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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 I"****. 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.

- Editorial Team

Frontiers in Life Science Volume I

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CONTENTS

Frontiers in Life Science (Volume I)
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EXPLORING THE USE OF MARINE PRODUCTS IN THE MANAGEMENT OF NON-COMMUNICABLE DISEASES CANCER

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Abstract:

Non-communicable diseases (NCDs) have been found as the foremost cause of death and disabilities all over the globe. It includes cardiovascular diseases (CVDs), cancer, diabetes mellitus and chronic respiratory diseases. Out of all these NCDs, cancer is the leading cause of fatality and as per WHO, 10 million people were died in year 2020 due to cancer. Current treatment of cancer is associated with several limitations and there is need to find out some alternatives for betterment of patient suffering from cancer. Drugs obtained from marine source are now the new hope for cancer treatment. Many in vitro and in vivo studies showed the promising effects of marine drugs. Review summarizes the prominent marine drugs in useful in management of cancer.

Introduction:

Non-Communicable Diseases (NCDs) are the *major contributors in global death which* affects millions of people. Cardiovascular diseases (CVDs), cancer, diabetes mellitus and chronic respiratory alignment are the major identified NCDs. Cancer is foremost cause of death worldwide (Andress *et al.,* 2015; WHO, 2021). As per WHO survey cancer leads to 10 million deaths in 2020. It has been predicted that around 14.6 million people will die in 2035 if Cancer not treated properly (WHO, 2021).

Cancer which is major killer of mankind arises when orderly process that controls the multiplication and life span of normal cell go awry. It results in development of abnormal tumor

mass in any organ and tissue of body (Arun *et al.,* 2013; AACR, 2014). In last two decades several classes of anticancer drug have been developed. This conventional cancer treatment get suffers from several limitations which includes i) insolubility of drugs in aqueous medium, ii) delivery of sub therapeutic dose to targeted cells, iii) lack of bioavailability, iv) non-specific toxicity to normal tissue so called 'lack of selectivity', v) development of virulent multi drug resistance (MDR) (Arun *et al.,*2013; Schleich *et al.,* 2014). There is imperative need to have new and better way to prevent, detect and treat cancer. Researchers have focused on various novel strategies to address these problems like marine drugs (Schleich *et al.,* 2014).

Marine site acts as gold mine of bioactive natural product having variety of structural/chemical features which is not available anywhere else (Sarfaraj *et al.,* 2012). Research on marine organism as a potential source for treatment of life threatening diseases was started early in 1940 (Adil *et al.,* 2019). As a result of which seven marine based pharmaceuticals have been approved for marketing, 23 compound are in clinical trials between phase I & II and over one thousand compound are undergoing preclinical Studies (Gerwick and Moore, 2012). Bioactive compounds generated by different marine sources possess immense therapeutic property. Majority of these compounds are the protective chemicals generated by marine organism for their protection.

Overview on marine floras:

Marine floras consists of

Microflora (bacteria, actinobacteria, cyanobacteria and fungi)

Microalgae

Macroalgae (seaweeds)

Flowering plants (mangroves and other halophytes)

Marine area is loaded with biodiversity which occupies almost 71% area of globe. It has been proved that the marine microfloras remain nature's best source of chemicals (Kaur and Kapoor, 2002). The ocean organism generates unique chemicals to withstand in extreme environmental conditions. Researchers now also considering the microbial flora presents in the invertebrates can be responsible for production of therapeutic agents. Marine floras have been used for medicinal purposes in India, China, the Near East and Europe, since ancient times. Also, the peoples from China and Japan consuming seaweeds because of health benefits (Cragg *et al.,* 1997). Moreover; many research study have also reveals the usefulness of seaweeds as a anti-cancer, anti-oxidant, anti-inflammatory as well as anti-diabetic. Trabectedin isolated from the

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Ecteinascidia turbinate, got approval as an anti-cancer agent in Europe (Kathiresan and Duraisamy, 2005).

It clearly indicates that marine floras are the new hope for treating of many life threatening diseases like cancers and many more.

Anticancer Agents from Marine Product:

1. Bacteria

Microbes presents in marine environment can leads to development of new chemical moiety possesses therapeutic activity. Marine bacteria produces secondary metabolites have proved pharmacological activity.

Table 1: Compounds with therapeutic activity

Property	Name of compound
Anti-inflammatory	Pseudopterosins, topsentins and manoalide
Anticancer	Bryostatins, discodermolide, eleutherobin, and sarcodictyin
*A*ntibiotics	Marinone

Pseudopterosins:

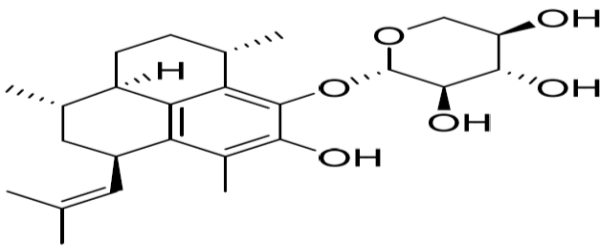
These are a group of marine diterpene glycosides obtained from *Pseudopterogorgia elisabethae* which is gorgonian soft coral. Potency of it depends on position of glycosylation on terpene skeleton and type of sugar moiety. These compounds possess variety of biological activities including anti-inflammatory, analgesic, wound-healing, anti-bacterial, anti-cancer, anti-viral, anti-malarial and anti-tuberculosis (Kathiresan *et al.,* 2008; Fenical *et al.,* 1987).

Pseudopterosin A (PsA), which contains a non-acetylated xylose sugar subunit showed following mechanism;

i. Cell membrane stabilization properties

ii. Alters the intracellular calcium

iii. Inhibit phagocytosis in free living ciliates (Mayer *et al.,* 2010).



Structure of Pseudopterosins (PsA)

2. Actinomycetes:

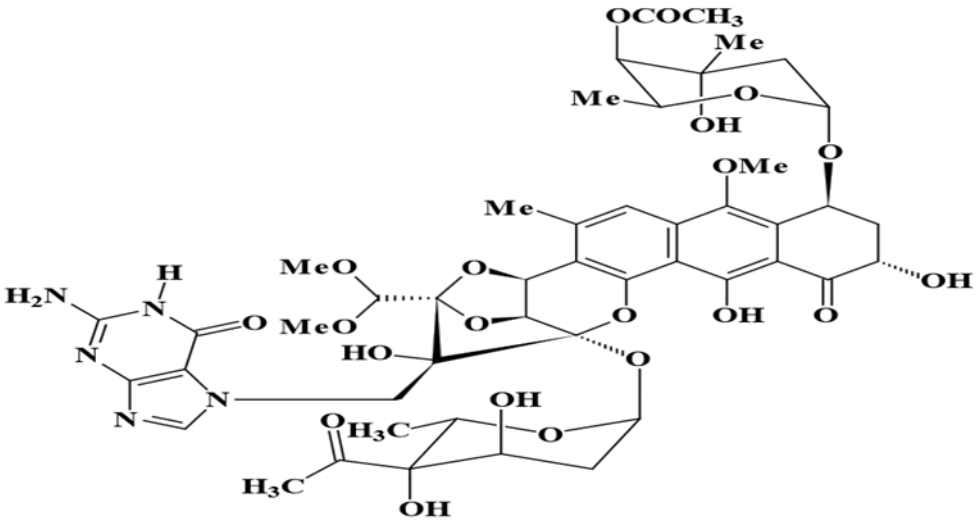
From several decades soil based actinomycetes of terrestrial origin have been used for discovery of antibiotics and related bioactive compounds.

Gutingimycin:

It is isolated from marine Streptomyces. It was obtained from sediment of the Laguna de Terminos, Gulf of Mexico. It is trioxacarcin derivative (Mydlarz *et al.*, 2004). It has proved its anticancer property.

Thiocoraline:

This bioactive depsipeptide compound is obtained from marine microorganism Micromonospora marina. Compound has proved the cytotoxic activity against lung and colon cancer. It also showed anti-proliferative effects in colon cancer cell lines.



Structure of Gutingimycin

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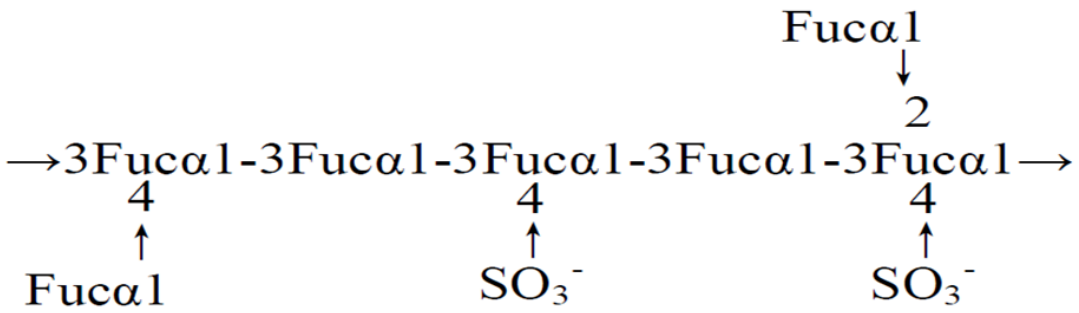
3. Fucoidan:

It is polysaccharide compound. It contains L-fucose as well as sulfate ester groups in ample proportion. Brown seaweed and some marine invertebrates acts as the main source of it (Bilan *et al.,* 2002; Bo *et al.,* 2008). Initially, in 1913 it was named as "fucoidin" as when isolated from marine brown algae. However; as per IUPAC now it is named as "fucoidan".

Fucoidans obtained from brown seaweed, Fucus vesiculosus, possesses simple chemical compositions, mainly being composed of fucose and sulfate and it is aalso available commercially. It is consist of 44.1% fucose, 26.3% sulfate and 31.1% ash along with little aminoglucose (Song *et al.,* 2000).

Polysaccharides have proved role in management of cancer. Fucoidans are found to be effective against sarcoma 180 (Aisa *et al.,* 2004).



Structure of Fucoidan

4. Didemnin B:

It is a cyclic antiproliferative depsipeptide isolated from the Caribbean tunicate Tridemnum solidum. It is the first marine product which enters in clinical trial for cancer management (El-Said *et al.*, 2013). It showed antitumor activity against a variety of models.

It showed following mechanisms of action:

i. Obstruct the synthesis of RNA, DNA and proteins

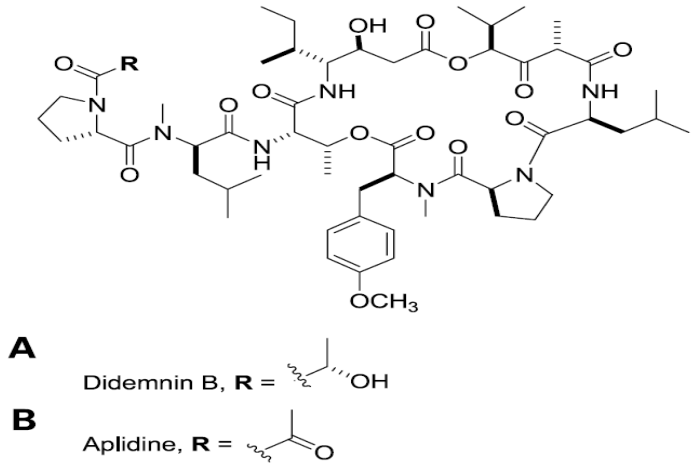
ii. Activation of the FK-506 apoptotic pathway (Fitzner *et al.* (2008).

Didemnin B, aplidine showed following mechanism of action;

i. Interfere the synthesis of DNA and proteins

ii. Induces cell cycle arrest

iii. Inhibition of ornithine decarboxylase enzyme which plays crucial role in tumor growth.



Structure of Didemnin B and aplidine

5. Cytarabine:

Cytarabine is in the antimetabolite and nucleoside analog families of medication. It works by blocking the function of DNA polymerase. Cytarabine was patented in 1960 and approved for medical use in 1969. It is first marine derived anticancer drug isolated from a marine sponge (Dyshlovoy and Honecker, 2019).

Conclusion:

As current treatment of cancer is associated with several side effects, use of marine medicines is a new hope for betterment of health of patient suffering from cancer. There is need to use advantages of available gold mine of marine drugs.

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THE TECHNIQUES OF HYBRIDE PRODUCTION THROUGH THE FUSION OF ISOLATED SOMATIC PROTOPLASTS

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Abstract:

In somatic hybridization the nucleus and cytoplasm of both parents are fused in the hybrid cell. Sometimes, nuclear genome of only one, parent but cytoplasmic genes (plastome) from both the parents are present in the fused hybrid, which is known as cybrid or cytoplasmic hybrid (Nagata, 1971; Zimmerman, 1981, 2016). Thus, protoplast fusion technique can be used to overcome the barriers of incompatibility and acts as a method for the genetic manipulation of plant cells. Sexual hybridization between closely related species has been used for years to improve cultivated plants Milchers, 1974; Kao, 1990). Unfortunately, sexual hybridization is limited in most cases to cultivars within a species or at best to a few wild species closely related to a cultivated crop. Species barriers thereby limit the usefulness of sexual hybridization for crop improvement. Somatic cell fusion leading to the formation of viable cell hybrids has been suggested as a method to overcome the species barriers to sexual hybridization. Plant protoplasts offer exciting possibilities in the fields of somatic cell genetics and crop improvement (Venkateshwarlu et al., 2019, 2020).

Keywords: Somatic Hybridization, Fusion, Hybrid production, Isolated protoplasts.

Introduction:

The techniques of hybrid production through the fusion of isolated somatic (body) protoplasts under in vitro conditions and subsequent development of their product (heterokaryon) to a hybrid plant are known as somatic hybridization (Roset, 1989). This procedure eliminates sex altogether hybridization. It provides us with an opportunity to construct hybrids between taxonomically distant plant species beyond the limits of sexual crossability (Schell, 1987; Fernanda, 2000). It also creates cells with new genetic, nuclear as well as cytoplasmic consti-

tutions that otherwise cannot be obtained. Somatic hybridization involves the fusion of protoplasts, selection of hybrid cells and identification of hybrid plants.

Techniques of Hybrid production:

Protoplast fusion

It involves mixing of protoplasts of two different genomes and can be achieved by either spontaneous or induced fusion methods.

Spontaneous fusion method

Cell fusion is a process integral to plant development. The most prominent process is egg fertilization. The breakdown of cell wall during protoplast isolation led people to believe that there would be spontaneous fusion leading to the formation of homokaryons because multinucleate cells were detected as soon as enzymatic protoplast isolation techniques were applied. The argument that cell wall degradation would permit dilation of plasmodesmata, fusion and complete mixing of cells was supported by Electron Micrographic studies (Ramezan *et al.,* 2011). Spontaneous fusion of protoplasts is observed when protoplasts are isolated from callus cultures. However, spontaneous fusion products do not regenerate in to whole plants except for undergoing a few divisions (Carlson, 1972; Solvey *et al.,* 1994). Later studies revealed that isolated protoplasts are usually characterized by smooth surfaces and fusion has to be induced by one of a variety of treatments.

Induced fusion method

Spontaneous fusion is of no value as fusion of protoplasts of different origins is required in somatic hybridization. To achieve this, a suitable agent (fusogen) is added to fuse the plant protoplasts of different origins. Some of the methods that have been employed are explained below. Protoplast fusion method has been outlined in Fig. 1.0.

Treatment with sodium nitrate

Induced fusion by $NaN0_3$ was first reported by Power *et al.* (1970). Isolated protoplasts are suspended in a mixture of 5.5% sodium nitrate in a 10% sucrose solution. The solution containing the protoplasts is incubated in a water bath at 35^0C for 5 min and then centrifuged for 5 min' at 200x g. Following centrifugation, most of the supernatant is decanted and the protoplast pellet is transferred to a water bath at 30°C for 30 min. During this period, most of the protoplasts undergo cell fusion (Harms, 1992). The remaining aggregation mixture is gently decanted and replaced with the culture medium containing 0.1% $NaN0_3$. The, protoplasts are left undisturbed for sometime after which they are washed twice with the culture medium and plated.

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This technique results in low frequency of heterokaryon formation, especially when mesophyll protoplasts are involved.

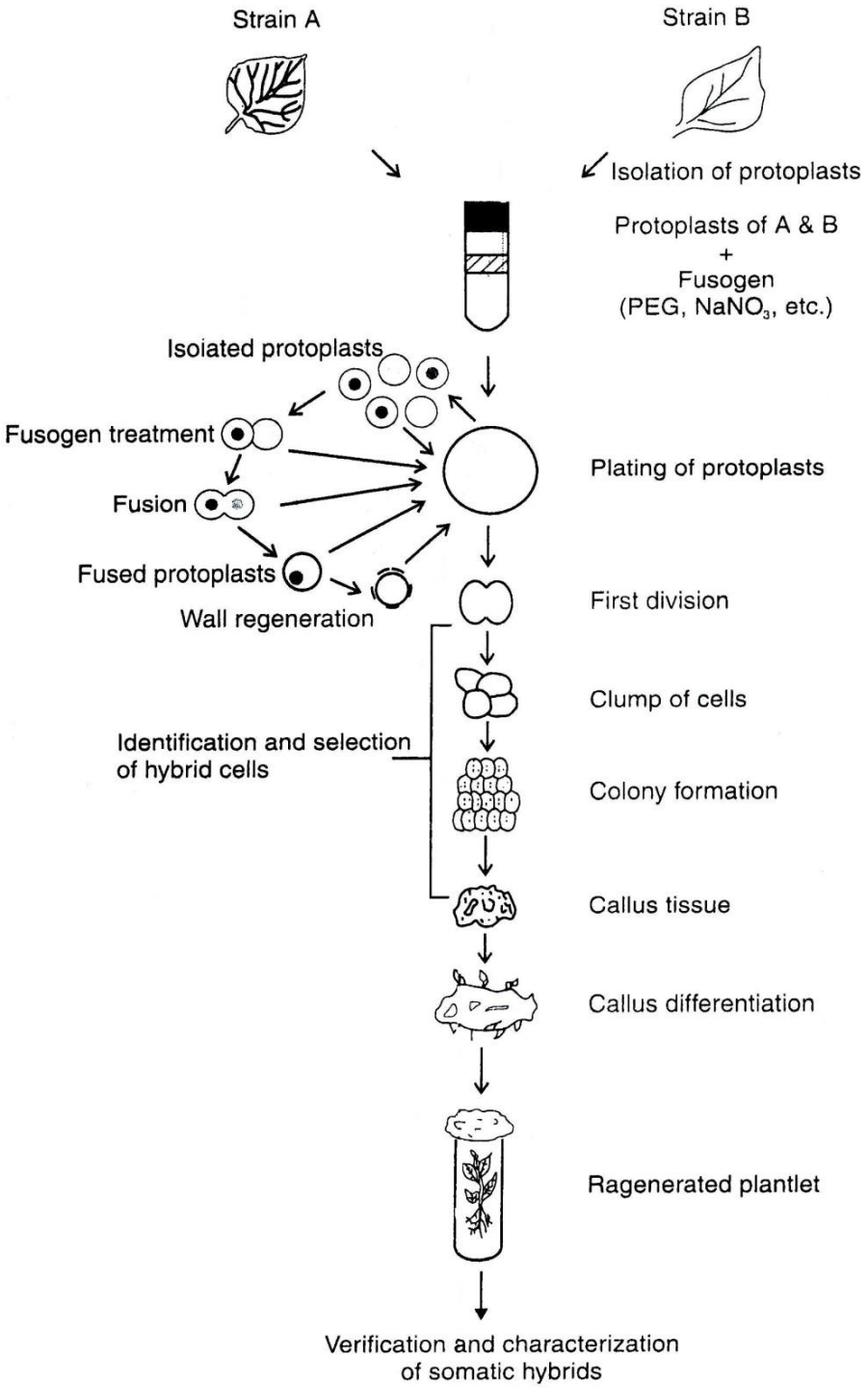


Figure 1: Schematic illustration of protopast fusion and regeneration of plants

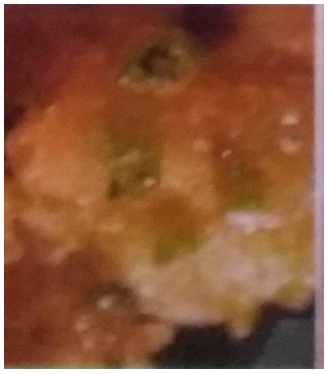
Calcium ions at high pH

Studied the effect of high pH and calcium ions on the fusion of tobacco protoplasts. In their method, isolated protoplasts are centrifuged for 3 min at 50x g in, a fusion-inducing solution of 0.5 M mannitol containing 0.05 M $CaCI_2$-$2H_zO$ at a pH of 10.5. The centrifuge tubes containing the protoplasts are then incubated in a water bath at 37°C for 40-50 min. After this treatment, 20-50% of the protoplasts were involved in fusion.

Polyethylene glycol method

Developed PEG method of fusion of protoplasts. This is one of the most successful techniques for fusing protoplasts (Puite, 1992). The protoplasts are suspended in a solution containing high molecular weight PEG, which enhances agglutination and fusion of protoplasts in several species. When sufficient quantities of protoplasts are available, 1 ml of the protoplasts suspended in a culture medium are mixed with 1 ml of 28-56%; PEG (1500-6000 MW) solution (Drew, 1993). The tube is then shaken for 5 sec and allowed to settle for 10 min. The protoplasts are then washed several times by the addition of protoplast culture medium to remove PEG. The protoplast preparation is then resuspended in the culture medium (Handley *et al.,* 1986). The PEG method has been widely accepted for protoplast fusion because it results in reproducible high-frequency heterokaryon formation, low cytotoxicity to most cell types and the formation of binucleate heterokaryons. PEG-induced fusion is non-specific and is therefore useful for interspecific, intergeneric or interkingdom fusions (Vasil, 2002).

(Plate-1, *In vitro* Somatic emryogenesis A, B *Solanum nigrum* Table-1)



A: Callus with Somatic Embryos **B: Regenerated Somatic Hybride Plants**

In Vitro production in the present study, Adenine Sulphate when used in combination with Benzyl Amino Purine induced multiple shoots. Among the combinations tested, Benzyl Amino Purine NAA 2.0-5.0 mg/l with 5.0 mg/l Adenine Sulphate produced maximum number of shoots with intermittent callus at the basal cut end. Of the various concentrations of Adenine Sulphate tested, 15 mg resulted in maximum number of shoots followed by 20mg/l (9.8), 10mg/l

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(6.5) and 5mg/l (6.8). Average number of shoots generated per explant on medium with 2.0 mg/l Benzyl Amino Purine and 15 mg/l Adenine Sulphate is an improvement of almost 3 fold in the multiplication rate as compared with shoots induced on MS medium with 2.0 mg/l Benzyl Amino Purine alone. Addition of 5 and 10mg/l Adenine Sulphate had no significant effect on number of shoots produced per explants. The presence of Adenine Sulphate initiated friable callus and suppress the formation of new shoots. A similar observation was reported in *Hemidesmus indicus* using 5-20 mg/l Adenine Sulphate with Benzyl Amino Purine and naphthalene acetic acid. The desired product yield but does not support the cellular growth of regenerated Somatic hybride plants.

Table 1: Regenerated Somatic Hybride Plants in *Solanum nigrum*

Sr. No.	BAP+KN+L-Glutamic acid-(GL) concentration (mg/l)	Growth Response
1	BAP+Kn 0.5 mg/l	Callus with Embryos
2	BAP+Kn 1.0 mg/l	Callus
3	BAP+Kn 2.0 mg/l	Callus + Embryos 2-4
4	BAP+Kn 3.0 mg/l	Callus Shoots 2-4
5	BAP+GL 1.0 mg/l	Callus with embryos
6	BAP+GL 2.0 mg/l	Callus with embryos
7	BAP+GL 3.0 mg/l	Shoots 1-2
8	BAP+GL 4.0 mg/l	Shoots
9	BAP+GL 5.0 mg/l	Shoots 2-4

Electrofusion

Protoplasts are placed in to a small culture cell containing electrodes, and a potential difference is applied due to which protoplasts line up between the electrodes. If now an extremely short wave electric shock is applied, protoplasts can be induced to fuse. In this fusion method, two-step procedure is followed beginning with application of an alternating current (AC) of low intensity to protoplast suspension (Tudses *et al.,* 2014; 2015). Dielectrophoretic collectors adjusted to 1.5V and 1 MHz and an electrical conductivity of the suspension medium less than 10^{-5} sec/cm generate an electrophoresis effect that make the cells attach to each other along the field lines (Szarka *et al.,* 2002). The second step of injection of an electric direct

current (DC) field pulse of high intensity (750-1000 V/cm) for a short duration of 20—50 µsec leads to breakdown of membranes in contact areas of adjacent cells resulting in fusion and consequent membrane reorganization. This electrofusion technique has been found to be simple, quick and efficient. Cells after electrofusion do not show cytotoxic response. However, this method did not receive much acceptance because specialized equipment is required.

Mechanism of fusion

Protoplast fusion consists of three main phases

i. **Agglutination or adhesion:** Two or more protoplasts are brought into close proximity. The adhesion can be induced by a variety of treatments, e.g. concanavalin A, PEG, high pH and high Ca^{+} ions.

ii. **Plasma membrane fusion at localized sites:** Membranes of protoplasts agglutinated by fusogen get fused at the point of adhesion.

It results in the formation of cytoplasmic bridges between the protoplasts. Plant protoplasts carry a negative charge from -10 to -30 mV. Due to common charge, the plasma membranes of two agglutinated protoplasts do not come close enough to fuse. Fusion requires that membranes must be first brought close together at a distance of 10 A or less. The high pH-high Ca^{++} ions treatment has shown to neutralize the normal surface charge so that agglutinated protoplasts can come in intimate contact (Xhu *et al.,* 2003). High temperature promotes membrane fusion due to perturbance of lipid molecules in plasma membrane and fusion occurs due to intermingling of lipid molecules in membranes of agglutinated protoplasts. PEG agglutinates to form clumps of protoplasts. Tight adhesion may occur over a large or small localized area. Localized fusion of closely attached plasma membranes occurs in the regions of tight adhesion and results in the formation of cytoplasmic bridges (Grosser, 2011). It has been further suggested that PEG, which is slightly negative in polarity can form hydrogen bonds with water, protein, carbohydrates, etc. which are positive in polarity. When the PEG molecule chain is large enough it acts as a molecular bridge between the surface of adjacent protoplasts and adhesion occurs,

iii. **Formation of heterokaryon:** Rounding off of: the fused protoplasts due to the expansion of cytoplasmic bridges forming spherical heterokaryon or homokaryon.

Identification and selection of hybrid cells

Following fusion treatment, the protoplast population consists of parental types, homokaryotic fusion products of parental cells, heterokaryotic fusion products or hybrids and a variety of other nuclear cytoplasmic combinations. Despite efforts to increase the efficiency of

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protoplast fusion, usually not more than 1-10% of the protoplasts in a treated population have actually undergone fusion (Barbara *et al.*, 2015). The proportion of viable heterospecific binucleate A + B type fusion products may be even lower. In the absence of systems that would fuse different protoplasts specifically to produce heterokaryocytes at a very high rate, there is an obvious need to select the products of fusion amongst the unfused and homofused parental cells (Tiwari *et al.*, 2010; 2011). Identification and recovery of protoplast fusion products have been based on observation of visual characters, or hybrid cells display genetic complementation for recessive mutations and physiological complementation for *in vitro* growth requirements. In complementation, fusion of two protoplasts each carrying a different recessive marker, will generate afusion product which is functionally restored since each parent contributes a functional allele that corrects the respective deficiency of the other parent (Ahloowalia, 1991; Amberger *et al.*, 1992).

