, B.K., Mishra Suchismita and Babu, B.V., Knowledge and perceptions about lymphatic filariasis: a study during the programme to eliminate lymphatic Filariasis in an urban community of Orissa, India. Tropical Biomedicine 23(2): 156–162 (2006).
17. *Melrose WD (2002). "Lymphatic filariasis: New insights into an old disease".* Int J Parasitol. ***32*** *(8): 947–60.* doi*:*10.1016/S0020-7519(02)00062-0. PMID 12076624
18. Parasites-Lympatic Filariasis https://www.cdc.gov/parasites/lymphaticfilariasis/biology_w_bancrofti.html accessed June 2022.
19. Lympatic Filariasis https://www.who.int/news-room/fact-sheets/detail/lymphatic-filariasis#:~:text=The%20MDA%20regimen%20recommended%20depends,mg)%20in%20countries%20with%20onchocerciasis accessed June 2022.
20. Ivermectin: Uses, Side Effects and More https://www.webmd.com/drugs/2/drug-1122/ivermectin-oral/details accessed July 2022.
21. Albendazole https://en.wikipedia.org/wiki/Albendazole accessed July 2022.

Frontiers in Life Science Volume VI

ISBN: 978-93-91768-01-0

About Editors

Dr. Shakun Mishra is working as Head Department of Botany, Govt. S. N.P.G.College, Khandwa (M.P.) INDIA. She did her Ph. D. in the year 2011 from D.A.V.V. Indore (M.P.) India. She has 36 years of teaching experience at U. G. level and 26 years at P.G. level. She is actively engaged in research from two decades. Dr. Mishra published 56 research papers in various journals and proceedings, 08 book chapters and associate editor for four books published by Bhumi Publishing, India. She attended and presented 45 research papers in various national and international conferences and also guest lecturer and chairperson in two national seminars. Presently, she is Fellow/Life Member of 10 National and International research societies such as Society of Life Sciences, Satna (M. P.), Indian Hydrobiology, Chennai and I.A.A.T., etc. She received some prestigious awards like F.I.C.C.E. in 2004, F.S.L.Sc. in 2005 and F.E.S. in 2013. She completed one M.R.P. funded by U.G.C. and Ex-Chairman of BOS in subject of Botany, Soil Sc., Seed Tech. and Horticulture in D.A.V.V.Indore and also same in Central University Bhopal. Typhonium flagelliforme (Lodd.) Blume(Araceae), is reported by Mishra for the first time for Burhanpur District from Madhya Pradesh forms an addition to the Araceae, Flora of Madhya Pradesh.

Dr. Balaji Govindrao Rajbhoj has been working as Assistant Professor in Department of Botany Sundarrao More Arts Commerce and Science College Poladpur Dist Raigad. Maharashtra, India. He has obtained his M.Sc. Degree with specialization in Plant Pathology He had completed his research work for Ph.D. on the topic Developing location specific Bio- fertilizers from Swami Ramanand Teerth Marathwada University Nanded. He had a brilliant academic career from the beginning. He is recognized as a research guide in Botany from University of Mumbai. He has published twenty seven Research papers in peer reviewed / refereed and indexed journals of National and International repute, 03 book chapters and presented twenty two research papers in various National and International conferences and also conducted guest lectures and chairperson in seminars. He also worked as NSS Area Co-ordinater in the University of Mumbai.He has teaching experience of over thirteen in the years at degree level. He has completed two Minor Research Projects sanctioned by University of Mumbai.

Dr. (Mrs.) Suchita C. Warangkar is working as an Assistant Professor at Dept of Microbiology, Netaji Subhashchandra Bose College, Nanded. She was awarded with Ph. D. in December 2009 from School of Life Sciences, Swami Ramanand Teerth Marathwada University, Nanded with title as “Studies on Production, Purification and Characterization of L-asparaginase from Erwinia Carotovora”. She has 13 years of teaching experience at UG and PG level. Dr. Warangkar has published 15 research papers in Scopus and SCI journals and 1 book. She has attended 28 seminars, conferences and workshops and presented her research work in 10 research events. She is Annual member of some renowned societies like Microbiological society of India, American Society of Microbiology, European Biotechnology Network and Anaerobic Society of Microbiology. She was worked as coordinator for 4 seminars, conferences and workshops

Sujith R. is working at Department of Microbiology, SRM Medical College Hospital and Research Centre, SRM Institute of Science and Technology (SRM IST), Potheri, Chengalpattu Dt., Tamilnadu, India. Sujith R. is Medical Microbiologist - Biotechnologist - Bioinformatician – Alternate Medicine – Educational Administration & Supervision – Medico-legal (Bioethics) and Advisor. He was honored with many awards such as Custodians of Humanity Award received from Sh. Ashwini Kumar Choubey – Minister of State for Health and Family Welfare, Government of India, Title Honorary Professor of Wellness Studies conferred as an honorary recognition by L.A.D.C., Los Angeles, Dr. of Science in Alternate System of Medicine, bend mark certificate with a tile as “Emperor of Laboratory Medicine”, etc. He is Founder Member & President of Governing Council, Research India Foundation (RIF), Member of Forum of Ethics Review Committee of India (FERCI), Consortium of Accredited Healthcare Organizations (CAHO), Indian Academicians and Researchers Association (IARA) and Indian Association of Tropical Parasitology (IATP).

Bhumi Publishing

ISBN 978-93-91768-01-0

9 789391 768010