Chlorophyll deficiency complementation

First used genetic complementation to isolate green somatic hybrids following fusion of two distinct homozygous recessive albino mutants of *Nicotiana tabacum.* They used chlorophyll deficient light sensitive varieties "sublethal" and "virescent". After two months of incubation under high light conditions, green colonies develop. This is the most frequently used method to isolate somatic hybrids (Fig. 1.1). Chlorophyll deficient mutants have also been used to raise somatic hybrids of *N. tabacum* + *N. sylvestris.*

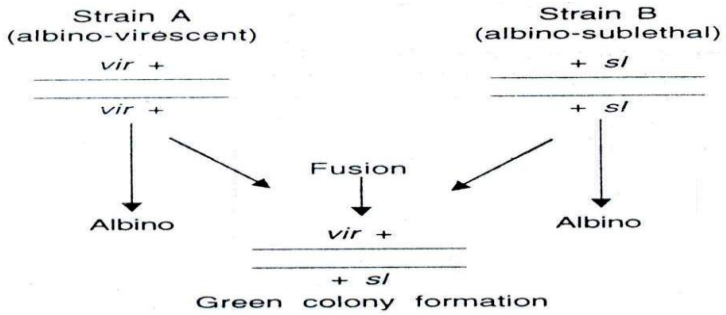

Figure 1.1: A selection scheme involving complemenatation of chlorophyll deficient mutations

In most cases, it has not been necessary to use two albino mutants to recover somatic hybrid shoots (Bellincampi, 1987). A single recessive albino mutation could be very useful in combination with a morphological trait or a growth response in the isolation of somatic hybrid

plants. Single recessive albino mutation marker in combination with morphological marker(s) to distinguish putative somatic hybrids in *Daucus* has been used (Fig. 1.1). Both hybrid protoplasts and *D. capillifolius* will regenerate into green shoots. However, the morphology of leaves of fused product resembles those of *D. carota* leaves and can be isolated (Beversdorf *et al.*, 1977)

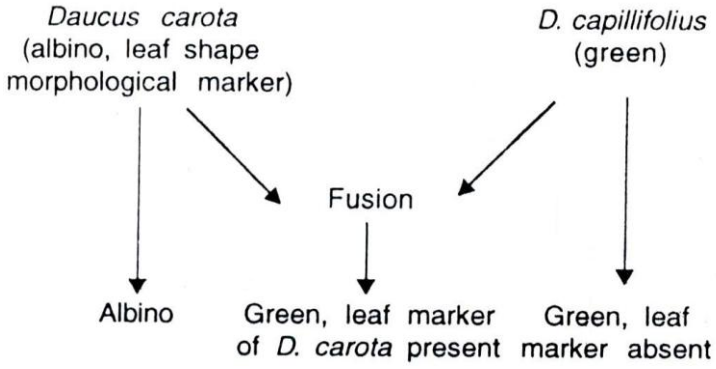

Figure 1.2: A scheme illustrating selection of interspecific hybrid plants in Daucus utilizing morphological marker with albino mutant

Auxotroph complementation

This method of selection has been applied to higher plants on a limited scale. The limitation is due to the paucity of higher plant auxotrophs. There is a report by Glimelius *et al.* (1978) where two parents were nitrate reductase deficient mutants of *N. tabacum* and could not be grown with nitrate as sole nitrogen source, while hybrids could regenerate shoots in the nitrate medium (Fig. 1.3). The lack of nitrate reductase (NR) activity causes an absolute requirement for reduced nitrogen and is caused by a deficiency either in the NR apoenzyme (nia-type mutant) or in the molybdenum cofactor (cnx^- type mutant) (Binding, 1974).

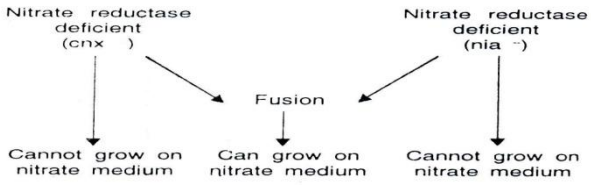

Figure 1.3: A summary of somatic hybrid selection based on auxotroph complementation

Complementation of resistance markers

Dominant characters such as traits conferring resistance to antibiotics, herbicides, amino acid analogues or other toxic compounds have been recognized as potent selectable markers. When protoplasts from two lines are being fused together, the sensitivity trait of each parent will be dominated by the respective resistance trait from the other parent and will grow on a medium

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containing both the metabolites because of double resistance as compared to single resistance of the parents. This is also referred to as biochemical selection (Cocking, 1960; Butenko *et al.*, 1980; Christianson, 1983). Selected double resistant somatic hybrids in *Daucus carota* following fusion of protoplasts from S 2-aminoethyl cysteine (2AEC) and 5-methyl tryptophan (5MT) resistant parental lines but were sensitive to other compound (Fig.1.4). Used a kanamycin resistant variant of *N. sylvestris*, KR103, isolated from the cultured cells, as a genetic marker to recover fusion products between *N. sylvestris* and *N. knightiana*. Complementation system involving antibiotics (e.g. methotrexate, streptomycin, cycloheximide, and chloramphenicol) resistance has also been used.

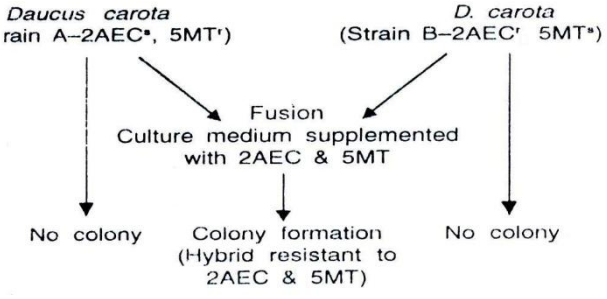

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Figure 1.4: Scheme illustrating selection of somatic hybrids utilizing complementation of resistance markers

Use of metabolic inhibitors

In this method, parental cells are treated with an irreversible biochemical inhibitor such as **o**doacetate or diethylpyrocarbamate and following treatment only hybrid cells are capable of division, lodoacetate pretreatment has been used to aid recovery of somatic hybrids between *N. sylvestris* and *N. tabacum* and *N. plumbaginifolla a*nd *N. tabacum.* In each case the parent protoplasts treated with iodoacetate were unable to reproduce, while the newly formed hybrid protoplasts continued to develop and yield hybrid plants.

Use of visual characteristics

The most efficient but the most tedious method TO select products of protoplast fusion is to visually identify hybrid cells and mechanically isoiate individual cells. The approaches used by various workers have been summarized:

- Use of morphologically distinct cells: Fusion of mesophyll protoplasts containing green chloroplasts with colorless cell culture protoplasts containing distinct starch granules due to growth on sucrose supplemented medium. Fused products can be seen

immediately after PEG treatment with one half containing chloroplast and other half with distinct starch granules.

- Use of petal protoplasts with leaf protoplasts and petal + cell culture protoplasts can be readily distinguished. The usually vacuolar petal pigment is originally separated within the fused cell but eventually becomes evenly distributed throughout the fused cell
- Fluorescent labeling: Parental protoplasts that are isolated from the same type of cells and hot sufficiently different for visual distinction may be loaded with different fluorescent dyes prior to fusion. Fluorescein isothiocyanate (fluorescing in the greerr) and rhodamine isothiocyanate fluorescing in the red have been used to label two separate batches of mesophyll protoplasts of *Nicotiana* (Duke, 1981). The labeling is achieved by adding 0.5 mg/l of the compound into the enzyme mixture during incubation period (Galbraith and Mauch, 1980). The double fluorescent label of fusion products can be recognized in a fluorescence microscope and fusion products then cultured after micro-isolation. The double fluorescence provides a possibility to separate fusion products from parental protoplasts when fusion mixtures are run through fluorescence activated cell sorter (FACS).
- The growth pattern of hybrid callus is different from either parental line. Hybrid callus is often more vigorous than parental callus.
- Differences in the morphology of callus: Parental type protoplasts as well as hybrid cells can develop to form different callus types (Fig. 1.5). No selection pressure is applied at the protoplast or cell level. There are differences in the morphology of callus in hybrid of *Datura innoxia* and *Atropa belladonna.*

Compound selection system

Most of the somatic somatic hybrid plants that are known today have been selected using compound selection strategies rather than straightforward marker complementation. Compound selection systems have used differential growth characteristics, regenerability, morphological features and complementation marker systems to distinguish somatic hybrids from parental cells/plants (Gaj, 2001).Whatever the system employed, the utility of a selective system lies in the fact that it provides enrichment for fused cells by reducing or eliminating the non-desired parental type cells. This enrichment thus greatly reduces the number of colonies or plants among which hybrids must be sought.

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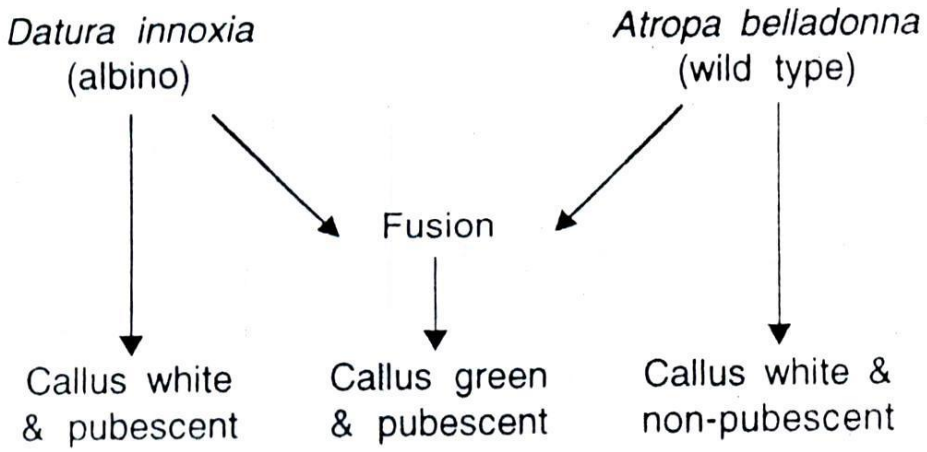

Figure 1.5: Selection system based on morphology of callus employed in intergeneric somatic hybridization

Verification and characterization of somatic hybrids

Protoplasts from any two species can be fused together. However, there are a number of limitations to widespread utilization of somatic hybridization in higher plants, including aneuploidy, species barriers to hybridization, and the inability to regenerate plants from protoplasts. Besides these limitations, hybrids have been developed and these must be verified as products of somatic fusion of two different protoplasts. Successful passage through a selection system provides the first circumstantial evidence for the somatic hybrid nature of selected plant materials. Further evidence must be added based on the traits not involved in the selection (Ghazi, 1986). Proof of hybridity requires a cleat-demonstration of genetic contribution from both fusion partners and hybridity must be sought only from euploid and not from aneuploid hybrids.

1. **Morphology:** When plant regeneration has been accomplished from protoplast fusion, products a wide range of morphological features can be drawn upon for hybrid verification. In most cases morphological characteristics of either somatic or sexual hybrids are intermediate between the two parents. Both vegetative and floral characters such as leaf shape, leaf area, petiole, size, root morphology, trichome (hairs on the leaf surface) length and density, flower shape, color size and structure, corolla morphology are considered, intensity of flower pigment and seed capsule morphology. The genetic basis for most of these morphological traits has not yet been elucidated, but the intermediate behavior in hybrids suggests control of traits by multiple genes. Sometimes intermediate morphology is not observed in the somatic hybrids because these traits behave as dominant I single gene traits as they are present only in one parent, but are also expressed

in the somatic hybrids. Such traits include stem anthocyanin pigment, flower pigment, heterochromatic knobs in interphase cells and leaf size. Pollen viability character in general shows a decrease in viability in the hybrids. Whenever possible, additional genetic data should be presented to support hybridity.

2. **Isoenzyme analysis:** Electrophoretic banding patterns of isoenzymes have been extensively used to verify hybridity. Isoenzymes are the different molecular forms of the enzyme that catalyze the same reaction. For isoenzymic studies, both starch and polyacrylamide gels are employed where electrical properties of proteins (enzymes) are used for separation into different bands. Somatic hybrids may display isoenzyme bands of certain enzymes specific to one or the other parent or to both parents simultaneously. Enzymes that have unique banding patterns versus either parental species and that have been used for identification of somatic hybrids include esterase, aspartate aminotransferase; amylase, isoperoxidase phosphatase, alcohol, malate, and lactate dehyrogenase and phosphodiesterase. If the enzyme is dimeric, the somatic hybrids contain a unique hybrid band intermediate in mobility in addition to the sum of parental bands. This probably represents formation of a hybrid dimeric enzyme unique to the somatic hybrids. Since isoenzymes are extremely variable within plant tissues, it is important to use the same enzyme from each plant and to use plants/ tissue at identical developmental ages when comparing parental plants with somatic hybrids. For comparison, zymograms (a diagrammatic representation of isoenzyme banding pattern) should therefore be prepared and interpreted with caution.

3. **Chromosomal constitution:** Counting chromosomes in presumed hybrid cells can be an easy and reliable method to verify hybrid cells and it also provides information on the ploidy state of the cells. Cytologically the chromosome number of the somatic hybrids should be the sum of chromosome number of two parental protoplasts used for fusion. Variation in chromosome number (aneuploidy) is generally observed in hybrids but frequently chromosome number is more than the total number of both the parental protoplasts. Besides numerical number, structural and size differences of their chromosomes should be studied. The presence of marker chromosomes can greatly facilitate the analysis of genetic events in hybrid cells. Extensive use of banding techniques can be made to identify specific chromosomes and investigate rearrangements in somatic hybrid cells.

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4. **Molecular techniques:** Recent progress in the development of molecular biological techniques has greatly improved our capabilities to analyze the genetic constitution of somatic plant hybrids. Specific restriction patterns of chloroplast and mitochondrial DNA has been used to great advantage to characterize the nature of plastoms and chondrioms of somatic hybrids and cybrids. Species-specific restriction fragments of nuclear DNA coding for ribosomal RNA have been shown to verify somatic hybrids of *Nicotiana glauca + N. langsdorffii* (Uchimiya *et al*., 1983). With the availability of numerous molecular markers such as RFLP, AFLP, RAPD, microsatellites etc., the hybrid identification can be done using these techniques. These are more accurate methods of analysis based on DNA, so no environmental effect is there. RFLP markers have been used for the identification of somatic hybrids between potato and tomato. PCR technology has been utilized for hybrid identification (Baird *et al*., 1992). RAPD markers established the hybridity between *Solanum ochranthum* and *Lycopersicon esculentum* (Kobayashi *et al*, 1996).
5. **Genetic characterization:** Somatic hybrid plants that produce fertile flowers can be analyzed by conventional genetic methods. Results obtained from segregation data of selfed F_2 generations are generally in good agreement with the expected values and have provided a sound confirmation of the somatic nature of the plants analyzed.

Chromosome number in somatic hybrids:

Variation in chromosome number (aneuploidy) is generally observed in hybrids but frequently chromosome number is more than the total number of both the parental protoplasts, Interspecific and intergeneric somatic hybrids are mostly polyploids. The chromosome number of these hybrids indicates that only few hybrids have the sum total chromosome number of both the parents as expected in an amphiploid (Hansen, 1999). There is an indication that closely related species would yield true amphidiploids through somatic hybridization. Even sexually compatible parents show deviation in the number of chromosomes in their somatic hybrids (Henry, 1998). The chromosome number of parent species and somatic hybrids obtained has been listed in Table 1.1. The variability in chromosome number of hybrids could be due to following reasons:

i. Multiple fusions give a higher chromosome number.
ii. Fusion of more than two protoplasts with subsequent mitotic irregularities.

iii. In PEG and electro-induced fusions about one-third of fusion products result from fusions among more than two protoplasts.

iv. Asymmetric hybrids result from fusion of protoplasts isolated from actively dividing tissue of one parent and quiescent tissue of the other parent.

v. Unequal rates of DNA replication in two fusing partners may also give asymmetric hybrids,

vi. Somaclonal variation in cultured cells used for protoplast isolation may also lead to variation in chromosome number.

Table 1.1: Chromosome number in some of the interspecific hybrids produced through protoplast fusion

Plant species with their	**number**		**Chromosome in hybrid**
Brassica oleracea (2n =	. +	*B. campestris* (2n = 18)	Wide
Brassica napus (2n = 38)	+	B. *juncea* (2n = 36)	Wide
Datura innoxia (2n = 24)	+	*D. stramonium* (2n = 24)	46, 48, 72
Nicotiana tabacum (2n =	+	*N. glutinosa* (2n = 24)	50 - 58
Nicotiana tabacum (2n =	+	*N. nesophila* (2n = 48)	96
Nicotiana tabacum (2n =	+	*N. sylvestris* (2n = 24)	72
Lycopersicon esculentum = 24)	+	*L. peruvianum* (2n = 24)	72
Petunia parodii (2n = 48)	+	*P. hybrida* (2n = 14)	44 - 48
Solanum tuberosum (2n = 48)	+	S. *chacoense* (2n = 14)	60

Cybrids:

Sexual hybridization is a precise mixture of parental nuclear genes but the cytoplasm is or interkingdome maternal parent only, while in somatic hybrids the cytoplasm is derived from both the parents. Venkateshwarlu (2020). However, somatic hybrids can be obtained where nucleus is derived from one parent and cytoplasm is derived from both, thus producing cytoplasmic hybrids, also called as cybrids (Fig. 6.8). Venkateshwarlu *et al.*, (2019).Early segregation of nuclei in a fused product can be stimulated and directed so that one protoplast contributes the cytoplasm while the other contributes the nucleus alone or both nucleus and cytoplasm. There are different ways of inactivating the nucleus of one protoplast. Thus, there will be fusion between protoplasts containing the full complement of nucleus, mitochondria and chloroplasts with functional cytoplasmic component of second parent. The various approaches to achieve this type of fusion are:

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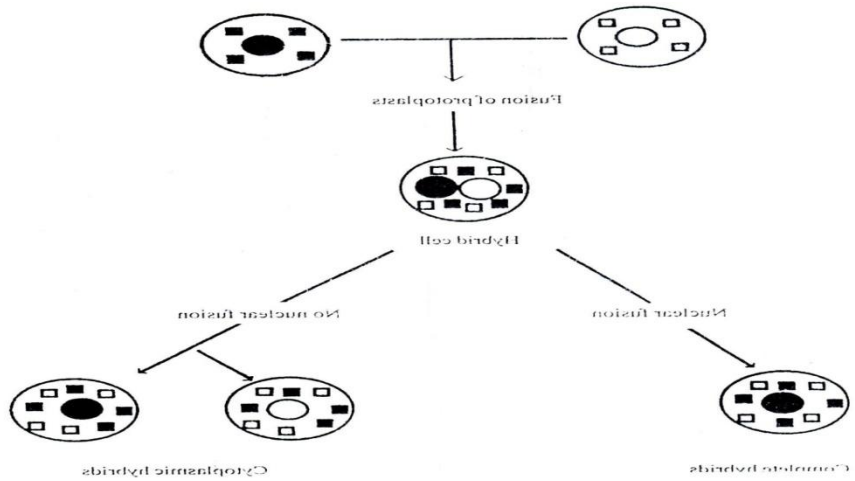

Figure 1.6: Schematic illustration of somatic hybridization which can produce complete hybrids (left) or cytoplasmic hybrids (right)

Conclusion:

This opportunity will undoubtedly lead to the production of new genetic variation and thereby widen the genetic base for plant breeding. More over in case of vegetative reproducing plants, the genetic variation can be induced through protoplast fusion. Thus, in spite of having some limitations protoplast culture and somatic hybridization have immense potentialities and prospect in future plant biotechnology especially for the improvement of different plant species. Protoplast fusion and somatic hybridization have opened up a new avenue in plant science. Protoplast fusion provides a method of combining the different genomes of different genera and species with the potential of overcoming sexual incompatibility barrier between plants. Besides this, studies of fusion product can give information about compatibility or incompatibility of the nuclei or cytoplasm. By protoplast fusion, it is possible to transfer some useful genes.

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QUALITY OF PLANTING MATERIALS IN GREENHOUSE

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Introduction:

Use of high quality planting materials is critical for success in greenhouse plant production. Good propagation capacity must develop together with expanding greenhouse crop production. Some farmers grow their own transplants, while others purchase them from a specialized nursery. When and where to get planting materials must be identified before planning greenhouse production. Proximity to nurseries which might supply transplants is a factor in the site selection of greenhouse production facilities: a long distance from the supplier may preclude the purchasing of transplants. The supply of planting materials (seeds and transplants) must be precisely scheduled for each production cycle. Good coordination skills are required to effectively work with nurseries, especially commercial nurseries, as timing of production and timely delivery of transplants are critical. Whether transplants are produced in-house or purchased from commercial nurseries, care must be taken to follow good agricultural practices to avoid introducing diseases and pests to the production greenhouse through seeds and transplants.

A good transplant is usually defined by the grower's specifications. According to the grower's preferences, different management techniques may be required. For example, home gardeners may favour robust, succulent plants, while commercial farmers may select more hardened plants. No simple procedure can be followed in growing vegetable transplants, and only through experience can you begin to produce a consistent product. In general, vegetable transplants should be stocky, green and pest-free with a well-developed root system. Once transplanted, they should tolerate environmental challenges and continue growing to achieve optimum yield. Overly hardened or under fertilized transplants may not establish quickly, resulting in delayed maturity and reduced yields. Insufficiently hardened or over-fertilized plants

may succumb to disease or abiotic stresses. The ideal technique for growing transplants is to raise the plant from start to finish by slow, steady, uninterrupted growth and with minimal stress. Since ideal growing conditions rarely exist, plant growth needs to be controlled through the manipulation of water, temperature and fertilizer.

Seeds

Potential seed problems are: unexpected low germination rate, contamination with different species and introduction of seed-borne diseases. For example, bacterial canker of tomato (*Clavibacter michiganensis* subsp. *michiganensis*) is a notorious seed-borne pathogen and outbreaks occur annually in tomato production areas worldwide (ASTA, 2009). For early detection, attention must be paid to the seed source as well as to the seedlings during propagation.

Seed source

- Keep records with key information: purchase date, source (vendor name), variety name, seed lot number, seed treatments and other seed quality parameters.
- Test the germination rate before planting: it is recommended to follow the standardized protocol used by the country's organization relevant to seed quality and trade; the germination rate must be recorded and kept with the other seed-related information for potential future track-back needs.
- Use seeds from a reliable source: this is the only way to avoid unintentionally buying adulterated or "fake" seeds or improperly disinfested (therefore contaminated) seeds.
- For genetically modified organisms, follow the relevant national or international regulations.

Handling of seeds

Seed storage

Understand seed type. Seeds may be classified according to their tolerance level to drying or temperature: orthodox, recalcitrant and intermediate. Most greenhouse grown species produce orthodox seeds. However, under similar storage and harvest conditions, seeds exhibit different inherent longevities depending on the species (Walters and Towill, 2004). Relative life expectancy under favourable storage conditions for certain crop groups is: legumes (beans) 3-4 years; crucifers (broccoli, cauliflower) 4-5 years; lettuce, endive and chicory 4-5 years; spinach, beets, carrots and chard 2-3 years; cucurbits (melons, squash) 4-5 years; tomatoes 4 years; peppers 2 years; onion, parsley, parsnip and salsify 1 year. As seeds age, the germination

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percentage declines at varying rates depending on conditions and species. Guidelines for storage behaviour (orthodox vs recalcitrant) are presented in Table 1.

Table 1: Guidelines to identify storage behaviour of seeds

Trait	Guideline	Some exceptions
Growth habit	Most herbaceous plants produce orthodox seeds	Aquatic species
Habitat	Many aquatic species, tropical rainforest species and temperate climax forest species produce recalcitrant seeds.	Most native Hawaiian species, temperate conifers, some maples
Water content at harvest	Most orthodox seeds naturally dry on the Parent plant.	All immature seeds, Solanaceae, Cucurbitaceae
Seed size	Recalcitrant seeds are often large.	Some aquatic species, Rutaceae, some Rubiaceae
Desiccation sensitivity	Orthodox seeds can survive complete water loss; recalcitrant seeds cannot.	Orthodox seeds dried very slowly (for > 2 weeks) can be severely damaged

Table 2: Recommendations for relative humidity (RH) and moisture content, together with approximate longevity of selected species

Species	Optimum RH	Optimum moisture content of seed (g H_2O / g dw)	Time to 50% loss in viability
Lettuce (*Lactuca sativa*)	20%	0.04–0.05	> 4 years at 5 °C > 20 years at -18 °C
Onion (*Allium cepa*)	20%	0.06–0.08	> 4 years at 5 °C > 20 years at -18 °C
Sunflower (*Helianthus annuus*)	20%	0.03–0.04	> 6 years at 5 °C > 25 years at -18 °C
Pea (*Pisum sativum*)	20%	0.09–0.12	> 10 years at 5 °C > 25 years at -18 °C
Tomato (*Lycopersicon esculentum*)	20%	0.05–0.06	> 12 years at 5 °C > 25 years at -18 °C

Store unused seeds following recommendations from the seed source. In general, orthodox seeds should be stored in dark, dry and low temperature environments, kept in a tight container to avoid moisture. When old seeds are used, a germination test must be performed to verify the germination rate; their viability depends on the type of crop. The rate of seed deterioration depends on the type of seed and on the storage conditions. High moisture content and high temperature will result in a very rapid decline in visibility. Therefore, the longer the seed storage period, the more important that the seed moisture content is low, and that the temperature is also low. The optimum storage humidity conditions and moisture content of seeds of some greenhouse-grown crop species are shown in Table 2.

Seed treatment

Seed treatments vary depending on the seed company. Seed priming can improve germination and emergence, resulting in better uniformity; indeed, seeds of some species are almost always primed, as germination is poor without. Pelletizing seeds produces better uniformity and improves handling in automated seeders.

Germination

- Keep germination facilities clean and free from algae and pests.
- Select conditions optimal for the crop species, thus improving uniformity and minimizing the time, reducing the overall costs for producing transplants.
- Some species require oscillating temperature: eggplant and their rootstocks *torvum* generally germinate faster under day-night oscillating temperature conditions.
- Monitor the media (not air) temperature during germination and control it in the optimum range; evaporation from wet media can reduce the temperature to a few degrees below the air temperature.

Transplants

For greenhouse crop production, it is recommended to use high-quality transplants with the following characteristics:

- Absence of infection from diseases or pests
- Ability to survive in unfavourable environments after transplanting
- Good morphology suitable for planting
- Well-developed root system (or higher root to shoot ratio)
- Absence of visual defects such as chlorosis (yellowing) or necrosis (dead tissue)
- The most important characteristic is disease-free and pest-free status (Doolan *et al*., 1999).

Organizational separation of transplant production from final crop production is a recent worldwide trend, especially for vegetable and floriculture/ornamental crops requiring special techniques, such as grafting or vegetative propagation, and specific facilities to produce desirable transplants. It is cheaper to buy such transplants than to produce them in-house, considering all the specialized facilities and skill-sets required. The decision needs to be made by each individual operation, considering all the relevant issues and cost analyses.

When commercial nurseries are not available, or purchasing transplants is not economically advantageous, growers choose to produce their own transplants using their own facilities. Environmental conditions and fertilizer requirements are often specific to transplant production. Transplants are often produced by a short cycle, and growth and development are subject to weather conditions. Good production planning is necessary to coordinate with the final crop production. Records should be kept, including seeding date, variety name, substrate name, tray type, chemicals applied etc.

It is important to avoid wetting foliage. Sub irrigation works better than overhead irrigation if the facility is available. If overhead irrigation is the only option, it is recommended to allow foliage to dry before the sun sets, as prolonged leaf wetness can lead to increased disease development (ASTA, 2009).

Inspections should be carried out to identify signs of diseases and pests. If plants exhibit signs of infection, they must be discarded or an appropriate control method applied.

Grafted seedlings

Grafting is widely applied in vegetable production. In some countries, the technology is relatively new and care must be taken to avoid failure or transmission of diseases during the grafting process.

- Keep grafting tools (razorblade, grafting tubes etc.) and working area clean with regular disinfestations. According to ASTA (2009), ethanol (70-75%) and other disinfectants are suitable for disinfecting cutting tools and hands, which should then be rinsed with clean water to avoid damage to the plant from residual disinfectant. Both disinfectants and rinsing water should be changed regularly.
- Choose the rootstock on the basis of the specific problems to be solved by grafting and the grafting compatibility between scion and rootstock. Seed companies have information on expected phenotypes including disease resistance, but it is recommended to test any new scion-rootstock combination on a small scale before starting propagation on a large scale.

Table 3: Guidelines for selecting grafting rootstocks

Type	Resistance	Other traits
Tomato		
Interspecific hybrid (hybrid between different tomato species, e.g. 'Maxifort', *Solanum lycopersicum* x *S. habrochaitaes*)	Different for different rootstock varieties but generally include *Fusarium*, *Verticillium* wilt, root knot nematodes. Some include bacterial wilt and higher race (race 3) of *Fusarium*.	Generally vigorous. Some rootstocks have chilling tolerance. However, less uniformity in plant growth at the seedling stage (germination and emergency).
Intraspecific hybrid (hybrid within the same cultivated tomato species, e.g. 'Aloha', *Solanum lycopersicum*)	Different for different rootstock varieties but generally include *Fusarium*, *Verticillium* wilt, root knot nematodes. Some include bacterial wilt and higher race (race 3) of *Fusarium*.	Very uniform growth. Less vigorous
Cucurbits		
Interspecific hybrid squash (hybrid between different squash species, e.g. 'Tetsukabuto', *Cucurbita maxima* x *C. moschata*)	*Fusarium*. Some also have vine decline, *Verticillium* wilt and anthracnose	Suitable for all cucurbits. Traits varied among different rootstock varieties (vigour, chilling heat or drought tolerance etc.).
Bottle gourd (*Lagenaria siceraria*)	*Fusarium*. Some also have resistance to vine decline, *Verticillium* wilt and Anthracnose.	For watermelon. Chilling tolerance.
Fig leaf gourd (*Cucurbita ficifolia*)	*Fusarium*	For cucumber. Chilling tolerance.

- Select the optimum grafting method on the basis of plant performance after grafting and success rate of grafting. Tube grafting is a standard procedure for tomato and eggplant, but there are several grafting methods used for cucurbits. When considering automated

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grafting, it is important to take into account the advantages (e.g. lower labour input) and challenges (high capital costs and limited flexibility in terms of size of plants or trays).

- Prepare scion and rootstocks to reach optimum graftable stage at the same time. Depending on the grafting method and species to graft, grafting must be done at the optimum growth stage of scion and rootstock seedlings. Use of overgrown or too young seedlings beyond optimal ranges reduces the grafting success rate. Good propagators must pay attention to seed germination timing and growing conditions to produce scion and rootstock seedlings at an optimal stage for grafting.
- Keep healing facilities clean and free from algae and pests. Healing conditions often include high humidity (nearly 100%) and warmth (28-29 °C) with lighting, conducive to the growth of algae and fungi and the rapid spread of disease.
- Choose between two-headed and single-headed grafted seedlings. For tomato, two-headed seedlings (pinched to induce two lateral shoots) are widely used to reduce the number of plants needed per cultivation area. However, an inappropriate combination of scion and rootstock may reduce the yield when they are two-headed. A small test to determine growth and yield capacity of two-headed plants must be conducted before using them on a large scale.

Purchasing transplants from commercial nurseries

- Use transplants from reliable nurseries. Long-distance transportation causes deterioration of transplant quality. Select nurseries not only for their propagation skills (product quality) but also for their proximity to the production site and the transportation methods used.
- Upon receipt of transplants, inspect carefully for signs of disease or pests. On finding signs of infection of notorious diseases and pests that could spread in the greenhouse (e.g. bacterial canker or TYLCV for tomato, bacterial fruit blotch for cucurbits), discard all the transplants and disinfect any trays and bench surfaces with which they have come into contact.
- Maintain records of any products used for controlling pests and diseases during the propagation period.
- For genetically modified organisms, follow the relevant national or international regulations.
- If possible, visit the nursery during the transplant production process, check the young plants and discuss the quality with the manager.

Production scheduling

In commercial propagation, production scheduling is critical to maximize profits.

- Schedule backwards, starting from the target shipping (delivery) window determined by customers or final crop production schedule. The time required to reach the growth stage suitable for transplanting is largely dependent on the crop species, climate conditions (solar radiation, day and night air temperature, and CO_2 concentration) and growing methods (substrate, fertilizer and tray types). Experience is required to forecast transplant finishing time.
- Understand the different facility requirements for the various stages of transplant production. For standard transplants, there are several stages, such as germination, transplanting, hardening and shipping. For grafted seedlings, there may also be sorting, grafting, healing and pinching. It is first necessary to establish how many trays (flats) one germination room can hold and how many workers are available for grafting in a given week.
- Understand two variables: crop time and production space. Scheduling production and analyzing facility use associated with transplant production can be a complicated process comprising multiple variables. To better schedule crops and turns, nursery propagators must develop their own computerized spreadsheets to better understand the facility use in any given week of the production period. This capacity is extremely important when propagation involves multiple species and different finishing timings.

Packing and transportation

- Select the packing and transportation method. Transplants are best transported when packed in trays inside cardboard boxes or on racks in trailers, but some growers prefer to receive “pull-and-pack” seedlings to reduce transportation costs. In either case, packing to accommodate rough handling is necessary, especially when a commercial freight service is employed.
- Avoid long distance transportation. Transplants should be transported over the shortest distance possible, to minimize costs as well as the damage associated with transportation. However, in some cases, such as grafted seedlings that are not widely available in some countries, transportation may be longer than the normal time for vegetable transplants. Normal transportation time is no longer than 10 hours.
- Select the timing of transportation to minimize environmental stress. Once scheduled, select exact timing to avoid the risk of exposing transplants to extreme heat or cold.

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During summer, overnight or early in the morning is preferable to midday to avoid heat stress, especially when plants are transported in a non-refrigerated truck. In contrast, midday transportation is more desirable when freezing temperatures are expected at night.

- Select the transportation route to minimize mechanical stress. Mechanical stress caused by vibration during transportation has a negative impact on the transplants. It can physically damage the transplants or promote ethylene production. Ethylene accumulation can induce adverse physiological impacts such as flower abortion or leaf yellowing, especially during long distance transportation.
- Use a refrigerated trailer at a controlled selected temperature for long distance transportation. Too high temperatures can produce adverse physiological effects such as flower abortion (Kubota and Kroggel, 2006).
- Assure ventilation to avoid ethylene accumulation during transportation. A commercial nursery truck (non-refrigerated) designed for transporting transplants. This type of truck has some ventilation and is suitable for relatively short distances (no more than several hours).
- Complete necessary importation paperwork for international shipping of transplants.
- Do not transport plants if there is any sign of disease or virus infection. Introduction of viruses such as TYLCV is often associated with transportation of plant materials. Accidental introduction of infected plants following inappropriate judgment by a careless propagator could cause a catastrophic outbreak affecting the entire production region.

Facilities and materials to grow plants

The facilities and climate conditions for transplants are different from those for final crop production. Young seedlings are generally more sensitive to abiotic and biotic environmental stresses and a growing facility must be carefully selected in order to achieve optimum growing conditions. There are several production stages, and each one has specific recommendations with regard to environmental conditions, fertilization and plant maintenance methods.

Production site selection

The transplant production facility should be located at a distance from the farming area, which is a potential source of insects and diseases that can easily reach the transplant production facility. The site should be levelled and well drained with ready access to an abundant supply of quality water. The greenhouse should be positioned sufficiently far from surrounding trees or

buildings so as to prevent shadows. Considerations concerning greenhouse location may be summarized as follows:

- Good drainage and water supply
- Sufficient distance from cultivation area
- Good proximity to shipping routes
- Easy access to utilities
- Local zoning for land use and tax laws
- Room for expansion and absence of shadows

Seedling trays

Seeds can be sown in a variety of ways (depending on their end use) in individual plant containers or plastic flats filled with various types of sterile growing media (substrates).

Choose trays or containers suitable for the production. Criteria include plant species, growing conditions (irrigation method), local availability and type of mechanical seeder used. There are various containers and trays:

- Individual containers may be more appropriate for foliage plants or mature seedlings (flowering stage) of vegetable species. They come in paper, plastic, clay, peat moss, Styrofoam etc.
- Individual plastic containers (called net pots or web pots) are used in floating or NFT hydroponic systems, filled with coarse substrates, such as perlite, clay pellets and rockwool.
- Moulded plastic or Styrofoam "plug" or cavity (multi-celled) trays are available in various sizes containing tens to hundreds of cavities, and can be filled with growing medium or cubes for the production of multiple seedlings in each tray.

Use steam or other disinfectants to sterilize reused trays. Plastic containers can be sterilized using 10 percent bleach, while Styrofoam containers and trays are steam-sterilized. In some countries in Europe, Styrofoam is recycled to be used for other purposes (Styer and Koranski, 1997). When disinfectant solution is used, soak the trays long enough to ensure efficacy; rinse containers thoroughly to avoid chemical toxicity; allow the trays to dry prior to use.

Choose tray type and size adaptable to the mechanical seeder, transplanter and other greenhouse propagation systems (benches and irrigation systems). Test candidate trays for plant performance as plant growth is affected by type of trays (cell size, volume, colour etc.).

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Limit the maximum reuse of seedling trays (or plug trays) to 2-3 times. Styer and Koranski (1997) suggest that the cost of labour for washing, disinfecting, stacking and storing used trays almost equals the cost of new trays. Reuse also increases the risk of disease introduction resulting from incomplete disinfection.

Substrate

- Select a substrate and understand its physical properties. There are various substrates available for horticultural use (e.g. sand, peat, moss, vermiculite, perlite, rockwool, rice hulls, coconut coir and compost). In general, a substrate needs to have good air porosity and water-holding capacity.
- Maintain the media pH in the optimum range (5.5-6.5 in general). Too high or too low pH can cause micronutrient deficiency or toxicity, respectively.
- Keep the initial level of EC (electrical conductivity) below 0.75 dS/cm at a 2:1 (v:v) dilution (Styer and Koranski, 1997). Some substrates have fertilizers mixed in (known as "starter charge") and the amount of starter charge needs to be taken into account in the fertilization schedule.
- Use substrate from a reliable source. Organic substrates (e.g. coconut coir) are often inconsistent in quality and vary depending on the source and origins.

Chemicals

- Ensure that any chemicals used during propagation do not violate the regulations of the country where the plants are to be grown for production
- Use products from reliable sources.
- Follow the application instructions on the product label; some chemicals require professional certificates for applicators (workers).
- Keep records of product name, dose, application method, operator name, date and time of application etc.

Fertilizers

- Use products from a reliable source; avoid low quality fertilizers as they may contain contaminants such as heavy metal.
- Keep good records when mixing fertilizers to make up stock nutrient solution. Record fertilizer product name, salt name, weighed amount, operator name, date and time etc.
- Check EC and pH of nutrient solution regularly; EC and pH meters need to be calibrated and maintained using methods recommended by manufacturers.

- Use appropriate nitrogen source based on plant performance, pH requirement and costs. For example, use of nitrogen in nitrate form at a higher ratio tends to keep the substrate more basic.
- Handle acid and base used for pH control cautiously with appropriate worker protection equipment (safety goggles and gloves). Store them in an appropriately designated acid cabinet.

Seeding machine and automation

Choose the seeding machine and other automation (tray filler etc.) on the basis of expected use and performance, as it could be a significant capital investment. Types of seed (size, coating and shape), trays (dimension, number of cells) and substrate need to be considered in order to select the best performing machine.

IPM facility

In order to reduce the risk of introduction into the greenhouse of insect pests or insect-transmitted viruses, it is recommended to:

- Use a double-door entrance;
- Cover the air intake (vents) with insect screens;
- Control weeds inside and outside.

Irrigation and fertigation

Irrigation

It is important to choose an appropriate irrigation method or system, taking account of the relative advantages and disadvantages. There are two basic irrigation systems for transplant production:

- Overhead irrigation systems are traditionally used for containerized transplants. However, they can contribute to pathogen attack when used in regions with high temperatures and humidity.
- Sub irrigation systems (flotation or ebb and flow) are designed to flood beds with nutrient solution. In sub irrigation, the water must contain disinfectants and algae growth protectants.

The advantages of sub irrigation include lower pesticide, water and fertilizer use in propagation, elimination of groundwater contamination, and reduced risk of foliar and soil-borne diseases (Thomas, 1993). Also, overhead watering does not guarantee uniform water flow throughout the medium and can induce drought stress in the roots, especially during hot seasons.

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Overhead irrigation enhances root growth, but it uses more fertilizer than sub irrigation (Nicola and Cantliffe, 1996).

To improve fertilizer and water-use efficiency, over-irrigation should be avoided. Transplant quality tends to be low (stem extension or tender tissue) when grown under conditions of over-irrigation. It is important to water transplants thoroughly until the entire substrate is moist, and then allow the substrate water content to reduce before the next watering. When overhead irrigation is used, late afternoon watering should be avoided as the plants remain wet overnight, increasing the likelihood of disease. A too-wet substrate also increases the incidence of damping-off disease.

Fertigation

Choose the fertilizer concentration, frequency and dose according to plant growth stage and climate conditions (solar radiation and temperature, which influence transpiration demand). For example, target concentrations for fertigation in the solution for tomato seedlings are 80-100 mg/litre total nitrogen (75-100% in nitrate form), 30-50 mg/litre phosphorus, 140-180 mg/litre potassium, 100-150 mg/litre calcium and 30-60 mg/litre magnesium, in addition to other micronutrients. The pH is normally adjusted to 5.5-6.5. Seedlings grown on high rates of nitrogen fertilization are succulent and less resistant to dry weather and solar radiation, leading to a low rate of plant survival after transplanting in the open field (Rosca, 2008). Transplant quality can be improved by applying higher concentrations of fertilizer less frequently (known as "pulse feeding"-Garton *et al*., 1994), resulting in thicker stem diameters. Limiting fertilizer is also used to harden transplants before shipping or transplanting. Carefully monitor the discharge (amount and EC) to minimize pollution. Direct discharge to the ground should be avoided as it contaminates the groundwater.

Growth control and hardening techniques

Hardening is a crucial step in transplant production. In general, transplants should have well-balanced shoot and root development. Young seedlings growing at high planting densities may have extended stems or excessively large shoot mass relative to the roots. Spindly tender plants are more vulnerable to mechanical damage during handling and transplanting. The quality of transplants affects stand establishment after transplanting to the final production greenhouse. Hardening preconditions transplants to tolerate transplanting stress by exposing them to, for example, water stress; the practice is usually applied to transplants to be used in open-field production or to be grown in environmental conditions harsher than those they were exposed to

during propagation. Excessive hardening should be avoided as it may exhaust the plant's energy reserves (Garton *et al*., 1994).

A typical hardening method involves restriction of the water supply and gradual exposure to conditions expected in the fields or greenhouse to which the plants are transplanted (light intensity, day-night temperature oscillation and relative humidity). This hardening process is performed over several days or for over a week, depending on the species and preferred nursery practice. Some vegetable seedlings (e.g. tomato) may also be hardened off with limited fertilization, as too much fertilization, especially nitrogen, tends to make seedlings soft. Some growers move the seedling trays to benches placed in open field in direct sunlight. However, it is recommended that transplants used for greenhouse production be hardened off inside the greenhouse to mitigate the risk of bringing in insect pests.

Day-night temperature difference (DIF)

Plant stem growth rates of some floricultural and vegetable species are positively correlated to the difference between day temperature (DT) and night temperature (NT), known as DIF (DIF=DT–NT) (Moe and Heins, 1990). A high DIF promotes stem elongation and the daily average temperature determines overall development rate (leaf emergence and flower initiation). Using DIF helps to keep the seedlings compact in size without using growth regulators. Keeping transplants cooler during the day than at night reduces plant height in the temperature range 10–30°C (Wien, 1997). High temperatures during the first 3-4 hours after sunrise can cause considerable elongation in vegetable seedlings (Bodnar and Garton, 1996). This excessive elongation can be mitigated by keeping the greenhouse temperature cooler (by 4-5°C) during morning hours than at night (Bodnar and Garton, 1996).

Irrigation deficit and water stress

When plants are subjected to mild water stress, the rate of stem elongation and leaf area expansion decreases, and carbohydrates accumulate in the leaves. Water stress therefore induces changes in plant growth that are helpful in preparing the plant for transplanting (Wien, 1997). However, as the plant transpiration rate is affected by environmental conditions, experience is required to determine irrigation timing without imposing too much water stress. A soil moisture sensor calibrated for the specific substrate offers an alternative approach.

Nutrition deficit

The growth rate of transplants can be regulated by controlling the concentration of nitrogen and other nutrients in the substrate. Reducing nutrient supply just before transplanting can slow down the growth rate during the hardening stage. As long as the transplants are not

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completely starved of the major nutrients by this procedure, there should be little problem with the resumption of growth after transplanting (Wien, 1997).

Shaking and brushing

Mechanical stress affects seedling growth, because it can enhance ethylene production. Brushing the tops of the transplants several times a day can have remarkable dwarfing effects (i.e. shortening stem and petioles; increasing chlorophyll content) (Wien, 1997). The effects of these mechanical perturbations vary among species and cultivars. Brushing has proved successful in solanaceous crops (including tomato, pepper and eggplant), but care should be taken with cucurbits, which are more fragile and may become damaged (Schrader, 2000).

With tomato, keeping transplant height short results in a reduction of stem elongation rate, especially important during winter, when light conditions are suboptimal (Fontana *et al*., 2003). Chemical growth regulators may not be used to control vegetable transplant height (it is necessary to check the registration status with the local authority). Therefore, mechanical conditioning is one way of controlling stem elongation in commercial greenhouse production.

Transplant age

When producing vegetable transplants, seedlings should be transplanted at the optimum age. Generally, as the age of the transplant increases, leaf number, height, leaf area and dry shoot weight of vegetable seedlings increase linearly, regardless of transplant cell volume. Avoid any delay in transplanting. Almost all vegetables may be transplanted as early seedlings with little effect on growth, but as they increase in age, this situation changes (Vavrina, 1998). Age strongly influences subsequent performance in the greenhouse. Although planting the largest seedlings possible might appear advantageous in terms of getting the crop off to a quick start, larger seedlings are also more prone to transplanting shock. In general, relatively young vegetable transplants provided with adequate growing space in the greenhouse produce the best stand and fastest crop development. The added stress associated with transplanting larger-than-optimal plants appears to substantially delay crop development.

Determine best growing practices for achieving optimum age of transplants. The optimum age depends on the crop, cell size to be used and conditions during the grow-out period. For example, 2-week-old transplants grown in the small cell volume may be the best option for muskmelon growers if their sole concern is total-season yields. However, if growers want to maximize early-season yields, then 2-week-old transplants grown in the large cell volume are the best choice (Walters *et al.*, 2005). Growers must adjust their growing practices and schedules for different crop species and cell sizes.

There is no single definition of the best seedling age or the most appropriate phenological stage of transplant age. In general, northern countries use older and further developed seedlings, as follows (OMAF, 2007):

- Tomatoes: first flowers showing
- Cucumbers: 4–5 true leaves visible
- Peppers: flower at first branching level opening

Nutrient deficiency and toxicity

- Optimize fertilization programme based on the plant's needs. Different species require different fertilization. Some commercial substrates for transplant production contain a starter charge of fertilizers, in which case no fertilization is required for the first few days. For nitrogen, many plant species perform better when both ammonium nitrogen and nitrate nitrogen are used. However, an excessively high rate of ammonium nitrogen may cause toxicity to the plants; furthermore, the form of nitrogen also affects the Ph in the substrate (nitrate makes it more basic and ammonium more acidic).
- Maintain adequate pH in the substrate to avoid nutrient deficiency and toxicity. Generally, a pH of 5.5–6.5 is considered optimum for many plant species: a too high pH can lead to iron deficiency inducing pale green newly emerged leaves; too low can cause micronutrient toxicity (e.g. boron). Some substrates (e.g. peat moss) are acidic and others (e.g. vermiculite) basic. The amount of bicarbonate ions determines the water alkalinity and influences the buffer capacity of the nutrient solution in the substrate.
- Use fertilizers from a reliable source to avoid contamination (heavy metals etc.).
- Analyze the water quality and design the fertilization programme accordingly. Water quality may change over time (seasons or years), and periodical analysis in a reliable laboratory is therefore recommended. Excessive amounts of sodium, soluble salts or bicarbonates can become problematic and growers may want to consider another water source or different water treatment.

Pests and diseases

Good agricultural practices relevant to plant propagation are described below.

- Inspect planting materials regularly. Early detection is critical to control biological problems and minimize damage. Propagation is usually conducted in short cycles, but because of the high density, pests and diseases spread very rapidly. Once any symptom is found, minimize access to the affected area and notify workers of the outbreak as soon as possible.

- Be familiar with the symptoms of commonly occurring pests and diseases to identify problems at an early stage and minimize plant loss.
- Apply appropriate control methods (chemical or biological) in consultation with a local extension agent or advisor.
- Do not apply foliar fungicides in high temperatures as foliage may get injured.

Disorders caused by growing environments

Pay attention to light contamination from neighbouring greenhouses and buildings at night. Street lights and worker's lights sometimes influence plant morphology and flowering. Be familiar with toxicity symptoms of air contaminants. Incomplete combustion of gases causes air pollution that can harm humans as well as plants. The concentrations that negatively affect plants vary according to whether exposure is short term or long term. Young transplants are especially tender and sensitive to by-products of incomplete combustion (Bodnar and Garton, 1996), and tomato is particularly sensitive to ethylene exposure. Problems are often associated with the first use of heating systems in the winter and they disappear as heating demand becomes less.

Traceability

Record key information for each lot of planting materials (seeds and transplants) including material information (source, type), dates, facility used, environmental data and workers' names. Consider the introduction of a tracing technology (e.g. barcodes or RFID – radio frequency identification), to identify each lot or tray of transplants (especially if a large number are grown under various schedules in the same facility) and to record the relevant production-related information. The successful introduction and use of such a system significantly reduces errors in boxing and shipping.

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TOBACCO ADDICTION: A HUMAN MADE EPIDEMIC

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Abstract:

Smoking and use of tobacco is a global epidemic causing death of more people than HIV/AIDS, malaria, TB combined. According to WHO; use of tobacco is single largest cause of disease and premature death, claiming one life every 8 seconds and killing 1 of 10 adults globally which is totally preventable. In India use of tobacco grows 2-3% per annum and account for 13% of all deaths. In this narrative article, I explored historical background of tobacco, its burden, various forms in which the tobacco is consumed, constituents in tobacco and effect of same on human body and various treatment options and measures to cease tobacco addiction. As MARK TWAIN quotes "Giving up smoking is the easiest thing in world. I know because I have done it thousands of time. Though tobacco quitting is hard; dentist are in a unique position to advise the tobacco users to quit the habit through various methods and effective counselling. Smokers who receive assistance-behavioural, pharmacologic or both can experience quit rates around 20% at least 6 months after quitting (CIHI, 2020).

Keywords: Tobacco, Smoking, burden of tobacco, pharmacological and non pharmacological treatment, contituents of tobacco

Introduction:

India is the fourth largest consumer of tobacco and third largest producer .WHO estimates that there are about 1100 million regular smokers in world today. 300 million in developed whereas 800 million in developing countries! (WHO, 2019). This global consumption of cigarettes has been rising since 20th century as people consider smoking to be a part of glamour. Nearly cigarettes are produced at the rate of five and half trillion a year which makes tobacco smoking a global epidemic. According to recent survey ASIA, AUSTRALIA and FAR

EAST are largest consumers (2715 cigarettes), followed by AMERICANS (754 billion) and WESTERN EUROPE (606 billion).

Historical background of tobacco:

Christopher Columbus reported a gift of strange leaves from Salvador; he noticed that these leaves were used in ceremony as well as in medicinal purpose, also he spotted the same leaves in India , these leaves were consumed by Indians in powdered form.in India tobacco was introduced in late 16^{th} and early 17^{th} century by Portuguese traders. In 1776 British East India Company began to grow tobacco as a cash crop in India, which later flourished and tobacco is still grown as an important crop in India (U.S. Department of Health & Human Services, 2019).

Burden of tobacco in India:

According to National household survey of drug and alcohol abuse conducted in 2002, 55.8% of males aged 12-60 years. According to National epidemiological oral health survey and fluoride mapping 23-24% smoke tobacco amongst which 45% smoke bidi; 42-46% chew paan masala .amongst smoked tobacco consumers 34% consume bidi whereas 31% consume cigarette

Tobacco preparations:

Tobacco is derived from species of plant of genus Nicotiana of potato family. Carl Linnaeus in 1753 had named the genus of tobacco plant NICOTIANA after the French ambassador Jean Nicot.

Tobacco is cured by various ways:

1. Piped warm air
2. Directly over an open slow burning fire
3. Sun curing

Now while consuming it is chewed, smoked, sniffed or chewed

Forms of tobacco used in India:

In India tobacco is either consumed in smoked or smokeless form

Smoked tobacco includes:

Bidi, chillum, chutta, cigarettes, dhumti, hookah, hookli

One of the most deleterious habits practised in Andhra Pradesh is reverse smoking; in which tobacco is smoked with lighted end inside the mouth. While practising reverse smoking as the lighted end of cigarette is inside mouth hence the temperature of palatal mucosa raises upto 58 degree celcius. Reverse smoking produces palatal patches reported to exhibit a malignant change of 12 per 1000 (U.S. Department of Health & Human Services, 2019).

Smokeless tobacco includes:

Khaini, Manipuri tobacco, mawa, mishri, paan, snuff, zarda, gutka, pan masala, gudakhu

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Constituents in tobacco:

Tobacco smoke is estimated to contain over 4000 compounds many of which are carcinogenic and mutagenic .Increased amount of carcinogens have been shown in snuff and chewing tobacco. It is partly derived from bacterial or enzyme action on nicotine during curing.

1. Nicotine:

Most toxic and acts with great speed. Average lethal dose of mere 30-60 milligrams can cause addiction. It triggers dopamine which is a chemical in brain associated with feeling of pleasure. It increases heart rate and blood pressure by constriction of cutaneous blood vessels and it also does effect on muscular, hormonal and metabolic effect. It also increases the platelet stickiness and aggregation which damages the lining of blood vessel .it is also a causative agent of coronary diseases and is a potent carcinogen.

2. Tar:

It is the particulate matter inhaled when smoker draws on lighted cigarette. Each particle is composed of nitrogen, oxygen, hydrogen, carbon dioxide and carbon monoxide and many volatile and semivolatile substances. In condensed form tar is sticky brown which not only stains fingers of smoker; but also lungs! Moreover it has some carcinogens or tumour initiators like benzopyrene.

3. Carbon monoxide:

Carbon dioxide in large amount may be fatal; as it has affinity for haemoglobin 200 times greater than oxygen; therefore it binds preferentially to blood reducing amount of oxygenated blood which may be reduced to about 15%. Other consequences caused by CO are –development of coronary diseases, interference with myocardial oxygenation, increase platelet stickiness promoting atherosclerosis, restricts the oxygen available to foetus.

4. Nitrogen oxide:

Cause lung damage as well as initiate emphysema.

5. Hydrogen cyanide and other ciliatoxic agent:

Interferes with mechanism of cilia and hence hampers the clearance mechanism in humans. It results in accumulation of toxic agents in lungs increasing the likelihood of developing disease.

6. Metals:

Thirty metals have been detected in tobacco smoke! Amongst which metals such as arsenic and cadmium are potent carcinogens.

7. Radioactive comounds:

Radioactive compounds present in high amounts are polonium -210, potassium -40, radium-228 which are established as carcinogen.

Health consequences of tobacco:

Tobacco is major contributor to oral diseases.

- It slows wound healing and promotes periodontal diseases, halitosis and oral infections.
- Smoking tobacco contributes to cancer of oral cavity and tongue, larynx, pharynx, oesophagus, stomach, uterine, cervix and lungs.
- There are some evidences which prove that smokeless tobacco also may contribute as causative agent of oral cancer.
- Smoking tobacco is a known cause for cardiovascular diseases, chronic obstructive lung disease, emphysema and chronic bronchitis.
- Exposure of non-smokers to second hand smoke is important cause of respiratory infections, worsening of asthma and poor lung function and amongst all the second hand smokers; most of them are women and children.
- According to newer study smoking is major risk factor for tuberculosis in India. Tuberculosis is 3 times more common among ever smokers than among never smokers and even mortality is 34 times greater amongst smokers.
- Pregnant women exposed to passive smoke may deliver low weight babies.
- According to a survey pregnant mothers who smoke give birth to a baby with cleft lip and palate and this is due to inability to differentiate and migration of neural crest cells.
- One major concern for people smoking tobacco is; tobacco along with alcohol account for 75% of disease burden of oral and oropharyngeal squamous cell carcinoma! This is because tobacco contains potent carcinogens including nitrosamines, polycyclic aromatic hydrocarbons, nitrosodi cthanolamine, nitrosoprolone and polonium.

Pharmacological and non-pharmacological approach for tobacco cessation:

Dentists are in a unique position to advice the tobacco users to quit habit through various methods and with effective counselling. The present review provides an outline of different pharmacological and non-pharmacological interventions for cessation of smoking. Smokers who receive treatment experience a quit rate of around 20%. Therapy is to be decided on nature of tobacco dependence. According to clinical practice guideline for treating tobacco use and dependence all smokers trying to quit the tobacco use , must be encouraged to use one or more effective pharmacological or non-pharmacological agents or in some circumstances where only

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pharmacological treatment fails to cease addiction; the patient is to be shifted on pharmacological treatment together with the non-pharmacological one (WHO, 2019).

Pharmacological treatment:

Following are the pharmacological agent used for tobacco cessation.

These are divided as first line agents, second line agents, combination therapy, herbal therapies and certain emerging trends.

First line drugs:

First line drugs include: NICOTINE REPLACEMENT THERAPY (NRT) NRT acts on nicotine receptors in ventral tegmental area of brain due to which dopamine is released into nucleus accumbens. First it reduces the physical withdrawal symptoms associated with nicotine abstinence among dependent smokers and secondly while the NRT takes care of nicotine dependence the smoker can focus on psychological aspects of quitting.

So various products are available in market which works as nicotine replacement; amongst which some are:

- Nicotine gum which include NICORETTE
- Nicotine lozenge which include COMMIT
- Nicotine transdermal patch including NICOTROL PATCH and NICODERM CQ
- Nicotine nasal spray which include NICOTROL NS
- Nicotine oral inhaler including NICOTROL CARTRIDGE

Second line drugs:

Second line drugs are only and only used in patients where patients are unable to use first line as these are not approved by FDA and are more prone to adverse effects. Some of the second line drugs are – nortriptyline and clonidine.

Nortriptylin is tricyclic antidepressant; whereas clonidine is centrally acting adrenergic agonist that reduces sympathetic outflow from central nervous system.

Combination therapy:

It uses long acting formulation that is patch in combination with short acting formulation. The long-acting formulation prevents onset of withdrawal symptom. Short acting drugs are helpful to control withdrawal symptoms that occurred during potential relapse condition. So the two combinations that are mostly used are NRT and bupropion or NRT and nortryptylin.

Emerging trends:

New compounds that have shown some promising results are Rimonabant and Varenicline.

Rimonabant antagonizes cannabinoid receptors in central nervous system. It improves abstinence among smokers.

Varenicline is acetylcholine receptor partial agonist.

Non pharmacological therapies:

The first smoking guideline was developed in 1996 and was updated in 2000 by USPHS. They introduced 5 H's and 5A's.

The 5 A's are

- Ask about tobacco use- identify and document tobacco use status for every patient at every visit
- Advise to quit- in a clear, strong and personalized manner urge every tobacco user to quit
- Assess willingness to make cessation attempt- is the tobacco user willing to make cessation attempt at this time?
- Assist in cessation attempt- for the patient willing to make cessation attempt, use counselling and pharmacotherapy to help him or her quit
- Arrange follow-up- schedule follow-up contact, preferably within the first week after cessation date

The 5R's are

- Relevance- Identify motivational factor that are relevant for patient such as risk of heart diseases, cancer, second hand smoke.
- Risk- Ask the patient about negative health effect of smoking
- Rewards- Ask the patient about potential benefits of smoking cessation
- Road blocks- Ask the patient to identify that will make a quiet attempt difficult
- Repetition- Repeat motivational interventional with each patient encounter

The 5 A's consist of steps or questions asked to screen and test the smoker but this approach may not be useful to patient who is not yet motivated to quit; hence this 5"s approach was introduced. It needs equal contribution of the practitioner and the patient to make this treatment successful

Conclusion:

According to many randomized trials and various studies; most efficient method for a smoker to successfully quit the habit is combination of pharmacotherapy and non-pharmacological interventions. Besides these; other measures such as increasing taxes on tobacco product, implementation of strict laws by government, awareness campaigns and workshops are very important for millions of individual to quit the habit

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ETHNOMEDICINAL PLANTS USED IN TREATMENT OF SKIN DISEASES IN DAKSHINA KANNADA DISTRICT, KARNATAKA, INDIA

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Abstract:

Skin diseases come in a variety of forms and frequently affect individuals of all ages. The skin acts as a physical barrier, preventing foreign pathogens from invading. However, certain microorganisms that live in the superficial skin layers of humans are referred to as the skin Microbiota or Dermatophytes. These organisms include bacteria, viruses, archaea, and fungi. These Dermatophytes are restricted to hair, nails, and superficial skin because they require keratin for growth (Keratinophilic microflora). These dermatophytes grow rapidly and become infectious under certain conditions, but not under others. Summer in Dakshina Kannada district is notorious for skin infections due to the high temperatures (nearly 35-40^{0}C), which result in increased sweating. Sweat conditions are conducive to the development of a variety of skin diseases, including rashes, boils, ringworm, nail infection, herpes, yeast infection, and skin itching. The majority of skin diseases in the district have been effectively treated by traditional healers through the use of ethno-medicinal plants. In this chapter, we discuss (we have discussed) various ethno-medicinal plants that are used by traditional healers in Dakshina Kannada to treat various types of skin diseases.

Keywords: Microbiota, Dermatophytes, Traditional healer, Ethno-medicinal plants etc.

Introduction:

Skin disease is a common ailment and it affects all ages from the neonate to the elderly and causes different types of infection in different ways. Human skin is the outer covering layer and the largest organ in the body. It plays a vital role in defence mechanisms against infection. Skin is divided into three main layers, viz. epidermis, dermis and hypodermis and also made up of many specialised cells. Each layer provides a distinct role in the overall function of the skin. The Epidermis is the external layer of the skin and varies in thickness in different parts of the body (Marks and Miller, 2006).

The Epidermis is home to millions of bacteria, fungi and viruses that compose the skin microbiota. As the largest organ of the human body, the skin is colonized by beneficial microorganisms and serves as a physical barrier to prevent the invasion of pathogens. When the balance between commensals and pathogens is disturbed, skin disease can result. The microbial composition of healthy adults was found to be different and primarily dependent on the physical and chemical nature of the skin (Byrd *et al.,* 2018).

Temperature and humidity are higher in partially occluded skin regions such as the groin, axillary vault, and toe web. This skin part favours the growth of moist microorganisms such as *Bacilli spp,Coryneforms spp* and *S. aureus*. The skin of the face, chest and back with a high density of sebaceous glands and in which lipophilic microorganisms such as *Propionibacterium spp*. and *Malassezia spp* thrive well. Other areas of skin, such as the arms and legs, are relatively dry and have a wide range of surface microclimate variations. Such areas were found to have fewer microorganisms than moist areas of the skin surface (Roth and James, 1988).

In the body, especially the umbilicus or navel part, is rarely exposed to UV light, soaps or bodily secretions and with high humidity and temperatures, so it has an almost undisturbed community of moist microbiome which largely comprises bacteria such as *Corynebacterium spp* and *Staphylococcus spp* (Hulcr Jiri *et al.,* 2012).

The variability of skin microbial flora depends on different factors such as age, location and sex, which are specific to the host (Somerville, 1969). Microbial colonizing in the skin microenvironment predominantly depends on age. Foetal skin is sterile but microbial flora colonization occurs immediately after birth. It may be vaginal delivery or caesarean section. Sebum production increases after puberty, promoting the growth of lipophilic bacteria on the skin.Ultimately, the composition of microbial flora changes according to the differences in physiological and anatomical variations in male and female skin microenvironments such as sweat, sebum and hormone production (Marples, 1982; Giacomoni *et al.,* 2009).

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In 100 young adults, 14 different genera of fungi were found between their toes. These microbial floras included yeasts, dermatophyte fungi and nondermatophyte fungi. *Candida albicans, Rhodotorula rubra, Torulopsis sp*. and *Trichosporon cutaneum* are all yeasts.The Dermatophytes (skin living fungi) include *Microsporum gypseum,* and *Trichophyton rubrum.* Nondermatophyte fungi (opportunistic fungi that can live on skin) include *Rhizopus stolonifer*, *Trichosporon cutaneum, Fusarium sp, Scopulariopsis brevicaulis***,** *Curvularia sp, Alternaria alternata*, *Paecilomyces sp, Aspergillus flavus* and *Penicillium species* (Oyeka and Ugwu, 2002).

Types of Skin Diseases:

Skin disorders vary greatly in symptoms and severity, such as temporary or permanent, painless or painful, situational causes or may be genetic, minor or life-threatening. More than a thousand conditions develop different types of skin diseases and these skin diseases are categorized into the following types.

Rashes:

It is changes in the colour and texture of skin that usually causes an outbreak of red patches or bumps on the skin. Potential causes of rashes are allergies, diseases, reactions, and medications. They can also be caused by bacterial, fungal, viral, or parasitic infections. Examples of rashes include acne, dermatitis, eczema, hives, pityriasisrosea and psoriasis (Thomson *et al.,* 2009).

Bacterial Infections:

Some skin infections are caused by a variety of bacteria such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Mycobacterium leprae*, *Streptococcus pyogenes* and *Corynebacterium minutissimum. Staphylococci sp* and *Streptococci sp*are the most common types among bacteria. Bacterial infections include cellulitis, impetigo, boils, and leprosy (Sukumaran and Senanayake, 2016).

Fungal Infections:

Several fungi are always present on the surface of the skin which is harmless, such as *Candida albicans, Trichophyton rubrum* and *Tinea corporis*. Sometimes such organisms under certain conditions develop superficial skin infection. Examples are athlete's foot, yeast infection, ringworm, nail fungus, oral thrush, diaper rash are the common fungal skin infections (Vandeputte *et al.,* 2012).

Viral Infections:

Viral skin infections occur when a virus penetrates and infects the different layers of skin. Some viral infections are shingles (herpes zoster), Chickenpox, Molluscum contagiosum, warts, measles, hand, foot, and mouth disease. Viruses are categorized into three groups, such as poxvirus, human papillomavirus, and herpes virus. (Charles Patrick Davis, 2020).

Study Area:

Dakshina Kannada district (South Canara) is a district of Karnataka state; it is bordered by Udupi district to the north, Chikamagaluru district to the northeast, Hassan district to the east, Kodagu to the southeast and Kasaragod district of Kerala to the south, i.e. 12.8438° N, 75.2479° E (Figure-1). The District with its headquarters in the port city of Mangalore. It covers an area nestled in between the lush green Western Ghats to its east and the Arabian sea to its west. It comprises seven taluks such as Mangalore, Putturu, Sillia, Bantwala, Belthangady, Kadaba and Moodbidri.

The people living in Dakshina Kannada were known as' Tuluvas' because they spoke the Tulu language. The different communities living together in Tulunadu such as Billava, Mogaveera, Bunt, Kulala, Tulu gowda and Devadiga are the largest ethnic group in the District (Acharya, 2021).

Map of Study area

Methodology:

Primary data was obtained from the key informants, such as patients who attending the healers and discussion with the elderly local village people. The information was collected from

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the informants (Healer) through semi-structured open-ended interviews with questionnaire (Martin, 1995).

The objective of the study was explained to the healers. Information was collected in the local Kannada language and then translated to English. The plants used to cure diseases, vernacular names, parts of the plants used, form of use, causes diet, other ingredients added during drug formulations, symptoms of the ailments they treat, average number of patients, and then their experience in the field of treatment were all recorded. During the survey, depending on the convenience of the practitioner, the guided field walk method was followed (Martin, 1995; Maundu, 1995). A walk through the forest with the healers allowed for both confirmation of the medicinal plants they use for the treatment and the detailed information gathering. Each informant was interviewed more than twice and only those formulations having consistency were considered.

Plants collected by the herbal healers during the guided field walk were authentically identified with the help of standard floras such as Flora of the Presidency of Madras (Gamble, 1984), Flora of Karnataka (Saldahna, 1984), Flora of Udupi (Gopalkrishna Bhat, 2003) and Flora of South Canara (Gopalkrishna Bhat, 2014). The recent names of the plants (APG system of classification) have been given according to the The Plant List (2010). All medicinal plants recorded for the treatment of different diseases were photographed in the field; voucher specimens were made and deposited in the Herbarium of the Department of Applied Botany at Mangalore University.

Result:

For this work 50 traditional healers of Dakshinakannada (Mangaluru, Bantwala, Belthangady, Putturu and Sullia) were interviewed and information was documented. Most of the traditional healer is belonging to different community of Hindu religion such as Nalike, Gowda, Billava, Vishwakarma, Brahmin, Bhants, Gauda Saraswat Brahmins (Konkani), Balyaya, Bhandaris (Kshourikas), Kudubi, Rajapur Saraswat Brahmins (RSB) etc. and 2 from Muslim community. No one was got from Christian's community. Most of the traditional healer given medicine for different types of skin diseases such as Herpes, Skin allergy, Itching, Ringworm, Boils, Red mumps, Nail infection, Skin ulcer, Chicken pox and Bumps by using different ethno-medicinal plants. The following plant species were documented for their use in traditional health care system against various skin diseases. The enumeration of plants contains botanical name, family name, local name, part used and uses.

Table 1: list of ethnomedicinal plants for skin diseases

SL.NO	PLANT NAME	FAMILY	LOCAL NAME	PART USED	USES
1.	*Acacia catechu* (L.f.) Willd.	Leguminosae	Khadira	Bark	Herpes, Skin diseases.
2.	*Alstonia scholaris* (L.) R. Br.	Apocynaceae	Halemara	Bark	Skin diseases
3.	*Andrographis paniculata* (Burm.f.) Nees	Acanthaceae	Kiratakaddi / Nelabevu	Leaf	Skin diseases
4.	*Aristolochia indica* L.	Aristolochiaceae	Eshwariberu	Root	Herpes, Chicken pox, Itching
5.	*Azadirachta indica* A.Juss.	Meliaceae	Kahibevu	Leaf and Bark	Chicken pox, itching, Skin ulcer, Skin disease
6.	*Breynia vitis-idaea* (Burm.f.) C.E.C.Fisch	Phyllanthaceae	Pallisoppu	Leaf	Chicken pox, Red mumps, Herpes, Skin allergy, Boils
7.	*Calophyllum inophyllum* L	Calophyllaceae	Honnemara	Fruit	Bumps
8.	*Careya arborea* Roxb.	Lecythidaceae	Daddala	Bark	Herpes
9.	*Cassia fistula* L	Leguminosae	Kakkemara / Kondemara	Bark and Root	Skin disease, Ring worm, Itching, Herpes
10.	*Catunaregam spinosa* (Thunb.) Tirveng.	Rubiaceae	Karekayi	Root	Herpes
11.	*Centella asiatica* (L.) Urb.	Apiaceae	Onelaga / Timare	Leaf	Herpes.Itching
12.	*Chassalia curviflora* (Wall.) Thwaites	Rubiaceae	Nirvisha / Kadupatalagaruda	Root	Herpes
13.	*Citrus limon* (L.) Osbeck	Rutaceae	Nimbe	Fruit Juice	Itching, Cheek swelling, Herpes, Boils,

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14.	*Clerodendrum infortunatum* L.	Lamiaceae	Ittehoovu	Leaf	Nail infection
15.	*Cocos nucifera* L. (red)	Arecaceae	Kendali	Mesocarp Juice and Baby nut	Herpes
16.	*Curcuma longa* L.	Zingiberaceae	Harashina	Rhizome	Itching, Chicken pox, Herpes, Skin ulcer
17.	*Cyclea peltata* (Lam.) Hook.f. & Thomson	Menispermaceae	HaadeBalli	Root	Skin diseases
18.	*Cynodon dactylon* (L.) Pers.	Poaceae	Garike	Aerial part	Herpes
19.	*Dregea volubilis* (L.f.) Benth. exHook.f.	Apocynaceae	PettaTajank	Leaf and Root	Cheek swelling, Skin diseases
20.	*Elephantopus scaber* L.	Compositae	NelaMucchiru	Leaf	Nail infection,
21.	*Getonia floribunda* Roxb.	Combretaceae	Enjiruballi	Leaf	Herpes
22.	*Hemidesmus indicus* (L.) R. Br. ex Schult.	Apocynaceae	Nannari / Namadaberu	Root	Skin allergy, Bumps
23.	*Holoptelea integrifolia* Planch	Ulmaceae	Rahubeeja	Leaf and Bark	Herpes, Skin diseses
24.	*Indigofera tinctoria* L.	Leguminosae	Neelisoppu	Leaf	Herpes, Nail infection, Itching
25.	*Ixora coccinea* L.	Rubiaceae	Kepula,Kiskara	Root	Itching, Herpes
26.	*Jasminum malabaricum* Wight	Oleaceae	Kadumallige / Edrolisoppu	Leaf	Herpes, Itching
27.	*Jasminum grandiflorum* L.	Oleaceae	Jajimallige	Leaf	Herpes
28.	*Justicia adhatoda* L.	Acanthaceae	Adusoge	Root and Leaf	Bumps, skin allergy

29.	*Macaranga peltata* (Roxb.) Müll.Arg.	Euphorbiaceae	Uppaligemara	Resin	Bumps
30.	*Manilkara kauki* (L.) Dubard	Sapotaceae	Renjemara	Bark	Skin disease
31.	*Memecylon malabaricum* Cogn.	Melastomataceae	Ollekodi	Tender leaf	Herpes
32.	*Mussaenda laxa* (Hook.f.) Hutch. ex Gamble	Rubiaceae	Taalisoppu / Bellote	Leaf	Herpes
33.	*Ocimum tenuiflorum* L.	Lamiaceae	Tulasi	Root and Leaf	Herpes, Red mumps
34.	*Olea dioica* Roxb.	Oleaceae	Bilisaroli	Leaf	Herpes
35.	*Phyllanthus emblica* L.	Phyllanthaceae	Nellikayi	Fruit	Skin allergy
36.	*Plectranthus amboinicus* (Lour.) Spreng.	Lamiaceae	Sambrani / Doddapatre	Leaf	Herpes, Boils, Skin allergy
37.	*Polycarpaea corymbosa* (L.) Lam.	Caryophyllaceae	Pare hoovu / Paadehoovu	Aerial part	Herpes
38.	*Pongamia pinnata* (L.) Pierre	Leguminosae	Hongemara	Bark	Skin diseases
39.	*Pterocarpus marsupium* Roxb.	Leguminosae	Bengemara	Bark	Herpes
40.	*Pterocarpus santalinus* L.f.	Leguminosae	Kempuganda / Raktachandana	Stem core	Herpes
41.	*Rauvolfia serpentina* (L.) Benth. ex Kurz	Apocynaceae	Sarpaganda / Patalagaruda	Root	Herpes, Red mumps, Chicken pox, Itching
42.	*Rubia cordifolia* L.	Rubiaceae	Manjishna	Stem	Skin diseases
43.	*Ruta graveolens* L.	Rutaceae	Nagadali	Leaf and Fruit	Red mumps, Herpes
44.	*Salacia reticulata* Wight	Celastraceae	Ekanaykanaberu	Root	Red mumps, Herpes, Skin ulcer

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45.	*Santalum album* L.	Santalaceae	Shree ganda	Stem core	Herpes,
46.	*Scleropyrum pentandrum* (Dennst.) Mabb.	Santalaceae	Naykuli	Leaf	Herpes
47.	*Senna alata* (L.) Roxb.	Leguminosae	AneTajank	Leaf	Itching, Ring worm, Skin disease,
48.	*Strychnos nux-vomica* L.	Loganiaceae	Kasarakka / kayira	Bark yellow powder, Root and Seed	Herpes, chickenpox, Skin ulcer
49.	*Tabernaemontana alternifolia* L.	Apocynaceae	Kokkekayi / Kodasige	Bark and Seed	Herpes, Skin diseases
50.	*Tabernaemontana divaricata* (L.) R.Br. exRoem. &Schult.	Apocynaceae	Nandi Battalu	Leaf	Boils, Red mumps, Skin allergy, Nail infection
51.	*Tectona grandis* L.f.	Lamiaceae	Tega / Saguvani	Tender leaf	Herpes
52.	*Terminalia bellirica* (Gaertn.) Roxb.	Combretaceae	Shanti / Taari	Fruit	Skin allergy
53.	*Terminalia chebula* Retz.	Combretaceae	Alalekayi	Fruit	Skin allergy, Herpes
54.	*Tribulus terrestris* L.	Zygophyllaceae	NeggilaMullu	Fruit	Herpes
55.	*Uvaria narum* A.DC.	Annonaceae	Kari Maadari	Root,	Herpes
56.	*Ventilago maderaspatana* Gaertn.	Rhamnaceae	Kempuberu /	Root,	Herpes
57.	*Vitex negundo* L.	Lamiaceae	Nekkigida / Lakkigida	Leaf and Root	Herpes, Nail infection

Conclusion:

A total of 50 traditional healers were interviewed using a questionnaire and semi-structured open-ended interviews. 96 percent (48 people) of the 50 traditional healers are Hindus from a variety of communities, including Nalike, Gowda, Billava, Vishwakarma, Brahmin, Bhants, Gauda Saraswat Brahmins (Konkani), Balyaya, Bhandaris (Kshourikas), Kudubi, and RajapurSaraswat Brahmins (RSB). Only 4% (2 people) are Muslim, while there are no Christians. This finding indicated that the traditional healers are members of the region's ethnic groups. Typically, they live outside of towns, closer to the forest region.

While documenting ethnobotanical information, 57 distinct ethnomedicinal plants were identified as being used to treat a variety of skin diseases, including herpes, skin allergies, itching, ringworm, boils, red mumps, nail infection, skin ulcer, chicken pox, and bumps. Among 57 ethno-medicinal plants, certain plants were repeatedly used by various traditional healers from various regions, and their percentage of use was higher, indicating that their curative capacity is significantly greater than that of other plants. Such plants are *Aristolochia indica, Azadirachta indica, Breynia vitis-idea, Cassia fistula, Chassalia curviflora, Clerodendrum infortunatum, Indigofera tinctoria, Jasminum malabaricum, Ixora coccinea, Memecylon malabaricum, Mussaenda laxa, Plectranthus amboinicus, Pterocarpus santalinus, Rauvolfia serpentine, Ruta graveolens, Santalum album, Senna alata, Salacia reticulat, Vitex negundo.* The majority of the 57 ethnomedicinal plants are used primarily for Herpes treatment, and plant parts such as leaves and roots, in addition to stem, bark, and fruit, are frequently used to treat skin diseases.

The aforementioned plants are readily available throughout the year in their natural habitats in Dakshina Kannada district, where they flourish during the rainy season. However, urbanisation is gradually engulfing village areas in Dakshina Kannada, resulting in a drastic reduction in wild habitat area. As urbanisation continues, plastic pollution (intentional carelessness) has completely destroyed wild habitat and water sources. Additionally, as a result of modern education, the younger generation is less concerned with traditional knowledge, which results in the erosion of traditional knowledge’sof our soil, as the majority of traditional healer families lack heirs. These are the major obstacles of ethno-medicinal knowledge. Therefore, document and incorporate traditional knowledge from our region into our modern educational curricula, educating the younger generation and preserving traditional knowledge. This knowledge will contribute to the nation's strength in a variety of ways.

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RANDOMLY AMPLIFIED POLYMORPHIC DNA IN FISH AND FISHERY

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Abstract:

In comparison to other branches of biology, the principles of animal genetics have been applied less in fisheries and aquaculture. However, the technique of Randomly Amplified Polymorphic DNA has proved its importance as a molecular marker tool in study of applied genetics and biotechnology in fish and fisheries like any other applied branch of biology i.e. agriculture, pharmacy, veterinary science etc. Amplification of DNA employing short sequences of arbitrary primers has proved its usefulness to discriminate species and to detect natural as well as cultured fish populations. It has solved several issues related to species identification, fishery management, and segregation of fish populations, identification and conservation of species, breeding and hatchery maintenance and many more aspects of fish and fish biology in various parts of the globe since last three decades. This review is an extensive research on various utilities of the technique with particular reference to fish and fisheries though it is quite significant in other non piscine models as well as in plants and microbes.

Keywords: Conservation; Phylogenetics; Population Genetics; Primers; Random Amplification

Introduction:

The technique of RAPD employs random arbitrary primers in form of 10 to 20 mer oligonucleotides synthesized artificially as molecular probes to find its complementary sequences for amplification of genomic DNA of experimental organisms. During past few decades, RAPD as a tool of molecular genetics has occupied significant position in fish and fisheries solving many issues related to species identification, fishery management, population segregation and many more. Discovery of the technique as a molecular tool has created significant impact on various research aspects involving genetic architecture of eukaryotes contributing to the development of diverse molecular markers. The markers are amplification products of anonymous DNA sequences. Hadrys *et al.* (1992) described this as a powerful tool of molecular genetics to analyze genetic diversity and relationship among organisms for various purposes to determine taxonomic identity, assess kinship relationship, analyze mixed genome

samples and create specific molecular probe as well as other studies involving molecular mechanisms of ecology at DNA level. It was described by Fuchs *et al*. (1998), that RAPD is an efficient tool for various analysis i.e.- differentiation of populations which are isolated geographically and genetically for multiple purposes to verify the existence of locally adapted populations within a species which were supposed to have arisen through genetic selection under different environmental conditions or might be due to genetic drift. As per Isabel *et al.* (1999), dominant RAPD fingerprints were preferred to other genetic markers in order to gain information about the structure of natural population of various eukaryotic species. RAPD markers reveal polymorphisms in non-coding regions of the genome, hence are useful for studying the genetic structure of populations (Isabel *et al.,* 1999). According to Brown and Epifanio (2003), these molecular markers are considered to be non-coding and selectively neutral. These have extensive use in population genetics to characterize genetic diversity and divergence within and among populations as stated by Brown and Epifanio (2003) and these are based on assumptions of Hardy-Weinberg equilibrium and selective neutrality.

Innovation of Polymerase Chain Reaction has contributed to development as well as application of various DNA markers (Marle-Koster and Nel 2003 and Schlotterer 2004) with major impact on research of eukaryotic genomes. These molecular markers are included under Type II category like microsatellites and AFLPs (Liu and Chordes, 2004). As per Liu and Chordes (2004), this technique amplifies DNA bands from anonymous genomic regions through PCR and is very useful for aquaculture genetics to identify species, strain and hybrid as well as analysis of in breeding and Quantitative Trait Linked markers. According to Liu and Chordes (2004) significance of these markers was not fully appreciated in the early stages of aquaculture, which later on became clear and extremely important with functions as markers in population studies, as well as other studies like QTL mapping, genetic linkage analysis, evolution of genome, comparative genomics and identification of candidate gene. The technique was recognized and got significance since 1990s as effective molecular marker tool in various animals including fishes (Alam and Islam 2005). As per Alam and Islam (2005), it is used to investigate genetic parameters of wild as well as inland stocks of fishes. Caccon *et al.* (1997) described this as a marker tool which allowed evolutionary biologists to determine the genetic characters of closely related animals. Implications of this molecular marker in various organisms can be easily understood from existence of vast literature where as this review is an extensive research on various utilities of the technique with particular reference to fish and fisheries though it is also quite significant in other non piscine models of different plants and microbes.

Random Amplified Polymorphic DNA: The molecular tool

The technique uses short oligo-nucleotide primers which are short as well as arbitrary and single primers and not require prior knowledge of any sequence. According to Welsh and Mc Clelland (1990), simple and reproducible fingerprints of complex genomes can be generated

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through PCR-RAPD which does not require any prior sequence as demonstrated in various microorganisms as well as plants like *Oryza sativa*. The technique of RAPD involves polymerase chain reaction (PCR), which is the backbone of the study to find appropriate marker at molecular level. The novel molecule which plays significant role in this technique is the DNA polymerase for *in vitro* synthesis of oligo nucleotides during random amplification. Amplification is achieved by various steps of denaturation and annealing followed by extension as described by Welsh and Mc Clelland (1990) and Williams *et al.* (1990). During interpretation of result, the randomly amplified oligonucleotide products are resolved on agarose/sepharose/polyacrylamide gel showing polymorphism on the basis of amplification of bands which are either present or not and serve as indicators of dominant genetic markers demonstrating Mendelian pattern of inheritance (Williams *et al.,* 1990). As per Williams *et al.* (1990) and Welsh and Mc Clelland (1990), random amplification of sequences requires selected primers involving denaturation by heating of the template DNA at higher temperature (90-96ºC) or more to separate complementary strands followed by primer binding at lower temperature (30-45ºC) by making hydrogen bonds between the complementary nitrogenous bases and this is known as annealing which occurs on both sides of target sequences. The final extensions of the DNA strands are achieved by higher temperature (70-80ºC) which enables the enzyme to extend the primer(s) and synthesize copies of target DNA sequences. Choice of suitable annealing temperature is the bottle neck of this technique which depends on calculations regarding presence of bond energy between double or triple bonds linking nitrogenous bases of complementary strands of the DNA double helix at the target region and those of primers. This calculation is usually twice the number of adenosine and thymine added with thrice the number of cytosine and guanine {2 x (A +T) + 3 x (G+C)} where each nucleotide is presumed to require a temperature of 1ºC during annealing.

Application in fish and fisheries

The contribution of PCR to RAPD became highly significant in fish and fisheries management and aquaculture. As per Williams *et al.* (1990) amplification of DNA employing short sequences of arbitrary primers have proved their usefulness to discriminate species to detect natural as well as cultured fish populations. By employing the technique, Bardakci and Skibinski (1994) analyzed and discriminated various species of *Oreochromis*. Tassanakajon *et al.* (1997, 1998); Garcia and Benzie (1995) and Dinesh *et al.* (1993) reported on the usefulness of different information generated through RAPD on the genetic structure of fish species. It is useful to breeding programs and management of sustainable yield as well as identification, optimization and enhancement of stock preserving their genetic diversity as stated by Tassanakajon *et al.* (1997, 1998); Garcia and Benzie (1995) and Dinesh *et al.* (1993). Dinesh *et al.* (1993) used this technique employing random primers of various lengths ranging from less

than 10mer to 20mer or more through PCR in fishes of 12 different species where the random primers generated unique fingerprints for each species of fish in terms of number and position of RAPDs. Johnson *et al.* (1993) identified RAPD primers in different strains of zebra fishes developed in laboratory conditions showing extensive genetic polymorphism in developmental stages. During 1994, Hansen and Loeschcke reported that, the aim of any genetic conservation program should be to develop an integrated strategy conserving as much genetic diversity within the species as much as possible. It should ensure the presence of utilizable fish resources (Hansen and Loeschcke, 1994) for this purpose. Foo *et al.* (1995) reported on the rapidity and efficiency of RAPD to generate series of molecular genetic markers in fishes. The method was employed as a tool for identification of potential fish species and analyzed more than 100 individuals from 08 species of fishes like barramundi, Nile perch, John dory, mirror dory, silver dory, spikey oreo, warty oreo and smooth oreo by Partis and Wells (1996). As stated by Patil *et al.* (2016), it is a very essential step to identify and discriminate a species accurately for its conservation where as genetic diversity is highly indispensable for adaptation and survival in various ecological conditions. Comincini *et al.* (1998) reported the utilities of RAPD technique to identify six sturgeon species. This technique has been applied for various purposes in fishes like gene mapping studies (Kazianis *et al.*, 1996 and Postlethwait *et al.*, 1999); gynogenetic fish identification (Corley-Smith *et al.*, 1996 and Chen and Leibenguth, 1995). It is also employed to identify various species and subspecies through studying their phylogeny as per Bardakci and Skibinski (1994); Partis and Wells (1996) and Borowsky *et al.* (2001). Caccone *et al.* (1997) reported genetic differentiation within sea bass (*D. labrax*) population revealed through RAPD. Liu *et al.* (1998) reported RAPD markers to reveal the inheritance through Mendelian segregation in channel catfish, blue catfish and their F_1, F_2 and backcrosses. Williams *et al.* (1998) used the technique for identification of largemouth bass subspecies. As stated by Bielawski and Pumo (1997), there existed exceptionally low levels of genetic variation in striped bass of Atlantic Coast revealed through RAPD analysis. Variations in samples of Hilsa shad were investigated by Dahle *et al.* (1997), employing PCR-RAPD where as two years later in 1999, Kuusipalo studied genetic differentiation of endemic populations of *Lates stappersi* employing the same tool in muscle tissue extracts stored in alcohol by a salting method, without organic solvents. Postlethwait *et al.* (1999) applied this technique for analyzing genome of zebra fish. The technique has also been applied by Bernardi and Talley (2000) and Cagigas *et al.* (1999) to detect similarity and genetic diversity in numerous organisms. Similarly, Bardakci and Skibinski (1994) applied this tool for construction of phylogenetic trees to resolve taxonomic problems. To mention other applications of RAPD in fishes encompassing broad utilities providing quite significant information like sex chromosomes differentiation (Iturra *et al.*, 1998), identification of genetic inheritance (Elo *et al.*, 1997), fish conservation (Dioh *et al.*,1997), gene mapping (Liu *et al*., 1999) and genotoxicity study (Sayed *et al.*, 2013) in aquatic environment.

Guo *et al.* (2001) reported it as effective in identification of species and cross-contamination test. Jong Man (2001) described this technique as a very useful tool to differentiate fishes at various levels like strains, subspecies and species. Yoon and Kim (2001) informed it as simple, quick and easy but sensitive having its significance as a molecular tool in fish and fisheries employed for numerous applications i.e.- genetic diversity studies, species identification, genetic differentiation of intra or inter-breeding population, and detection of DNA for various purposes. As per Yoon and Kim (2001) and Holsinger *et al.* (2002), RAPD markers were genetically linked to trait/traits of interest and those could be used for various objectives like improvement of traits in genetics and breeding programmes, identification of individual and their pedigree as well as pathogenic diagnostics. Marle-Koster and Nel (2003) informed that augmentation of PCR with RAPD contributed a lion share to the development and application of various molecular markers. Barman *et al.* (2003) employed this technique to study genetic diversities or relationships among IMC (Cyprinidae) through species specific markers among *Labeo rohita*, *L. calbasu*, *Catla catla* and *Cirrhinus mrigala* for identification of hybrids, assessment of genetic diversity and study of taxonomic relationships at a molecular level. Ali *et al.* (2004) presented an extensive review of RAPD as molecular markers used in fisheries research and its advantages as well as disadvantages. According to Ali *et al.* (2004), RAPD became an ideal tool due to its simplicity and ease for various programmes related to plant and animal breeding, DNA fingerprinting and genetic mapping which had very significant utilities in population genetics. Liua and Cordes (2004) also informed that, RAPD determined the effect of genetic contamination. Ambak *et al.* (2006) reported about the use of RAPD loci to determine genetic distance and diversity as well as the number of polymorphic loci for construction of an unweighted pair group method for the arithmetic mean to construct dendrograms using Nei's unbiased distance. Okumus and Ciftci (2003) and Yousefian *et al.* (2011) reported that, fisheries scientists use different tools and techniques of biotechnology to reveal the genetic diversity in cultured fish which also included RAPD. Different authors reported from time to time regarding the comprehensive use of RAPD to detect genetic diversity in animals, (Cushwa and Medrano, 1996). The same was also reported in various fish species of tilapia by Naish *et al.* (1995), in striped bass by Bielawski and Pumo (1997), in murrel by Nagarajan *et al*., (2006), in *Clarias batrachus by* Garg *et al*. (2010) and in *Eutropiichthys vacha by* Chandra *et al*. (2010). Tassanakajon *et al.* (1998), Mishra *et al.* (2009), Nagarajan *et al.* (2006), Lakra *et al.* (2010) and Saad *et al.* (2012) observed population specific RAPD bands in *Penaeus monodon, Metapenaeus dobsonii, Chaanna punctatus, Monoporeia affinis* and *Plectropomus leopardus* respectively. Investigation of Saini *et al.* (2011) in various species of bagrid catfishes (*Mystus*) employing RAPD markers to discriminate polymorphic characters from more than 500 fragments generated using 10 decamer primers of arbitrary nucleotide sequences. Tripathy *et al.* (2012 and 2013)

employed this tool using 10-mer primer to evaluate genetic parameters of backcross generations of catla and rohu and reported information on the basis of molecular marker analysis which indicated more genetic proximity of rohu with backcrosses than catla. Different molecular biomarkers like microsatellites, RAPDs, AFLPs, RFLPs etc. were used from time to time for various purposes in fish and fisheries to estimate genetic variation, to plan strategies to conserve fish population, to determine sex, to identify disease carriers and to analyse transgenesis (Basavaraju *et al.*, 2007, 2014 and Figueras *et al.*, 2016). RAPD markers in various fish and non fish models were reported from time to time to study genetic divergenece and interactions in *Anguilla* (Takagi and Taniguchi, 1995), *Penaeus monodon* (Tassanakajon *et al.*, 1997), *Salmo salar* (Elo *et al.*,1997), *Symphysodon (* Koh *et al*., 1999), *Artemia (*Yi *et al.*, 1999), snakehead fish (Ambak *et al.*, 2006), *Mugil cephalus* (Suresh *et al*., 2013) and catfishes and cichlids (Asagbra *et al.*, 2014).

As a molecular instrument, RAPD markers are useful to investigate genetic interactions and divergence in several aquatic species. Liu *et al*. (1998 and 1999); Yoon and Kim (2001); Chong *et al.* (2000) and Kovacs *et al.* (2000) used RAPD in catfishes to analyze various aspects of their genetic architecture. RAPD fingerprints in two varieties of guppy were generated by Foo *et al.* (1995) using three oligonucleotide primers and their paired combinations which were reported to be different and reproducible. Nxomani *et al.* (1999) analyzed color forms in *Tilapia guinasana* which were genetically distinct. Sun *et al.* (2000), applied this technique like AFLP as powerful DNA fingerprinting method to classify strains and species of *Artemia*. Zhou *et al.* (2000 a, b) studied the genetic architecture of triploid gynogenetic silver crucian carp (*Carassius auratus gibelio*) using RAPD. Borowsky and Vidthayanon (2001) assessed the genetic variations in species of Balitorids from Thailand where as Gouin *et al.* (2001) characterized the genetic diversities of *Austropotamobius pallipes*, a threatened freshwater crayfish native to Europe. Reproducibility and polymorphism of RAPD and AFLP fingerprinting techniques were assessed by Bagley *et al.* (2001) in pedigreed populations of rainbow trout *Onchorhynchus mykiss*. As per Shikano and Taniguchi (2002) the amount of heterosis and F_1 performance in four strains of guppy is positively correlated with both genetic distance and dissimilarity between strains which are used for crosses characterized by microsatellite and RAPD markers and found to be useful to investigate genetic diversity among strains. The technique was applied to examine the genetic variations in endangered Neotropical fish *Brycon lundii* (Wasko and Galetti, 2002), collected from two different regions with distinct environments in Sao Francisco River (Brazil). Yue *et al.* (2002) employed three different DNA markers systems (RAPD, AFLP and microsatellites) to estimate genetic variations in *Scleropages formosus* from three different sources. Feral (2002) reported the utility of RAPD to understand marine biodiversity. Dong and Zhou (1998) and Bartfai *et al.* (2003) studied genotypes of common carp and Barman *et al.* (2003) reported that in IMC by this technique. Hatanaka and Galetti Jr. (2003) detected genetic variation by this

technique in *Prochilodus marggravii* from three collection sites in the area of influence of the Tres Marias dam (MG). As stated by Ahmed *et al.* (2004), RAPD markers have great utilities in systematic investigation at species level of tilapia. Utility of RAPD as a molecular approach providing useful information for species management and genetic conservation of several freshwater as well as marine fishes were reported by Wasko and Galetti Jr (2002); Hatanaka and Galetti Jr. (2003) and Wasko *et al.* (2004). Wasko *et al.* (2004) accessed genetic variability in neotropical freshwater fish, *Brycon cephalus* (Amazonian matrincha) evaluating the genetic diversity in a breeding programme through RAPD. Das *et al.* (2005) employed this technique to comprehend genetic variations among six species of *Labeo*. Genetic variation among different populations of Hilsa Shad was reported by Brahmane *et al.* (2006). In the same year, Upadhyaya *et al.* (2006) reported high level of genetic diversity and variation between populations of yellow grouper (*E. awoara*). Asensio *et al.* reported in 2009 that, RAPD was used to identify species of fishes due to its simplicity, specificity and sensitivity to characterize genes. Garg *et al.* (2009) applied this technique for analyzing genetic variation of *Mystus vittatus* (Bloch) populations of Madhya Pradesh, India. Mostafa *et al.* (2009) reported genetic diversity of wild and farmed *Labeo calbasu* by RAPD analysis of the genomic DNA. Jamshidi *et al.* (2009), reported on the suitability and efficiency of RAPD to distinguish parents from F_1 hybrids of female grass carp *(Ctenopharyngodon idella)* and male bighead carp *(Hypophthalmichthys nobilis)* by studying genetic variation and inheritance. Rahman *et al.* (2009) used this method in Bangladesh to assess genetic variation in three wild and one hatchery population of *Catla*. Shivraman *et al.* (2010) employed this technique to assess genetic differences and phylogenetics of cold water fish species. Da Silva *et al.* (2010) authenticated genetic diversity in *A. brasiliensis* from two different places by examining RAPD markers and made a conclusion that their populations from southern Brazil displayed minimal dissimilarity and genetic configuration. As stated by Shair *et al.* (2011), the method is a highly significant one in fishes providing efficient and sensitive way to estimate genetic variability, relatedness, inbreeding levels, pedigree analysis, detection of economic traits and other marker based analysis. Genetic structure of four wild populations of two hill stream fish *Barilius bendelisis* and *B. barna* were reported by Mishra *et al.* (2012) using RAPD. As per Giri *et al.* (2012), characterization of germplasm is an important link for conservation and efficient utilization of genetic resources which can be performed through RAPD analysis. In 2013, Kumla *et al.* investigated the genetic structure of four sub-populations of *Mystus nemurus* in Thailand by employing RAPD and Inter-Simple Sequence Repeat (ISSR) markers. Mojekwu *et al.* (2013) assayed RAPD polymorphisms to characterize markers within and among *Tilapia* from different water bodies and hatcheries through selective breeding programs. Pandey *et al.* (2013) mentioned the essentiality of information on the genetic profile of fish species which are cultivable to study molecular systematic and optimize farming and

management of fisheries. They described, usefulness of RAPD as a potential tool to test the genetic relationship among four predominant fish species, viz., *Tor putitora, Schizothorax richardsonii,Garra gotyla* and *Barilius bendelisis* (Cyprinidae: Pisces) from two different geographical region of Kumaun and Garhwal of Uttarakhand, India. Suresh *et al.* (2013) used RAPD markers to analyze genetic structure of four populations of *Mugil cephalus* from different states of India. As stated by Nandini and Thakur (2014), genetic diversity is indispensable for long term survival of species as it provides raw material for adoption and evolution to changing environmental conditions. Genetic variation is necessary for natural stock of fish ensuring their availability (Xia *et al.*, 2014 and 2015). According to Neekhra *et al.* (2014), importance of RAPD for molecular taxonomy studies in diverse fish species of IMC in central India should be re-established. Patil *et al.* (2016) employed RAPD profiling of molecular markers in Pisces (Nemacheilidae) for various purposes like phylogenetic relationship studies to develop a molecular-based tool to identify taxonomic status and to make discrimination based on genetic identity and diversity of loaches. DNA markers are the most commonly used tool for fish stock conservation at a minimum level (Carlson *et al.*, 2015). Morphometric pattern and genetic variations in species of Cyprinus and *Onchorhynchus mykiss* from Kashmir were evaluated by Ganaie and Ali (2016) using RAPD. Olukolajo and Olawale (2017) described the usefulness of RAPD markers informing genetic variations of snappers and grunters from and high (Lagos) and low (Badagry) brackish lagoon of Nigeria. Almaaty *et al.* (2018) confirmed through RAPD analysis that four Synodontis species under study were genetically different form each other. Danish and Singh (2018) assessed genetic diversity of catfish *Clarias batrachus* using RAPD markers. Mahboob *et al.* (2019) calculated genetic polymorphism in tilapia (*O. niloticus*) by using RAPD markers for the purpose of evolving strategies to conserve their diversity in the country. Megbowon (2019) reported the use of this technique to evaluate genetic variation among chichilids of Nigeria.

As per Atienzar and Jha (2006), the technique detected genotoxicicity-induced DNA damage and mutations in different organisms including fish. Similar, applications of RAPD in fishes detected genotoxic potential of chemicals and metals as evidenced by Castano and Becerril (2004), Zhiyi and Haowen (2004), Mohanty *et al.* (2009), Rocco *et al.* (2010) Orieux *et al.* (2011) and Osman *et al.* (2012). Salem *et al.* (2014) reported on the utility of RAPD markers in genotoxicity studies involving various non-fishes as well fishes reporting impact of pollutants on fishes exposed to landfill leachates in *Rutilus rutilus*. Rocco *et al.* (2014) detected DNA alterations in environmental genotoxicity studies by using PCR-RAPD through molecular characterization of *Dicentrarchus labrax* embryonic cells (DLEC).

Advantages of the technique

RAPDs were quickly applied in fisheries research and became very rapidly growing tools because they did not require much resources, equipment or technical expertise. According to

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Williams *et al.* (1990), the technique is easy, leading to automation of gene mapping methods which extended to genetic analysis covering organisms lacking a sufficient number of phenotype markers for complete description of their genome. According to Welsh and Mc Clelland (1990); Williams *et al.* (1990) and Klinbunga *et al.* (2000), the advantage of this technique was that it did not require preceding information of the genome for its successful application. According to Capili (1990) and Seyoum and Kornfield (1992) it is a more sensitive technique in comparison to mt-DNA analysis as the later one failed to reveal molecular variations among various populations of tilapia. As informed by Lynch and Milligan (1994), application of RAPD approach is relatively straight-forward and it can examine unlimited number of loci. Zhivotovsky (1999) described RAPD as a technique widely used for studying DNA polymorphism between closely related species without pre-requirement of genomic information and it had gained considerable attention particularly in population genetics. The technique was extremely valuable due to its requirement for less sophisticated equipments, low expense and efficiency in developing a huge number of molecular markers in a short time, although its reproducibility was a under doubt (Bardakci, 2001). As per Bardakci (2001), the major cause of RAPD's success was the addition of large numbers of genetic markers requiring small amount of DNA. It did not require sophisticated technique like cloning or sequencing or any other form of molecular characterization of the genome (Bardakci, 2001). The analysis provided least expensive but reliable identification of species in absence of availability of prior molecular information where each species exhibited an unique RAPD profile banding pattern and their simple comparison by employing one or two primers were sufficient for identification of species (Liu and Chordes, 2004). Liu and Chordes (2004) also reported that, RAPDs encompassed all the advantages of markers based on PCR amplification with more benefits in addition to those provided by commercially available primers, which did not have prerequisite of genetic information regarding the sequence of the target DNA or that of gene organization. Callejas *et al.* (2004), informed that, RAPD-PCR method was widely employed in genetic research as a powerful asset in species identification, and the polymorphism detected in nucleotide sequence was used as a genetic marker. The technique did not require any preliminary work like isolation of probe, preparation of filter or sequencing of nucleotide but it required some universal sets of primers and provided a simple and reliable method for measuring genomic variation. Brahmane *et al.* (2006) reported the advantages of RAPD in fisheries to profile genetic variety of a population offering as an inexpensive and quick molecular tool, assisting in monitoring, distinguishing and managing genetic diversity of natural fish populations raised in hatcheries.

RAPD has the ability to reveal intra-specific variation which may be utilized to screen degree of inbreeding in commercial species to prevent an increase in the frequency of deleterious recessive alleles in a population (Govindraju and Jayashankar, 2004;

Rohfritsch and Borsa, 2005; Bay *et al*., 2006; Galetti, Jr. *et al.*, 2006 and Craig *et al.*, 2007). As per Lopera-Barrero *et al* (2006) and Shair *et al.* (2011) RAPD had been used in endangered species of fishes to study variations required for conservation and management of fish estimating genetic diversity. RAPD profiles among species or strains provided an efficient system to generate molecular marker for gene mapping in an intra specific mating plan (Jamshidi *et al.,* 2009). Kabir *et al.* (2012), suggested a combination of karyotype and RAPD analysis to provide sufficient data of an individual fish germplasm.

Disadvantages of the technique

The technique has many advantages for genomic DNA profiling of organisms. In contrast, it has many disadvantages with limited progress due to unavailability of useful genes or identifiable markers linked to important traits. As per Raboum *et al*. (1999), the number of fragments amplified and the degree of polymorphism in eukaryotic species depended on the nucleotide sequence, secondary structure and primer's number used for each assay, which could be used to distinguish homozygotes and heterozygotes. There are many limitations restricting practical application of this technique like artifacts, reproducibility, dominance and interference by homology. Riedy *et al.* (1992) reported excess non parental bands of DNA in this methodology in primates. Ayliffe *et al.* (1994) warned regarding artifacts in RAPD analysis that, formation of non-parental bands or hetero-duplex formation should be very less (0.16% to 10%). Ayliffe *et al.* (1994) also demonstrated that artifactual polymorphism can arise during RAPD analysis as heteroduplex molecules formed between products of different allele are potential source for such error. As per Wirgin and Waldman (1994), RAPD markers were reported to be less reproducible with limited application in fisheries due to low annealing temperature used in amplification through PCR. It is assumed that, the inheritance of RAPD markers follows Mendelian pattern in a dominant fashion by scoring their presence or absence. However, both homozygotes as well as heterozygotes produce such bands which might differ in their intensity and those variations in efficiency of PCR made scoring difficult due to such variable intensities. Due to this, it became generally impossible to distinguish homozygous dominant from heterozygous individuals. Lynch and Milligan in 1994 reported that, dominant markers like RAPD were not as efficient as co-dominant markers for population genetics studies. Skroch and Nienhuis (1995) reported poor reproducibility of this method. As per Elo *et al.* (1997), formation of non parental bands should be to be less than 0.03% and concluded in salmon and brown trout that, it is one of the most time-saving technique as well as least expensive and non-invasive method to detect inter specific hybridization. According to Liu and Chordes (2004), each band developed through PCR-RAPD is believed to be a bi-allelic locus indicating presence or absence of an amplified product. Their polymorphic information content (PIC) values are quite beneath those for microsatellites and SNPs, hence less revealing then AFLPs as they generate fewer loci. As per Ambak *et al.* (2006), RAPD is useful to identify DNA polymorphism, estimate of genetic

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diversity and differences of related species in fish but the technique has some lacunae like essentiality of pre-optimization of amplification conditions and ascertaining of reproducibility for individual taxa prior to application of DNA fingerprinting to any genetic analysis. Many factors can affect the changes in the RAPD profiles (Atienzar and Jha, 2006) as it requires proper optimization for reliable results. The absence of amplification of a band in two genotypes does not necessarily represent genetic similarity between them as homozygotes cannot be distinguished from heterozygotes by RAPD. Liu and Chordes (2004) informed that, whether bands represented different loci or alternative alleles of a single locus was difficult to determine and there might be erroneous assessment of the number of loci under study when RAPD amplification could be due to deletion or insertion of nucleotide within the locus instead of primer binding sites.

Conclusion:

Though an array of molecular markers like mini and microsatellites, EST markers, RFLPs, AFLPs, SNPs are available today for solving various issues of fish and fisheries science at molecular level, RAPD has not lost its importance completely till to date and is on use for various purposes in different parts of world in both captive and culture conditions of fisheries. The technique is very useful to collect data about the genetic variation in the wild stock of fish population of the same geographic region and the information may be helpful to plan strategies for improvement in the breeding program by fisheries biologists. The same is also expected to be equally important for different species of plants and animals for various purposes.

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ENZYME ADDITION BIOGAS POTENTIAL TEST FOR ENHANCEMENT OF BIOGAS PRODUCTION ON CO-DIGESTION OF INTESTINE SOLID WASTE FROM SLAUGHTERHOUSE WITH FOOD WASTE

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Abstract:

In general intestine waste are highly organic in nature, whereas, it contain high protein and lipid content, which reflect in less C/N ratio, it affect the biogas production in anaerobic process. To optimize the C/N ratio food waste is a more suitable co-substrate. In order to improve the biogas production, lipase and protease enzyme help to rate of hydrolysis process. The effect of protease enzyme addition (0.25, 0.50, 0.75 and 1%) in biogas potential test was studied with IWS, FW, 1:1, 1:2 and 1:3 mixing ratios. Maximum specific biogas production at the end of 30th day were 0.21, 0.37, 0.46, 0.54 and 0.56 L/g of VS added with 1% protease enzyme addition for IWS, FW, 1:1, 1:2 and 1:3 mixing ratios respectively. In the case of effect of lipase addition (0.25, 0.50, 0.75 and 1%) study, the maximum specific biogas production at the end of 30th day were observed in the following order: 0.58 (0.75% lipase 1:2) > 0.57 (0.75% lipase 1:3) > 0.51 (0.75% lipase 1:1) > 0.38 (1% lipase FW) > 0.26 (1% lipase IWS). Based on the overall protease and lipase addition biogas potential test study, 1:2 mixing ratio with 0.75% lipase addition showed enhancement in biogas production compared with IWS, FW 1:1 and 1:3 mixing ratio with lipase and protease addition.

Introduction:

One of the major solid waste from the MSW is slaughterhouse solid waste. Solid wastes generated from slaughterhouses are required to be treated by adopting methods like rendering, controlled incineration, burial, composting, anaerobic digestion,etc., (Rouf *et al.* 2016). In India, currently, there is no standard treatment method has been adopted for slaughterhouse solid waste,

(Ahmad, and Ansari, 2012), especially in municipal slaughterhouses. One of the major solid wastes generated from the slaughtering process is intestine waste (IW). In general, IW is being disposed along with municipal solid wastes in open dumps in most of the cities and towns. It leads to environmental nuisance i.e odor, groundwater contamination, and gaseous emissions such as CO2 and methane in to the atmosphere (Ingrid and Insam, 2013). Anaerobic digestion (AD) is a suitable method to treat intestine wastes as well as recover energy. Moreover, AD is a more benefited treatment technology, it requires less area and energy when compared to composting and controlled incineration and it is more suitable for the Indian scenario.

Intestine waste from the slaughterhouse (IWS) contains high protein and lipid with low C/N ratio of 2 - 5 (Cuetos *et al.,* 2008) but the optimum C/N ratio requirement for the anaerobic digestion is 25 - 35 (Guermoud *et al.,* 2009). Due tohigh protein and lipid content, it takes longer retention time to degrade. This low C/N ratio leads to ammonia toxicity in the anaerobic digestion due to the conversion of nitrogen rich substances to ammonia (Braun *et al.,* 2003; Ahring, 2003; Gerardi, 2003). Free ammonia is membrane permeable (Banks *et al.,* 1999) and it inhibit the methanogenic activity (Kovács *et al.,* 2013). Co-digestion is a suitable option to overcome the above problem associated with the AD of animal waste. Co-digestion improves the AD of organic wastes by providing several benefits such as dilution of toxic compounds and balancing the nutrients including the C/Nratio, which improves the biogas production (Hartmann and Ahring, 2005; Rajin, 2020). Porselvam *et al.* (2017a) reported that the co-digestion of Intestine waste from slaughterhouse (IWS) with food waste (FW), which improves the C/N ratio of optimum ratio of 25-30 in 1:2 mixing ratio and also reported the higher biogas production of 57% and 17% than Intestine waste and food waste alone. In order to improve the treatment efficiency at higher loading rates and for improved biogas production, pre-treatment processes are required. Pretreatment processes reduces the particle size of solid waste resulting in increase in the specific surface available to the medium, increase the rate of hydrolysis process and reduce the reaction time, which improves biogas production (Guangxue *et al.,* 2009; Javkhlan *et al.,* 2014). Porselvam *et al.* (2017b) investigated the effect of thermo-alkali pretreatment followed by AD using NaOH and KOH, on co-digestion of IWS and FW in different ratios (1:1, 1:2 and 1:3) in terms of VS basis. From this investigation, biogas yield of 0.55 and 0.45 L/g of VS added were observed in 3% KOH and NaOH respectively. In the thermo-chemical pretreatment followed by AD of IWS with FW, is the chemical and energy intensive process which requires higher operation and maintenance cost and also generation of higher chemical sludge at the end of process.

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According to above scenarios, in order to improve the treatment efficiency and biogas production enzyme addition pre-treatment has been more suitable. Enzyme is thermostable and performs accelerated biochemical conversions like hydrolysis, esterification, acidolysis and aminolysis. SriBalaKameswari *et al.* (2011) studied Co-digestion of fleshings and primary and secondary sludge generated during the treatment of tannery wastewater and addition of *Steapsin*, a commercial grade lipase, to enhance the hydrolysis in anaerobic co-digestion and the biogas generation was observed to increase by 15% compared to the control without adding lipase. Romano *et al.* (2009) studied that the effect of enzyme addition on anaerobic digestion of wheat grass. Maha Affes *et al.* (2017) was investigated the effects of bacterial lipase (neutrophilic *Staphylococcus xylosus*) on biogas production of anaerobic co-digestion of slaughterhouse wastewater (SHWW) and hydrolyzed grease (HG). Sutaryo *et al.* (2014) investigated the effect of mixture of enzyme addition in dairy manure on anaerobic digestion. Mixture of enzyme addition to substrate at mesophilic (35°C) or thermophilic (50°C) process temperatures, but there was a significant 4.44% increase in methane yield in dairy manure at 50°C.

On the basis of the major research gaps identified from the literature, the present study involved a novel approach of lipase / protease addition of biogas potential test on co-digestion of IWS and FW (VS/VS basis). The major objective of the study is to optimize the mixing ratio (IWS/FW), effect of enzyme addition, enhancement of biogas production and higher removal efficiency.

Materials and Methods:

Segregated intestine solid waste sample was collected from slaughterhouses, Chennai, India. Food waste (FW) was used as co-substrate and the same was collected from CSIR-CLRI canteen. Sludge from anaerobic digester was used as inoculum in batch reactor studies. It was collected from anaerobic digester in sewage treatment plant at Perungudi, Chennai, India. Analytical grade protease and lipase enzymes from Hi-media chemicals were used for enzymatic addition biogas potential test.

Batch Biogas Potential Test (BPT)

Experimental set up of batch biogas potential reactor is shown in Fig.1.

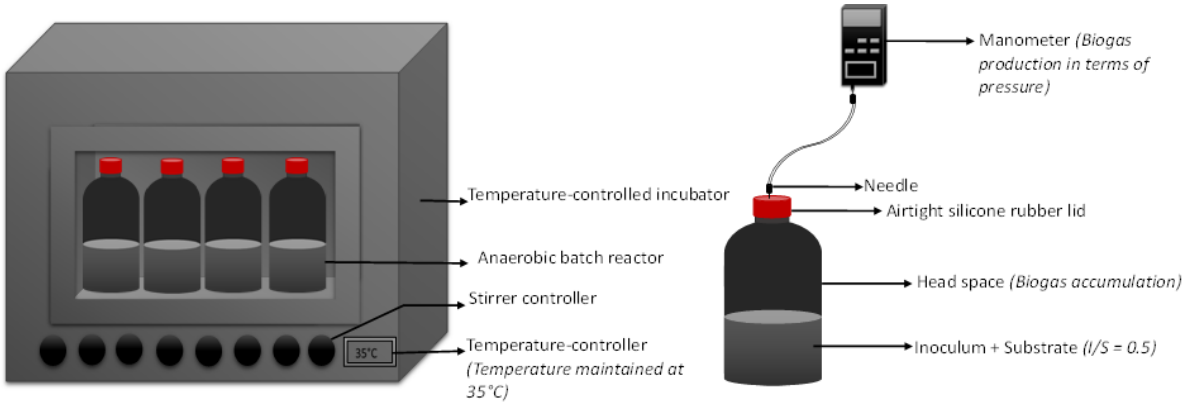

Fig. 1 Experimental set up of batch biogas potential reactor

Batch experimental set-up consists of 600 ml and headspace was maintained upto 400 ml. The reactor was closed with air tight silicon cap, which is suitable for inserting a needle for measuring the biogas and also to avoid leakage. In the batch study, the reactors were kept in 35°C, and maintained by temperature controller. The incubator had provisions for individual reactor with magnetic stirrer arrangement for variable speed and connected to a timer. The biogas produced was measured using manometer. Biogas production was calculated by using the following equation 1

$$V_o = V.\frac{(P - P_w).T_0}{P_0.T} \qquad (1)$$

Where, V_o - Volume of dry gas at standard temperature and pressure (STP)(ml), V – Actualvolume of the gas generated (ml); P – pressure of the gas phase at the time of reading (hPa); P_w – Vapour pressure of the water as a function of the temperature of the ambient space (hPa); T_o - Normal temperature (273 K); P_o – Normal pressure (1013 hPa); T – Temperature of the fermented gas or of the ambient space (K).

Effect of enzyme addition biogas potential test

The effect of enzyme addition in biogas potential test on IWS, FW and co-digestion of IWS and FW (i.e mixing ratios 1:1, 1:2 and 1:3) was carried out using commercial grade enzymes such as Lipase and protease with different concentrations (0.25, 0.5, 0.75 and 1 g of enzyme with 100g VS of the substrate). Details of protease and lipase enzyme addition biogas potential test reactor are given in Table 1. Graphical representation of enzyme addition biogas potential test on IWS, FW and different mixing ratio of IWS with FW are given in Fig 2.

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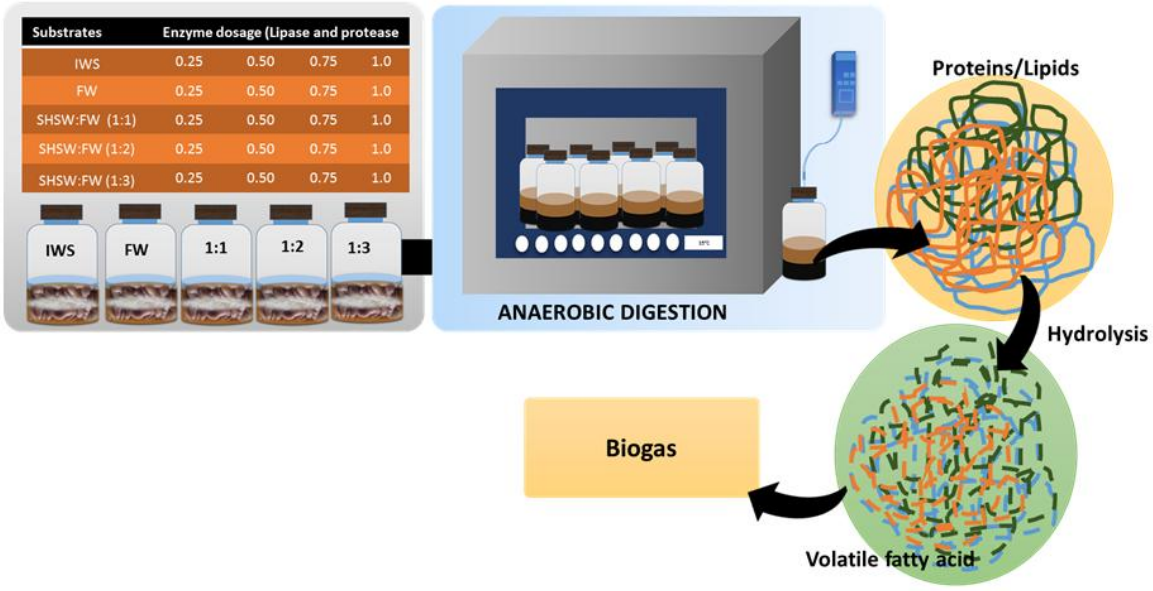

Fig. 2 Graphical representation of enzyme addition biogas potential test on IWS, FW and different mixing ratio of IWS with FW

Result and Discussion:

Biogas production

The cumulative biogas productions obtained during the addition of protease enzyme with IWS, FW and different mixing ratios of IWS and FW reactors are shown in Fig. 3(A). The maximum cumulative biogas productions was achieved in IWS, FW and different mixing ratios of IWS and FW reactors in the order of 6032 ml (1:3)> 5740 ml (1:2) > 4877 ml (1:1) > 3983 ml (FW) > 2222 ml (IWS) in 1% protease added reactors respectively.

The cumulative biogas production in IWS and FW gradually increased with time, whereas in the mixing ratios 1:1, 1:2 and 1:3 yielded considerable amount of cumulative biogas production within 10days by addition of 1% protease. Thereafter the amount of biogas production found to be marginal. The maximum specific biogas production protease addition IWS , FW, 1:1, 1:2 and 1:3 mixing ratios are shown in Fig.4 (A). In IWS and FW, maximum specific biogas production at the end of 30^{th} day was 0.21 and 0.37 L/g of VS added in 1% protease enzyme addition reactors respectively. Similarly, in the mixing ratio of 1:1, 1:2 and 1:3, the maximum biogas production at the end of 30^{th} day was 0.46, 0.54 and 0.56 L/g of VS added during 1% protease enzyme addition reactors. The biogas production in 1:3 mixing ratio with 1 % protease addition were 171 % and 64% higher than the biogas production in IWS and FW with 1% protease addition respectively.

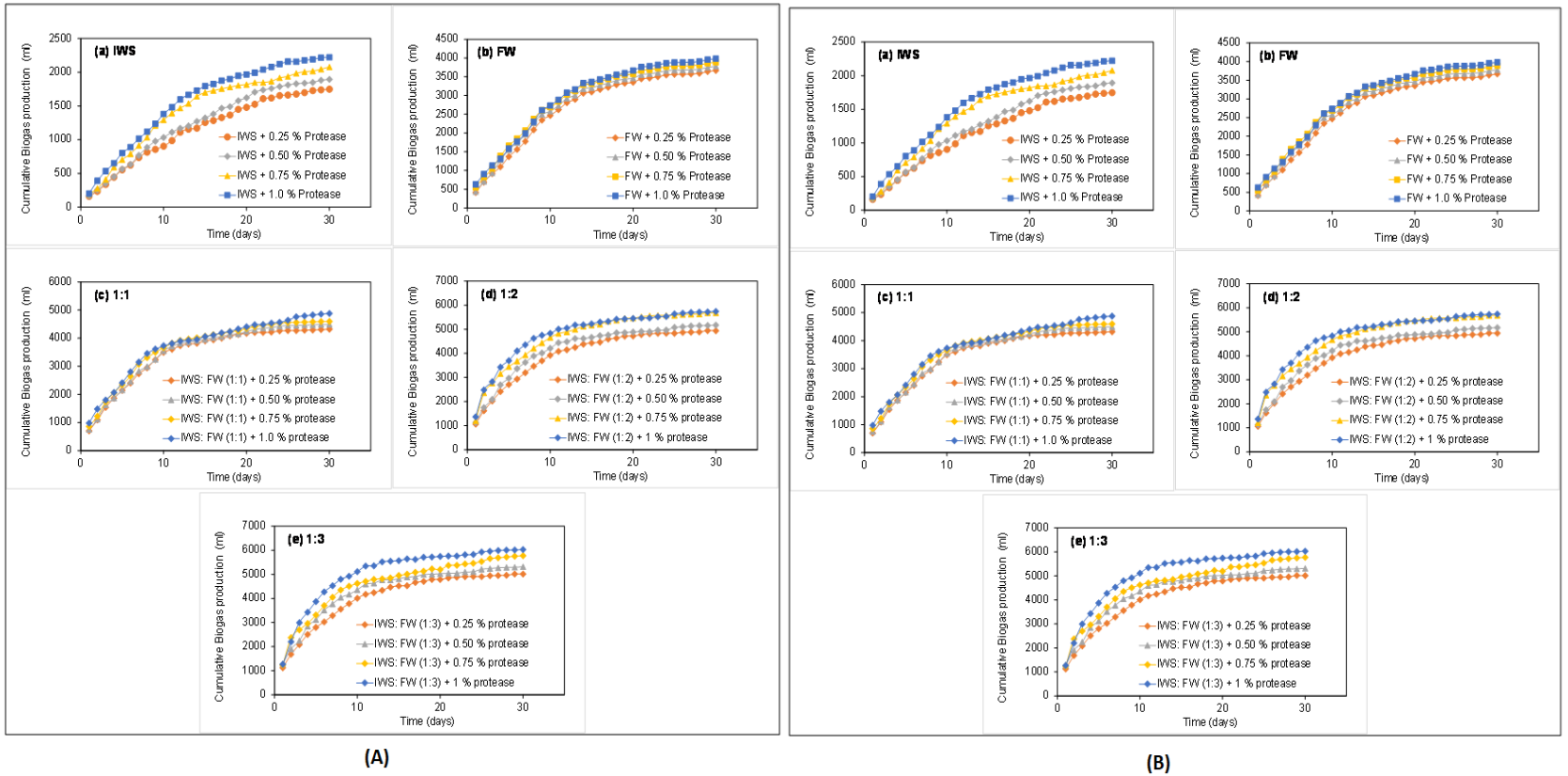

Fig. 3 Cumulative biogas production on (A) Protease (B) lipase enzyme addition biogas potential test of IWS, FW, 1:1, 1:2 and 1:3 mixing ratios

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Whereas in the case of mixing ratio 1:2, the biogas production at the end of 30th day i.e 0.54 L/ g of VS added, was 158, 44 and 14 % higher than the maximum biogas production achieved in 1 % protease addition of IWS, FW and 1:1 mixing ratio respectively. The maximum biogas production of 0.56 L/g VS added in 1:3 mixing ratio was also 211, 55.5, 47.5, 30.2 and 43.5 % higher than the biogas production without pretreatment of IWS, FW 1:1, 1:2 and 1:3 mixing ratio respectively. Based on the overall study, the 1:3 mixing ratio resulted in enhancement in biogas production when compared with IWS, FW, 1:1 and 1:2 mixing ratio.

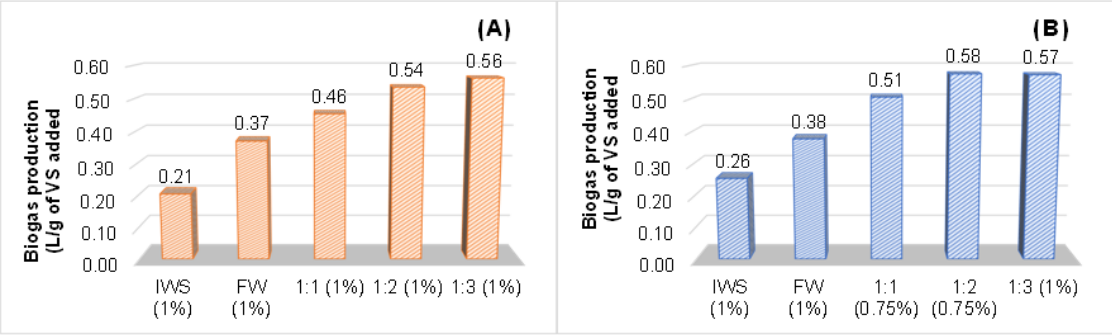

Fig. 4 Maximum Specific biogas production on(A) Protease (B) lipase enzyme addition biogas potential test of IWS, FW, 1:1, 1:2 and 1:3 mixing ratios

During lipase enzyme addition in batch biogas potential test, the cumulative biogas productions obtained for IWS, FW and different mixing ratios of IWS and FW reactors are shown in Fig. 3(B). The maximum cumulative biogas production were achieved in the order of 6018 ml (1:2) > 5967 ml (1:3) > 5358 ml (1:1) > 3944 ml (FW) > 2669 ml (IWS) in 1% lipase concentration respectively. Similar trends were observed for lipase as that of protease in biogas production during the study period.The maximum specific biogas production during lipase addition in batch biogas potential test for IWS , FW, 1:1, 1:2 and 1:3 mixing ratio are shown in Fig.4(B). During lipase addition in IWS and FW reactors, the maximum specific biogas production at the end of 30th day were 0.26 and 0.38 L/g of VS added in 1% lipase enzyme added reactors respectively. The maximum biogas productions from IWS and FW were 23.8 and 2.7 % higher than the corresponding biogas production from IWS and FW with 1% protease addition. Similarly in the mixing ratio of 1:1, 1:2 and 1:3, the maximum specific biogas productions of 0.51, 0.58 and 0.57 L/g of VS added at the end of 30th day were obtained with 0.75% lipase addition. The maximum biogas production of 0.58 L/g VS added was observed in 1:2 mixing ratio with 0.75% lipase addition, which was 123, 53, 14 and 1.7 % higher than the maximum biogas production of IWS, FW, 1:1 and 1:3 mixing ratio respectively. Based on the

overall study, it is observed that 1:2 mixing ratio with 0.75% lipase addition showed enhancement in biogas production compared with the individual biogas production of IWS, FW and 1:1 mixing ratio also.

VS removal efficiency

VS removal efficiency in biogas potential test of IWS, FW, 1:1, 1:2 and 1:3 mixing ratios with protease enzyme addition are shown in Fig. 5(A). During biogas potential test, maximum VS removal efficiencies of 50, 57, 64, 67 and 64 % were observed in 1% protease addition with IWS, FW, 1:1, 1:2 and 1:3 mixing ratio respectively. The VS removal efficiencies increased with increase in protease concentrations and found to be similar for 1:1 and 1:3 mixing ratios in concurrence with their specific biogas production as well. Based on the overall study, the maximum VS removal efficiency of 67% was observed in 1:2 mixing ratio with 1% protease addition.

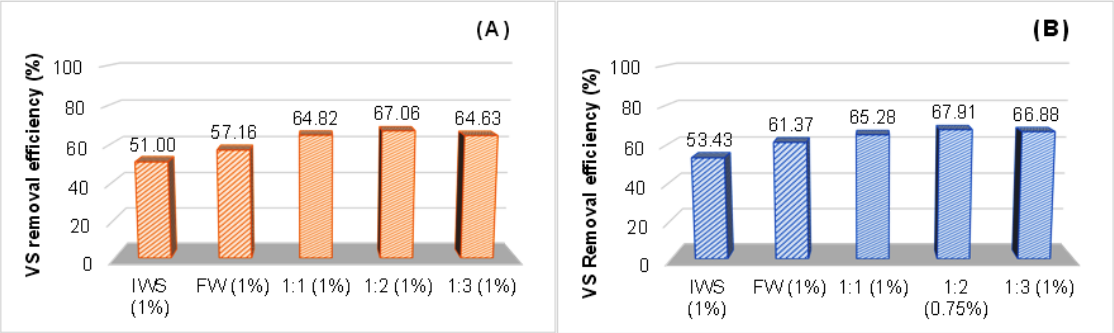

Fig. 5 Maximum VS removal efficiency on (A) Protease (B) lipase enzyme addition biogas potential test of IWS, FW, 1:1, 1:2 and 1:3 mixing ratios

The VS removal efficiency on lipase enzyme addition during biogas potential test with IWS, FW, 1:1, 1:2 and 1:3 mixing ratios are shown in Fig. 5(B). The maximum removal efficiencies at the end of 30th day were found to be 53.4 (IWS with 1% lipase), 61.4 (FW with 1% lipase), 65.3 (1:1 mixing ratio with 1% lipase), 67.9% (1:2 mixing ratio with 0.75% lipase) and 66.9 (1:3 mixing ratio with 1% lipase). Based on the overall study the maximum VS removal efficiency of 67.9 % was observed in 1:2 mixing ratio with 0.75 % lipase addition.

Profile of process parameters during AD

The profile of process parameter such as pH, VFA, Alkalinity, total ammonia nitrogen and free ammonia were monitored during protease and lipase addition in batch biogas potential test with IWS, FW and different mixing ratios. The influence of these monitored parameters and their impacts on the performance of the reactor during lipase and protease addition in batch

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biogas potential test with IWS, FW and different mixing ratios and the same is discussed in the section.

pH

Average pH profile duringprotease enzyme addition biogas potential test with IWS, FW, 1:1, 1:2 and 1:3 mixing ratios are shown in Fig. 6(A). In IWS at the end of 10th day, the pH was in the range 7.8 to 7.9, whereas in the FW, the pH was in the range of 6.5 to 6.7. Similarly in the mixing ratios of 1:1, 1:2 and 1:3, pH values were in the range of 7.4 to 7.6 ; 7 to 7.3 and 6.9 to 7.1 respectively. In the case of IWS, pH value reduced to the range of 5.4 to 5.8 at the end of 30th day. It is reported that the degradation of protein in IWS using protease enzyme resulted in high concentration of TAN accumulation inside the reactor and hence the FA concentration was reached as high as 133 mg/L. Edström *et al.* 2003 reported that free ammonia of 80 mg/L also inhibited the methanogenic activity during anaerobic digestion of slaughterhouse solid waste. Whereas in FW with less protein content, pH was around 6.5 to 6.7 at the end of 10th day and hence FA concentration in the reactor was 42 mg/L, which maintained self-buffering capacity of the reactor and at the end of 30th day, pH was in the optimum range of 7.2 to 7.5. Similar trend was observed in 1:3 mixing ratio due to higher quantity of food waste. Whereas in the case of 1:2 mixing ratio, pH was in the optimum range throughout the study period.

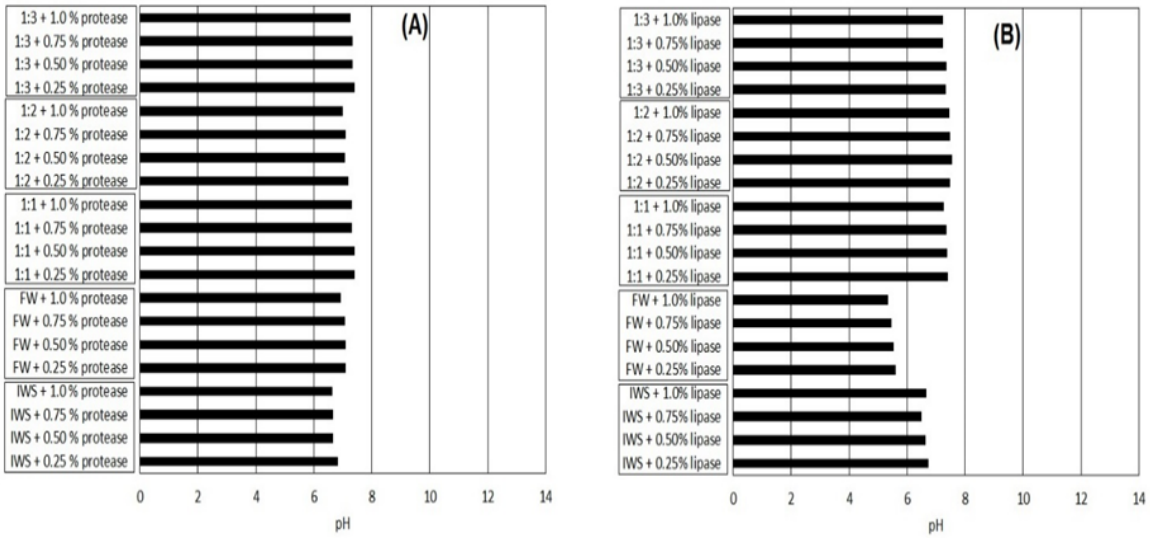

Fig. 6 Average pH of the reactor during (A) Protease (B) lipase enzyme addition biogas potential test of IWS, FW, 1:1, 1:2 and 1:3 mixing ratios

The pH profile of lipase enzyme addition in biogas potential test with IWS, FW, 1:1, 1:2 and 1:3 mixing ratios are shown in Fig. 6(B). The pH was in the range of 6.7 to 6.9 during lipase addition in biogas potential test with IWS, at the end of 10th day, whereas in the FW, pH was in

the range of 5.5 to 5.7. Similarly in the mixing ratios of 1:1, 1:2 and 1:3, pH was in the range of 7.1 to 7.3; 7.4 to 7.5 and 6.8 to 7.1 respectively. In the case of IWS and FW, pH was reduced gradually from 10th (6.7 to 6.9 and 5.5 to 5.7) to 30th day (6.3 to 6.5 and 5.2 to 5.5) due to increase in the VFA production of the reactor with lipase addition. At the same time, the lower pH resulted in reducing the FA inhibition obtained from protein degradation. In the case of mixing ratios 1:1, 1:2 and 1:3 the pH values were in optimum range throughout the study period.

VFA and Alkalinity

The VFA and alkalinity profile during protease enzyme addition during biogas potential test with IWS, FW, 1:1, 1:2 and 1:3 mixing ratios are shown in Fig. 7(A).

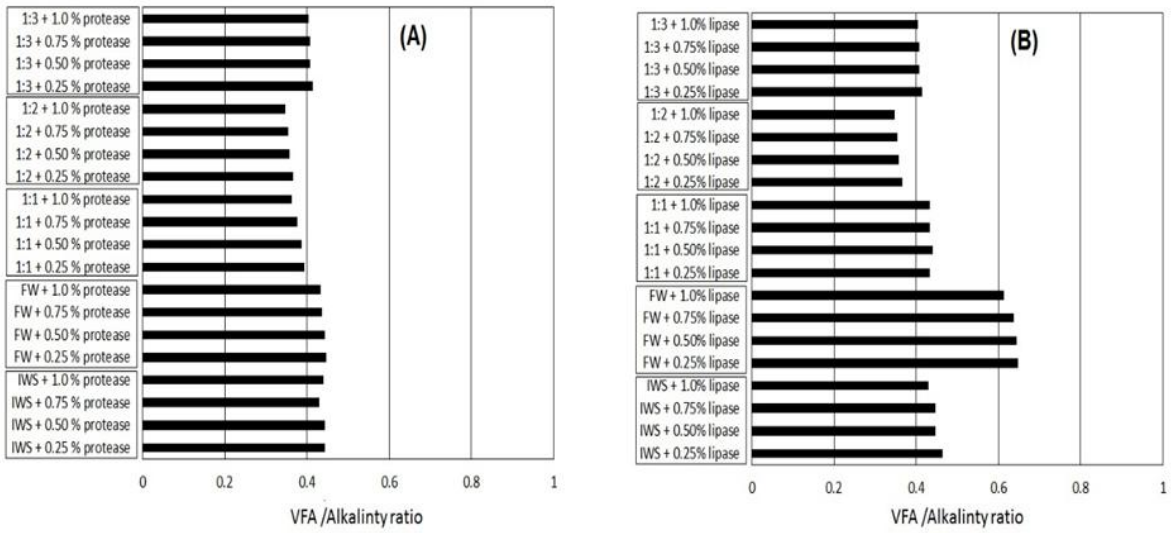

Fig. 7 Average VFA/Alkalinity ratio of the reactor during (A) Protease (B) lipase enzyme addition biogas potential test of IWS, FW, 1:1, 1:2 and 1:3 mixing ratios

In the IWS reactors with different protease concentration, the VFA concentrations at the end of 30th day were in the range of 2167 to 2217 mg/L respectively, which is very high compared with other FW and different mixing ratio (1:1, 1:2 and 1:3). VFA concentrations got increased from 10th to 30th day showing the VFA accumulation in the reactor due to protein degradation which in turn results in accumulation of TAN and FA concentrations in the reactor. The alkalinity in IWS reactor at the end of 30th day were in the range of 5040 to 5255 mg/L. However, the VFA/alkalinity ratio obtained were in the range 0.42 to 0.47, which means slight instability of the anaerobic process and hence reduction in biogas production was also observed when compared with FW and different mixing ratio of IWS with FW. In the case of FW, VFA concentration was reduced from 10th day to 30th day due to faster degradation of carbon source in the food waste to VFA and subsequent utilization of VFA for methane production. Based on the alkalinity concentration in the range of 2018 to 2677 mg/L at the end of 30th day and

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VFA/alkalinity ratios of the reactor were estimated to be in the range of 0.44 to 0.45. Similar trend was observed in mixing ratio 1:3 where larger amount of food waste portion leads to VFA accumulation. But in 1:3 mixing ratio the biogas production was a higher than FW. Similar trend as that of IWS reactor was observed in 1:1 mixing ratio. But the VFA/alkalinity ratio were in the range of 0.34 to 0.4, which is within the acceptable limit and in turn resulted in higher biogas production when compared with IWS. In the case of 1:2 mixing ratio, the VFA concentration were in the range of 922 to 995 mg/L at the end of 30^{th} day. It is observed that there was a gradual reduction in VFA concentration with time indicating conversion of VFA to methane. Similarly, the alkalinity concentration were of 1:2 mixing ratio in the range of 2500 to 3233 mg/L at the end of 30^{th} day, self-buffering capacity was observed in the reactor and VFA/alkalinity ratio were in the range of 0.34 to 0.37, which is suitable for anaerobic reactor thereby assuring the stability of the reactor.

VFA and alkalinity profile during lipase enzyme addition in biogas potential test with IWS, FW, 1:1, 1:2 and 1:3 mixing ratiosare shown in Fig.7(B). In IWS reactor with different lipase concentration, VFA concentrations at the end 30th day were in the range of 3007 to 3078 mg/L respectively. The VFA/alkalinity ratios were in the range of 0.42 to 0.48. While in FW reactor, VFA/alkalinity ratio was in the range of 0.6 to 0.65 indicating instability in the reactor.In case of 1:1, 1:2 and 1:3 mixing ratio, VFA concentration was found to get reduced gradually and resulted in enhanced VS removal efficiency and biogas productions of the reactor. Due to degradation of protein present in IWS, 1:2 and 1:3 mixing ratios, which increases the TAN concentration and improved self-buffering capacity, VFA/ alkalinity ratio was maintained in the acceptable range of 0.34 to 0.37 and 0.39 to 0.41 respectively. Whereas in the case of 1:1 mixing ratio, VFA/ alkalinity ratio was a little higher than the acceptable value of 0.4 i.e 0.42 to 0.45.

Theoretical retention time

Theoretical retention time for 1 % protease addition with IWS,FW, 1:1,1:2 and 1:3 mixing ratios were estimated to be 15, 13, 14, 8 and 9 days respectively. It was observed that the lowest theoretical retention time of 8 days was observed in the mixing ratio of 1:2 with 1% protease addition reactor with a biogas production of 0.46 L/g of VS added (80% of 0.58 L/g of VS added). Similarly, theoretical retention time of lipase enzyme addition with IWS, FW, 1:1,1:2 and 1:3 mixing ratios were estimated as 16, 13, 7, 10 and 11 days respectively. The lower theoretical retention time of 7 days was observed during 1 % Lipase addition with 1:1 mixing ratio, with a biogas production of 0.41 L/g of VS added.

Conclusion:

The effect of protease enzyme addition (0.25, 0.50, 0.75 and 1%) in biogas potential test was studied with IWS, FW, 1:1, 1:2 and 1:3 mixing ratios. Maximum specific biogas production at the end of 30th day were 0.21, 0.37, 0.46, 0.54 and 0.56 L/g of VS added with 1% protease enzyme addition for IWS, FW, 1:1, 1:2 and 1:3 mixing ratios respectively. The maximum specific biogas production of 0.56 L/g VS added in 1:3 mixing ratio during 1% addition of protease enzyme was found to be higher than the respective biogas productions in IWS, FW, 1:1 and 1:2 mixing ratios with 1% protease by 166, 51, 21.7 and 3.7 %. In the case of effect of lipase addition (0.25, 0.50, 0.75 and 1%) study, the maximum specific biogas production at the end of 30th day were observed in the following order: 0.58 (0.75% lipase 1:2) > 0.57 (0.75% lipase 1:3) > 0.51 (0.75% lipase 1:1) > 0.38 (1% lipase FW) > 0.26 (1% lipase IWS). The maximum specific biogas production of 0.58 was observed in 1:2 mixing ratio with 0.75% lipase addition, which was 123, 52.6, 13.7, and 1.7 % higher than IWS, FW, 1:1 and 1:3 mixing ratio respectively. The maximum biogas production 0.58 L/g VS added in 1:2 mixing ratio with 0.75% lipase addition was found to be 177, 55, 26, 7.4 and 2.2 % higher than the maximum biogas production from 1% protease addition with IWS, FW, 1:1, 1:2 and 1:3 mixing ratios respectively. It is concluded that over all protease and lipase addition biogas potential test study, 1:2 mixing ratio with 0.75% lipase addition showed enhancement in biogas production compared with IWS, FW 1:1 and 1:3 mixing ratio with lipase and protease addition.

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BACTERIA: AN OVERVIEW

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Abstract:

Bacteria are unicellular microorganisms which present in everywhere. They play both harmful and beneficial role in the earth. In this chapter, all the characteristic of bacteria were reviewed, collected and compiled from various resources. After reading this chapter, we able to describe the general characters of bacteria, explain the structure of bacteria, gain knowledge about bacterial classification, describe the nutritional types of bacteria, know the growth phases of bacteria, and understand the physical chemical factors which affect bacterial growth, explain the types of reproduction of bacteria and describe the harmful and beneficial role of bacteria.

Keywards: Flagella, Growth curve, Reproduction, Harmful and Beneficial of bacteria

Introduction:

Several types of microbes are living in the earth with peculiar morphology. Depend on the presence of cell organelles and biochemical properties, the microorganisms are divided into two important groups namely prokaryotes and eukaryotes. Micro algae, bacteria and viruses are comes under prokaryotic group. Fungi, Bryophytes, Pteridophytes, Gymnosperms and Angiosperms are comes under Eukaryotic group [1]. Many unicellular organisms like bacteria and viruses that have a variety of sizes, shape, and envelope structures. The minimal requirements are cytoplasm, a cell membrane that surrounds the cytoplasm, and a DNA chromosome. Most bacteria are present in every place like soil, water, air, hot and cold area. Among them, few groups of bacteria depend on other living organism for their survival. In human body, bacteria commonly occur in the digestive system. The soil living bacteria play an important role for cleaning the environment by decomposing the dead matters. Among the various group of bacteria, some genus cause diseases in plants, animals and human. But few groups of bacteria play a vital role in food and dairy industry. Plant pathogenic bacteria are very dangerous to

crops, because they cause severe damage in products and yield loss to the farmers [1]. Likewise, some animal and human pathogenic bacteria are also cause highly poisonous diseases in human.

General characters of bacteria:

Bacterial cell wall gives a definite shape to the bacteria. Bacteria contain a single cell. They range in size from 0.5 µm - 10µm. Bacteria are individual living cells. Many bacterial species exhibit similar characters. Hence it is very difficult to identify them. They are commonly separated by their staining properties and morphological characters. Based on its staining properties, the bacteria are classified into two major group like Gram positive and Gram negative. A very few common characters are found in bacteria i.e, microscopic in size, Single cellular and presence of nucleoid (Nucleus like structure) [2].

Bacteria are considered as pioneer organism of all the advanced life forms in earth [3]. Because they have simple structure and cellular organization. All the membrane bound organelles i.e., nucleus, mitochondria, chloroplast and golgi bodies are absent in bacteria. Instead of nucleus, nucleoid (false nucleus) is present. It is considered as bacterial genetic material. Apart from these an extra chromosomal circular DNA is present in the bacterial cell. It is called as plasmid. Every bacterium has mesosome and ribosome (70 S) for protein synthesis. The bacteria can locomote with the help of flagella, from one place to another place. The number and arrangement of flagellum may vary from one to another species. Bacterial cell wall is covered by hair like appendages called as pili [3]. During conjugation, the sex pili form the conjugational tube between two bacteria. Some bacteria contain a smooth covering on its cell wall. It is called capsule. The bacteria which contain the capsule are called capsulated bacteria. They can form smooth colonies in growth medium.

Morphology and Size:

The external and internal structure of bacteria can be studied using Ultra electron microscope.

Size of the Bacteria

Bacteria are smaller which can be visualized only under magnification. Bacteria of medical importance generally measure 0.2 – 1.5 µm in diameter and about 3-5 µm in length [4].

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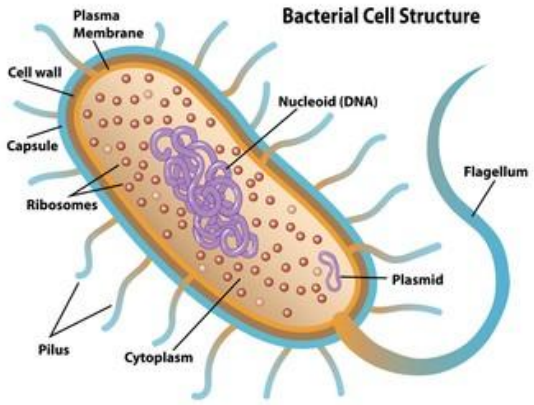
Ultra structure of bacteria



Cell wall

The cell wall is the outermost layer which gives shape and protection to the bacterial cell [4]. It is composed of many layers of peptidoglycans. Numerous N-acetyl muramic acid molecules are joined together to form peptidoglycan layer. They are cross linked. The peptidoglycon layer carries some chemicalcompounds like mucopeptides,teichoie acid and protein etc. The cell wall contains peculiar antigens of particular species [5].

Capsule

Many bacteria have a thick outer covering is called capsule. It is firmly attached to the cell wall and made up of polypeptides. Based on its presence, bacteria are classified into two groups namely capsulated and non capsulated bacteria. Bacteria with capsule can produce smooth colonies. The main function of the capsule is giving protection against antibiotics, drying and phage infection to the bacteria[5].

Flagella

In bacteria, one or more long unbranched tail like appendages present in the outer surface of the cell wall is called flagella. They are used for locomotion. They are present at one or both side of the bacterial cell. The flagellum is made up of three components namely basal body, hook and a shaft. The basal body consists of four rings namely L-ring, P – ring, S- ring and M ring. The hook connects the basal body with the shaft. The flagellum is made up of a specific protein called flagellin [5].

Fimbriae and pili

The hair like appendages arising from the surface of the bacteria called pili. They are otherwise called as fimbriae. They are very short when compared with the flagella. In common pili are present in gram negative bacteria. Pili are made up of specific protein called pilin or fimbrin. Some bacteria contain sex pili. It is long other than other pili. The bacteria with sex pili

are called F^+ cells and other group called F^- cells. The pili involved in the clumping of cells and transfer of genetic material between F^+ cells and F^- cells [5].

Cytoplasmic content

The cell membrane encloses a colloidal material is called cytoplasm.it does not exhibit streaming movement. It contains ribosomes, mesosomes and nucleoid. Other membrane bound organelles (mitochondria, chloroplast, lysosome, and endoplasmic recticulum) are absent.

Ribosomes

Ribosomes are the centres of protein synthesis. The bacterial ribosomes are 70 S type. They are made up of two subunits namely, 50 S and 30 S. the ribosomes are free floating organelle in the cytoplasm. Sometimes, the ribosomes are arranged in a linear series and attached to mRNA. Such ribosomes are called polyribosomes [6].

Mesosomes

Mesosomes are the invaginated structures formed by the localized infoldings of the plasma membrane. They contain vesicles, tubules and lamellar coils. Mesosomes are involved in cell plate (septum) formation in binary fission. They also play an important role in DNA replication, transfer of DNA to the daughter cells, exchange of exocellular enzymes, create a connection between plasmamembrane and nucleoid [7].

Genetic material

The bacterial chromosome is considered as nuclear material. It is not surrounded by nuclear membrane. The nuclear material without a nuclear membrane is called nucleoid. The bacterial chromosome is made up of double stranded circular DNA. In addition to the nucleoid, extra chromosomal genetic elements are present in the cytoplasm. They are called as plasmids. Plasmids are small, circular and self replicating double stranded DNA molecules. They are the boon of recombinant DNA technology (Genetic engineering). Some plasmids can integrate with bacterial chromosomes. Such plasmids are named as episomes [8].

Classification of bacteria:

Based on their shape of the cells, bacteria are classified into four types namely, Coccus, Bacillus, Spirillum and Vibrio.

1. Coccus - Coccus means berry. All the spherical shaped bacteria are called as Cocci. According to the arrangement of cells they are classified as Micrococcus (Single cell), Diplococci (Pair of cells), Tetracocci (Four cells), Streptococci (Chain of cells) and staphylococci (Cluster of cells).

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2. Bacillus – All the rod shaped bacteria are named as bacilli. Based on the arrangement of cells they are classified as Bacilli (Single cell), Diplobacilli (Pair of cells), Streptobacilli (Chain of cells) and staphylobacilli (Cluster of cells).
3. Spirillum – All the spirally coiled shaped bacteria are come under this group.
4. Vibrio – All the curved shaped bacteria are called as Vibrio.

Based on the presence of flagella, bacteria are classified into following types.

1. Absence of flagellum – Atrichous
2. Presence of single flagellum – Monotrichous
3. Presence of flagella at both ends – Amphitrichous
4. Presence of bunch of flagella at one end – Lophotrichous
5. Presence of flagella at all over the surface - Peritrichous

Growth phases of bacteria:

Growth phases of the bacterium are denoted by Growth curve [9].The shape of the growth curve is S. This growth curve is called as Sigmoid curve. It consists of four phases.

Lag phase – This phase is called as preparatory phase. In this phase no cell division occurs.

Log phase – This phase is called as exponential phase. The cells are actively divided and the number of cells increases very fast. More amount of nutrient medium is utilized by the bacteria.

Stationary phase – In this phase, the number of cells remain constant. The growth rate is very slow. The amount of nutrient is decreased and the amount of metabolic wastes is increased in this phase. As the constant number of cells, this phase is called steady phase.

Death phase – In this phase, the number of dead cells is greater than newly produced cells.

Nutritional types of bacteria:

Bacteria need enough suitable nutrients for its growth and reproduction [10]. Suitable moisture, temperature, pH and nutrients are necessary for bacterial growth. Based on the nutrition, bacteria are divided into four groups.

1. Photoautotrophs 2. Photoheterotrophs 3. Chemoautotrophs 4. Chemoheterotrophs.

Photoautotrophs

This group of bacteria can synthesize their own food from carbon source with the help of sunlight. These bacteria are otherwise called as photosynthetic bacteria. Ex: Green sulphur

bacteria. It contains a photosynthetic pigment called bacteriouridin. With the helpof this pigment, the bacteria can trap the sunlight and convert it into chemical energy.

Photoheterotrophs

These bacteria require sunlight but do not require carbon. They utilize organic compounds as carbon source and synthesize their food. These are otherwise called as photo-organotrophs. Ex: Non- sulphur purple bacteria.

Chemoautotrophs:

In the absence of light synthesis of organic food from inorganic substances by utilizing chemical energy is also known as chemosynthesis. The chemoautotrophic bacteria play a very important role in recycling inorganic nutrients.

(a) Nitrifying Bacteria

(b) Sulphur Bacteria

(c) Elemental Sulphur Oxidising Bacteria

(d) Sulphide Oxidizing Bacteria

(e) Iron Bacteria

(f) Hydrogen Bacteria

(g) Carbon Bacteria

Chemoheterotrophs

These heterotrophic bacteria use organic compounds (sugars and amino acids) as the source of carbon as well as energy. These are otherwise called as chemo-organotrophs.

Parasitic:

The bacteria which live in the living cells (host) and nourishes by them are called parasitic bacteria. These bacteria can cause diseases to the host. Hence these groups of bacteria are called pathogens. Ex: *Vibrio colerae*

Saprophytic:

The bacteria which live in dead organic matter are called saprophytic bacteria. they can draw the energy and carbon source from the dead cells.

Symbiotic:

The bacteria which live association with other organism are known as symbiotic bacteria. [9]. These bacteria take food and shelter from the host and give some benefits to the host. Ex: *Rhizobium.* The *Rhizobium* bacteria live in the root nodules of leguminous plants and fix the atmospheric nitrogen to nitrate in the soil. This nitrate is utilized by the leguminous plants.

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Factors affecting bacterial growth:

The bacterial growth is affected by a variety of factors both physical and chemical parameters [11].

Physical factors

Oxygen

Oxygen is one of the environmental factors that affect the growth of bacteria. Based on their oxygen requirement, bacteria are divided as,

1. Aerobes - Bacteria require Oxygen.
2. Anaerobes - Bacteria does not require Oxygen
3. Aero tolerant anaerobes - Bacteria does not require Oxygen, but not affected by Oxygen
4. Obligate anaerobes - Bacteria killed by Oxygen
5. Microphilic aerobes - Bacteria require low amount of Oxygen
6. Facultative anaerobes - Bacteria may grow either presence or absence of Oxygen

Temperature

Temperature is an important factor which affects the growth of bacteria. Each bacterium has a range of temperature where it can grow. Based on it, bacteria are classified in as

1. Psychrophiles - 0^{o} C to 15^{o} C
2. Mesophiles - 20^{o} C - 40^{o}C
3. Thermophiles - 55^{o}C - 80^{o}C
4. Hyperthermophiles - 80^{o}C to 100^{o}C
5. Extremophiles - 100^{o}C to 200^{o}C

pH

Each bacterium has an optimum P^{H} at which it grows best. Most bacteria grow best in a medium with P^{H} of 7. They have been classified as,

1. Acidophiles - pH - 5-6
2. Neutrophiles - pH - 7-8
3. Alkatophiles - pH - 8 - 11

Osmotic Effect

The Osmotic pressure also affects the growth of bacteria [11].

1. Cell in a hypertonic solution --- Plasmolysis/shrinkage of cell
2. Cell in a hypotonic solution --- Bursting of cell

Radiation

Radiations have very harmful effects on bacterial growth.

1. Ionizing radiations - Mutation in bacteria
2. UV radiations - Breaking of DNA in bacteria

Chemical factors:

Nutrients

The growth of the bacteria is also affected by the availability of nutritional factors. Bacteria require Carbon, Nitrogen, Sulphur, Phosphorus and some trace elements (Iron, zinc, Cobalt etc) for their growth. If the amount of nutrients is decreased in the medium it will affect the bacterial growth.

Salt concentration

Bacteria require ions that are given by salts for their growth. But high salt and high sugar in the culture medium or environment leads to loss of water from cell and death. But some bacteria can tolerate high salt concentration and they are called halophiles.

Reproduction of bacteria:

Bacteria reproduce mainly by asexual and sexual methods [12].

Asexual reproduction

1. Binary Fission
2. Conidia formation.
3. Budding formation.
4. Cysts formation.
5. Endospore formation.

Binary fission

most of the bacteria reproduce by transverse division called binary fission. in this method, a bacterial cell divides into two daughter cells by the formation of a septum. first the bacterial enlarges its size and the nucleoid is divided into two. then a constriction is formed in the centre of the cell wall. next the constriction is slowly moved inwards and a septum is formed in the centre of the cell. so that two daughter cells are formed and nucleoid move into both the cells.

Conidia formation:

The bacteria which have mycelia produce rounded spores at the tip of the hypha. These spores are called conidia. In suitable environment, the spore gives rise to new bacterium.

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Budding:

The development of small buds at one end of the parent bacterium is called budding. The buds enlarge and develop into a new bacterial cell. The new cell detaches from the parent cell and lives as a new bacterium.

Endospore formation:

During unfavourable conditions, the nucleoid of bacterium duplicates and the protoplast secretes a thick wall around the nucleoid. This thick walled structure is called endospore. During favourable condition it will grow into new bacterium.

Sexual reproduction

In bacteria, the sexual reproduction takes place through three methods [12].

1. Conjugation 2. Transduction 3. Transformation

Conjugation

The union of two bacterial cells in which the genetic material transfer from one bacteria to other is called conjugation [12].This method of reproduction was first reported in *E.coli* by Lederberg and Tatum. Of the two bacteria, which donates the genetic material is considered as male and F^+ cell. Because it has the genes for sex pili and able to form the conjugation tube. Another bacterium which receives the genetic material is called female cell and F^- cell. A conjugation tube is formed between F^+ cell and F^- cell. One strand of plasmid enters into the F^- cell from F^+ cell. Then the complementary strand of plasmid is formed in both cells. Finally the F^- cell becomes F^+ cell.

Transduction

The transfer of genetic material from bacteria to another through bacteriophage is called transduction. The phage infects the bacterium and multiplies within it. During this process, the new phages pick up certain bacterial genes also. After the lysis of bacterium, the new phages come out and infect another bacterium [12].

Transformation

The process of acquiring new genetic material from the medium by the cultured bacteria is called transformation. When the two strains of bacteria namely avirulent and heat killed virulent cultured in a growth medium, the avirulent strain acquires some genetic material from the heat killed virulent strain present in the medium and become virulent strain [12]. This transformed virulent strain can cause the typical disease. This kind of genetic transformation is

also called Griffith effect. Because this process was first discovered by Griffith in the culture of *Diplococcous pneumonia*.

Beneficial role of bacteria:

Bacteria play vital role in agriculture, medicine, industries and sewage treatment [13]. The important beneficial roles of bacteria are highlighted here.

Soil fertility

The saprophytic bacteria decompose all the dead matters and excreta of animals into simple organic compounds in the soil. This compounds are used as nutrients for plants.

Nitrogen fixation

The bacterial species like *Rhizobium, Clostridium, Azotobacter* etc., fix the atmospheric nitrogen in the soil in the form of nitrate. This fixed nitrogen is utilized by the green plants.

Biopesticides

Some of the bacteria like *Bacillus thuringiensis* are used to kill the insects which affect the crops. Such bacteria are called biopesticide. These bacteria affect only the harmful insect, but not beneficial insects.

Medicines

Many antibiotics and vitamins are derived from the bacterial culture in the industries. Ex: Streptomycin, Tetramycin, Chloromycin, Riboflavin, Ascarbic acid etc.

Industrial uses

Bacteria play an important role in the following industries

1. Dairy industry – Fermented milk, butter milk, curd, butter
2. Biogas industry – Methane production
3. Enzyme production – Amylase, Protease and Glucose isomerase
4. Leather industry - Remove fat from animal skin
5. Textile industry – Retting (Softening of hard fibres)
6. Biomining – leach out copper, cobalt and nickel etc.,

Harmful role of bacteria

Many bacteria cause diseases in living organism and food spoilage [14]. Some harmful activities of bacteria are listed here.

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Food spoilage and poisoning

Food like vegetables, fruits, fish, meat, bread and canned meat items are contaminated by many bacteria. Among them *Clostridium botulinum* causes death to the consumer by causing the disease Botulism.

Plant Diseases

Many bacteria affect the cultivated crops and cause severe diseases in them. They can cause heavy yield loss to the farmers. Some harmful plant diseases which affect the cultivation are listed below.

1. Citrus canker – *Xanthomonas citri*
2. Bacterial Blight disease – *Xanthomonas oryzae*
3. Leaf spot – *Xanthomonas vesicatoria*
4. Black rot – *Xanthomonas campestris*
5. Bacterial wilt – *Erwinia sp.*

Human diseases

Some bacteria infect human beings and cause harmful diseases. Some diseases are listed below.

1. Cholera – *Vibrio chloreae*
2. Typhoid – *Salmonella typhi*
3. Diphtheria – *Corynebacterium diptheriae*
4. Tuberculosis – *Mycobacterium tuberculosis*
5. Whooping cough – *Bordetella pertusis*
6. Leprosy - Mycobacterium *leprae*

Conclusion:

We have concluded that, bacteria are microscopic prokaryotes. They are made up of single cell with nucleoid. All the membrane bound organelles are absent. They are classified based on their shape and flagella. Bacterial cell has cell wall, inner protoplasm and other components. Bacterial growth phase has a lag phase, log phase, stationary phase and a decline phase. Many physical and chemical factors affect the bacterial growth. Based on their nutrition, they are classified as phototrophs, chemotrophs, autotrophs and heterotrophs. Bacteria reproduce mainly by asexual and sexual methods. Bacteria exhibit both beneficial and harmful effects in the surrounding environment.

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DIVERSITY OF PHYTOPLANKTON AND ZOOPLANKTON OF WARDHA RIVER FROM KAUNDANYAPUR AND PULGAON REGION, VIDHARBHA

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Abstract:

The present communication deals with the variations of phytoplankton and zooplankton in the Wardha River of Kaundanyapur, Pulgaon region, Vidharbha. The area Kaundanyapur, Gunjkheda, Panchdhara and Vitala were selected for the sampling. The samples were collected during the period of one year (September 2018 to December 2019). The samples under study were evaluated to study the diversity of phytoplankton and zooplankton. Phytoplankton diversity was shown in the four groups like Chlorophyceae, Cyanophyceae, Euglenophyceae, and Bacillariophyceae. Chlorophyceae were dominant as compare to the other reported groups. Zooplankton shows the variations in groups Viz. Cladocers, Protozoa, Rotifera, Nematoda. The members of Rotifers were dominant over other reported groups.

Keywords: Phytoplankton, Zooplankton diversity, Kaundanyapur, Pulgaon.

Introduction:

Planktons are the group of free floating microorganism. They move at the mercy of currents of winds as well as water. Phytoplankton are chlorophyll bearing suspended microscopic organism mainly algae. Phytoplankton plays an important role in aquatic ecosystem for development and growth of zooplankton and appeared as a paradox (Hutchinson, 1967). Phytoplanktons liberate oxygen during photosynthesis and aid in energy exchange process (Khan, 2003). Phytoplankton forms lowest trophic level in the food chain of fresh water ecosystem (Manoj Kumar and Khare, 2015). The number and species of phytoplankton determine the water quality. Diversity is the important ecological indicator to assess the quality of water. Several researchers have been worked on this issue (Khanna *et al.*, 2012; Budhlani and

Musaddiq, 2014; Kadam *et al.*, 2014; Belkhode and Sitre, 2016; Rawat and Trivedi, 2018; Mithani and Dahegaonkar, 2020).

The river wardha is a major river in Vidarbha region of Maharashtra.Wardha River originate at an attitude of 777 meters in Satpura range near Khaiwani village, Multai tehsil Baitul having length of 528Km. Kaundanyapur and Pulgaon are situated on the bank of River Wardha. The industrialization, urbanization increased waste discharge in the river. The environmental parameters and their consequences on phytoplankton productivity are important. Therefore the study undertaken was to analyse the phytoplankton diversity in river Wardha.

Material and Methods:

The investigation was carried out during (September 2018 to December 2019). Samples were collected from four sampling station Kaundanyapur, Gunjkheda, Vitala, Panchdhara region Pulgaon (Fig.1 – 6); monthly from fresh water at morning between 8.00 am to 10.00 am by using plankton net and collected samples were shifted into the liter plastic bottles.

Figures showing study sites:

Figure 1: Gunjkheda

Figure 2: Kaundanyapur

Figure 3: Kaundanyapur

Figure 4: Vitala region

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Figure 5: Gunjkheda region

Figure 6: Panchdhara region

The collected samples were allowed to centrifuge to concentrate and made up to 100ml after removing the surface water in the centrifuge tube. The population of plankton present in the centrifuge tube was transfer to other bottle and preserved in Lugols Iodine solution of further investigation (Kumara, 2018). The identification of plankton was done by the relevant literature (Adoni, 1985; Batish, 1992).

Result and Discussion:

The four sampling sites Kaundanyapur, Gunjkheda, Vitala, Panchdhara region shows seasonal diversity phytoplankton belongs to 34 species of 14 genera various groups like Chlorophyceae (15 species of 9 genera), Cyanophyceae (9 species 4 genera), Euglenophyceae (4 species) Bacillariophyceae (6 species 2 genera*). Oedogonium* and *Spirogyra* were dominant among other member of Chlorophyceae. The zooplankton in the area investigated shows 21 species and 10 genera of various groups Cladocers (3 species 2 genera), Protozoa (5 species 2 genera), Rotifers (9 species 4 genera), Nematoda (5 species 2 genera). Among these Rotifers were dominant over all reported groups. Few genera act as bioindicators of organic pollution. Majority of protozoans were found in the interval of June and July. The number of planktons was more in summer and gradually minimise in rainy season.

The present communication reveals that the water of Kaundanyapur was found to be more polluted during November to December, when most of the people from all over region come for prayer on the site. The various antropogenic activities cause the disturbance of the plankton diversity. The plankton diversity of Gunjkheda, Panchdhara and Vitala region of Pulgaonwere also changed during the month of September to October. The area is known for the immersion of ashes after funaeral. The ashes immersion cause adverse effect on the

phytoplankton as well as zooplankton diversity. During this period water was so polluted and not safe for drinking.

Table 1: List of Phytoplanktons recorded during the study

Chlorophyceae	
Genera	**Species**
Ankistrodesmus	*Falcatus*
Chlorella	*Vulgaris*
Chlorococcum	*Infusionum*
Cladospora	*Fracta*
Cosmarium	*Tenue*
Hydrodictyon	*Reticulatum*
Pediastrum	*Tetras*
Oedogonium	*Leave, plagiostomum, tapeinosporium, pisanum (Shiv kumar Rai 2012)*
Spirogyra	*chungkingensis, comdensata, longata, mirabilis*
Cyanophyceae	
Anabaena	*Fertilissimia*
Lyngbya	*Magnifica*
Nostoc	*Sp.*
Ocillatoria	*Limosa*
	Anacystis, botrycoccus chrococcus phormidi, rivularia
Euglenophyceace	
	acus, viridis, caudatus, gracilis
Bacillariophyceae	
Navicual	*Viridula*
Synedra	*Ulna*
	Diatom sp.amphore.stauronesis, cyclotella

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Table 2: List of Zooplanktons recorded during the study

Cladocera	
Genera	Species
Bosmina	*Longirostirs*
Daphnia	*Carinata*
Protozoa	
Arcella	*arenaria, conica, dentata, rota*
Balantidium	*Coli*
Rotifera	
Asplanchna	*Intermedia*
Brachionus	*calyciflorus, caudatus, falcatus, plecatilis, rubens*
Filinia	*Longiseta*
Keratella	*Tropica, cochlearis*
Nematoda	
	Salasi sp.
	Javanica, hapla, americanus, ascaris
	Ostertamia, Tylenchus (L.B.Chanu *et al.*, 2014)

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A PERSPECTIVE REVIEW OF *HELICOBACTER PYLORI*

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Abstract:

Helicobacter pylori are one of the commonest bacterial pathogen in humans. *H. Pylori* is a micro-aerophillic, small curved highly motile flagellated gram negative spiral bacilli that colonizes in the mucosal layer of stomach. *H. Pylori* is an important human pathogen causing type B gastritis, which is one of the most wide spread infection throughout the world. Usually *H. Pylori* gets transmitted and colonized in the mucus of gut by its unique morphological features and may activate gastrin, which activates parietal cells to hyper secrete acid in the stomach. This increased acid secretion likely plays a key role in pathophysiology of duodenal ulcers[1, 2] *H. Pylori* also decreases duodenal bicarbonate secretion weakening the protective mechanisms of the duodenal mucosa and infects the lower part of the stomach antrum. *H. Pylori* cultured from vomitus, diarrheal stools and saliva demonstrates that the bacterium is potentially transmissible by these routes. Both invasive and noninvasive diagnostic tools help to diagnose the invasion of this bacterium. Combination therapy of proton pump inhibitors and gastro protective coat forming suspension, along with anti-bacterial agents makes up an *H. Pylori* combo kit to treat *H. Pylori* infection. This article mainly focuses on the epidemiology, pathophysiology, and diagnosis and treatment strategy followed to combat *H. Pylori* infections.

Introduction:

The Nobel Prize in physiology and medicine 2005 was awarded jointly to Barry J. Marshall and Robin Warren, two Australian researchers who discovered the bacterium in 1982, *H. Pylori* and its role in gastritis and peptic ulcer disease. The discovery of *H. Pylori* offered the etiologic agent for initiating the inflammatory cascade of gastro intestinal tract.[3, 4] *H. Pylori* infection is followed by chronic active gastritis, glandular atrophy and intestinal metaplasia. It has been confirmed that the development of gastric cancer started with the acquisition of higher rates of histologically and serologically detected *H. Pylori* positivity it has been reported in early stage of cancer than in advanced cancer.[5, 6]

H. Pylori was found in 66.5% of men and 63.2% of woman worldwide. The common risk factors for the spread is by drinking water, lack of toilet facilities during childhood, lower family income, lower literary level and previous Gastro intestinal problems.

The gastric inflammatory process (Gastritis) is variable and independent of the presence symptoms of H.Pylori[7]. Currently *H. Pylori* is considered as a main cause of chronic gastritis.[8, 9] the inflammatory process is associated with the development of ulcers, atrophy, intestinal metaplasia, dysplasia, gastric adrenocarcinoma and MALT lymphoma (Gastric mucosa associated lymphoid tissue lymphoma.)

Both invasive and non invasive tests are carried out, Methods by invasive test are classified based on infection and atrophy of gastric mucosa, *H. Pylori* can be detected by histology culture or urease test. Non invasive methods include ELISA antibody or latex agglutination tests since, *H. Pylori* infection elicit a local mucosal and systemic antibody response. Non invasive methods are simple, reproducible, and can be done in stored samples. A Combination of proton pump inhibitors (PPI) based triple therapy has a first line treatment choice; a combined therapy of PPI, Antibiotics and antimicrobial agents are used to eradicate H.Pylori.

This article mainly focused on scientific perspective of H.Pylori, via epidemiology, pathophysiology, diagnosis and treatment.

Epidemiology:

H. Pylori is a curved spiral shaped highly motile gram negative bacillus with flagella, whose colonies are available, only on the mucus layer of the human stomach. It causes chronic gastritis, peptic ulcer disease and lympho proliferative disorders and is major risk factors for gastric cancer.10 the common risk factor therefore includes crowding, type of drinking water, and lack of toilet facilities during childhood, neglecting basic hygiene rules and Low socio-economic status[11].Human stomach is considered as the reservoir of this pathogen. Routes of transmission are Fecal-oral route and Gastro oral route (water could be a vehicle.)

H. Pylori could be considered as a waterborne pathogen. Water is risk factor for *H. Pylori* transmission. *H. Pylori* has been cultured from vomitus, diarrheal stool and saliva, demonstrating that the bacterium is potentially transmissible by these routes[12, 13, 14]. Infected household member with gastroenteritis carried a 4-8 fold increased risk for transmission. Such “close contact infection” depends on the degree of mixing and age distribution between susceptible as infected individuals.

H. Pylori infection leads to an increased risk, in the order of 4 to 9 folds of developing precancerous gastric conditions especially when the infection occurs in childhood. In 1994 the International Agency for Research on Cancer (IARC) monograph committee classified *H. Pylori* as a class I carcinogen to humans.[15, 16]

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Reproduction and Life Cycle:

H. Pylori reproduces and multiplies through binary fission, as with most bacteria. During binary fission, bacterial chromosomes replicate as the cell enlarges. This is then followed by division of the chromosome and formation of a septum that ultimately divides the cell into two.

The two halves of the chromosome ultimately separate and as the cell divides into two it allows each daughter cell to remain with the chromosomes and other cell components (cytoplasm, ribosome etc). This process creates two daughter cells.

During binary fission, enzyme helicase breaks the bonds that hold the nucleotide bases together to unzip the molecule. Other protein such as DNA polymerase, then play an important role in adding new nucleotides in order to build a double stranded DNA molecule for each of the daughter cells.

As mentioned, the bacteria inhabit part of the gastric mucosa where they live and multiply. This may occur outside the cells (epithelial cells) of the gastric mucosa or inside the cells (as invasive organisms). As they grow and multiply, they may be excreted along with fecal matter and into the environment (Water sources etc). By ingesting contaminated food or water etc, the bacteria may enter human beings (fecal-oral route) allowing them to pass down the esophagus to the gastric mucosa where the life cycle continues. Through this mode of reproduction and life cycle, the bacteria can continue to thrive as it spreads from one host to another.

Morphology:

The morphological form of this H-Pylori is curved to spiral; [17, 18]that resembles a corkscrew, short (or) tapered rod S- Shaped bacterium with 1 to 3 turns, 0.5*5 micro metre in length, and 30 micro metre in diameter. They also have branched/threads of 5-7 flagella attached to one end of the bacteria (on one polar end). The flagellum is covered in a sheath that protects their cells and structures. All helicobacter use flagella and taking advantage of its shape, play an important role in helping bacteria move easily in their environment. *H. Pylori* is able to rapidly move from one location to another.

Structure of the Flagella:

A basal body- Compared to the other parts, the basal body is the most complex structure that serves as the anchor to the flagellum. It is composed of concentric rings on different layers of the cell wall and a protein rod, which contributes to its complexity.

The filament- is the outer most part of the flagellum (tail-like) and is comprises of flagellin (globular protein)

Hook-is the part that connects the filament to the basal body.

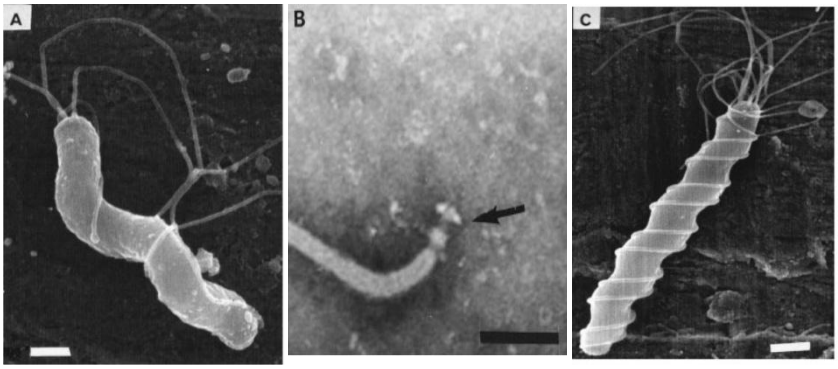

Figure 1: Morphology of helicobacter.

(a) S-Shaped *H. Pylori* with 5-7 sheathed polar flagella. (b) Detail of the flagella hook.

(c) Helical shaped H.Felis with periplasmic fibres in pairs and bipolar tufts of sheathed flagella.

Pathogenesis of *H. pylori* infection:

The gastric mucosa is well protected against bacterial infections due to the acidic pH of the lumen, [21, 22] *H. Pylori* lives in an acidic environment of the stomach; the initial infection is transmitted by the oral route. The microaerophilic bacterium uses its flagellae to move in the gastric mucus layer. Flagellum is used for swimming and its ability to secrete urease enzyme makes it possible for the bacterial to survive in the antral surface. The organism moves from one location to another within the gastric antrum and to more favorable areas when required. *H. Pylori* bacterium causing peptic ulcer disease. This infection is caused by secreting large amounts of enzyme urease. Using this enzyme, the bacteria can effectively breakdown endogenous urea to ammonia (as well as bicarbonate which is a base) thus creating favorable conditions for survival. By converting urea to ammonia, the ammonia buffers the H+ and forms ammonium hydroxide, creating an alkaline cloud around the bacterium, *H. Pylori* causes the pH level in its environment to rise and protect it from the acidic environment of the stomach. Production of the enzyme urease is largely dependent on the pH level.

When the pH level around the bacteria is low, channels on the bacteria cell membrane are activated, which stimulates production of the enzyme. Apart from producing ammonia which increases the pH level around the bacteria, the enzyme has also been shown to have toxic effects on the cells of gastric epithelia. It contributes to the infection and thus stimulates an immune response causes gastric epithelial cell injury[23].

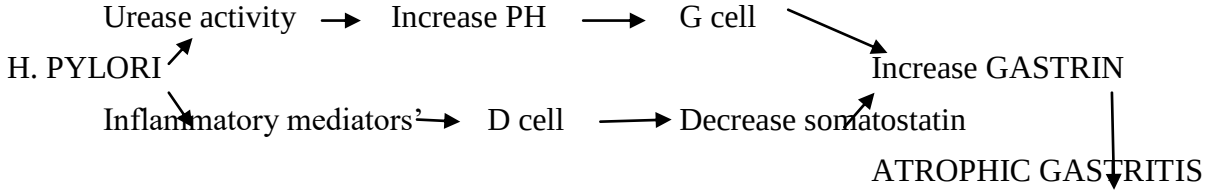

Figure 2

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Due to *H. Pylori* colonization in the antral mucosa, G endocrine cells in the distal antral region of the stomach are activated to release the hormone gastrin, which circulates and stimulates parietal cells in the corpus (body) region of the stomach to hypersecreation acid. By gastrin elevated mechanisms, ammonia generated by *H. Pylori* produce alkaline environment near G cell, thus stimulate gastrin release. Lower antral D cell in presence of *H. Pylori* infection decreases somatostatin production that increases gastrin release. *H. Pylori* also decreases duodenal bicarbonate secretion, thereby weakening protective mechanisms of the duodenal mucosa Shown in Figure.2

Recent studies have shown that *H. Pylori* bacteria mainly release specific **cytotoxin** causing duodenal ulcer. Cytotoxicity caused by *H. Pylori* has also been linked to gene proteins (Exotoxins) in epithelium cell. Cag A, a homologue of the Bordetella pertussis, Cag A, induces IL-8 in epithelial cell (Chemokines response), producing inflammatory immune response and vacuolating cytotoxin (VacA), associated to gastric mucosal surface, induces vacuolating toxin leading gastric mucosal injury and the induction of disease[27, 28] Other virulence factors include lipopolysaccharide (adhere to host cell inflammation). -Endotoxins, which are components of the bacteria's outer membrane as well as a lipase, mucinase and protease that are secreted by the bacteria.

A gastric ulcer is a peptic ulcer in the stomach. A duodenal ulcer is a peptic ulcer in the duodenum. *H. Pylori* infects the lower part of the stomach antrum. Gastric inflammation may lead to duodenal or gastric ulcer. Severe complications include bleeding ulcer and perforated ulcer[38, 39, 40.]represented in Figure.3

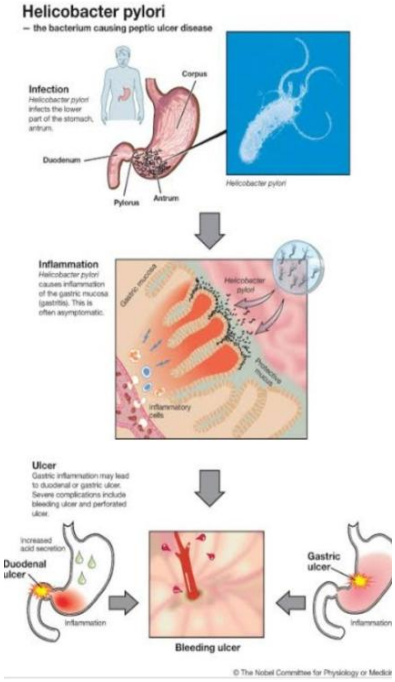

Figure 3: *H. Pylori* colonization of the Antral region gastritis

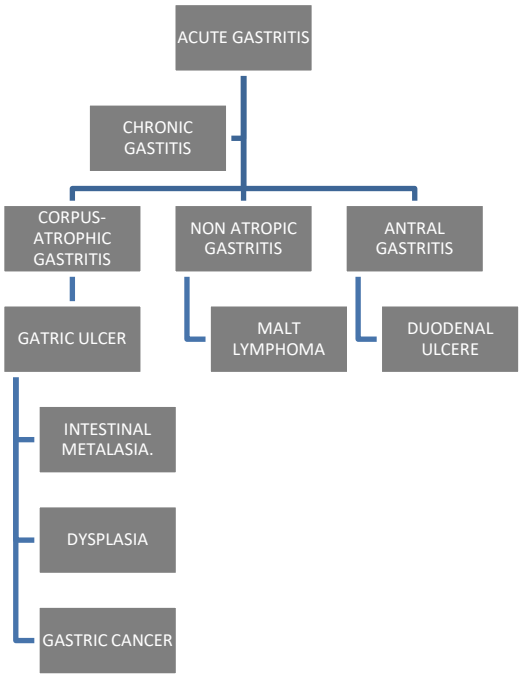

Figure 4: Representation of gastritis and related gastric diseases

Mechanisms of gastric inflammation in H. *pylori*

H. Pylori may produce substances that degrade mucin and injure epithelial cells, thereby reducing the resistance of the mucosa to acid injury. Alternatively, some suggest that *H. Pylori* produces **chemotactic** substances that attract and activate neutrophils and other inflammatory cells, with the activated leucocytes, causing tissue injury**.**[24,25,26] Considering the *H. Pylori* induced mucosal inflammation. There are two main mechanisms.

First, direct mucosal injury as the organism interacts with gastric epithelial cell accompanied by loss of microvilli, irregularity of the luminal border and intracellular changes including loss of cytoplasm, oedema and vacuolation. Surface epithelial degeneration correlates with the number of *H. Pylori* in close contact with the epithelial plasma membrane, a finding that support a direct toxic effect of bacterial products on epithelial cells. This in turn leads to direct cell damage (or) epithelial infection liberates pro-inflammatory mediators (**chemokines messengers)**. *H. Pylori* infection rapidly regulates the expression of IL-8 in human gastric epithelial cells[29]**.**

Secondly, *H. Pylori* – derived products may gain access to the underlying mucosa, directly stimulating host **non specific** immune response (the microbial attack usually involves a polymorph) {PMN – Polymorphs nuclear -Neutrophils, Basophils, eosinophil's} and Specific immune response**. (**T-lymphocytes and plasma cell). Specific and non specific immune responses involve the liberation of a variety of **cytokine** messengers. (Figure:5, Table:1). These cytokine messengers (IL-1 beta & TNF alpha) cause gastritis[30, 31, 32, 33, 34, 35].

IL-1 beta - Decrease acid secretion.

TNF alpha - Increase COX 2, Gastrin and growth factor.

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These messenger Cytokines produces chronic gastritis followed atrophic gastritis.

Table 1: Messengers for *H. Pylori* gastritis

CHEMOKINES	CYTOKINES
IL-8 (Neutrophil recruitment and activation)	TNF Alpha (pro inflammatory) Activation of leukocytes
GRO-Alpha	IL-1Alpha/Bet(pro inflammatory) Activation of leukocytes
RANTES	IL-6
MIP-1 Alpha	IL-7
	IL-10
	IL-12
	INF-Gamma
	GM-CSF

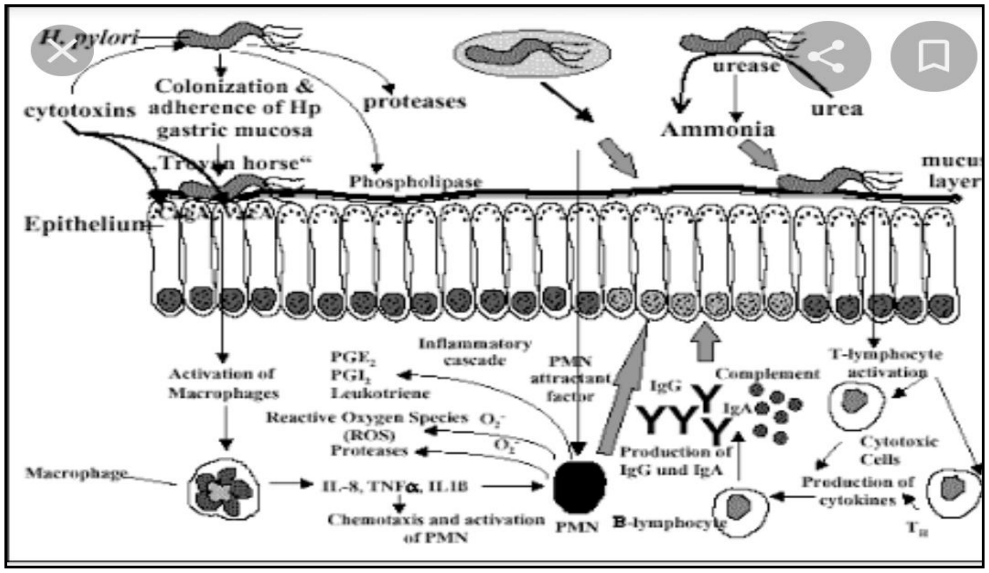

Figure 5: Mechanism of gastritis induced by *H. Pylori* in stomach

The mucosal immune system serves as the body first line defence from antigens and infections. T-cells play a vital role in antigen-specific immune responses and infection. T-helper cells are classified into 2 main types, Th1 lymphocytes, Th2 cells and antibody production including mucosal IgA. The secretion of IgA provides an immune response to potential antigen in food[36, 37].By secretion of IgA antibody (activation of lymphocytes and production of Th), *H. Pylori* elicits Th1 response with the release of cytokines leading to inflammation and epithelial cell damage. *H. Pylori* causes over 90% of duodenal ulcers and up to 80% of gastric ulcers. Infected persons have a 2 to 6 fold increased risk of developing gastric cancer and Mucosal Associated Lymphoid Type (MALT) lymphoma.

***H. pylori* causing diseases**

a. Non- malignant diseases

H. pylori plays a role in non–malignant diseases, such as peptic ulcer disease (PUD), Gastro oesophageal reflex disease (GERD), non ulcer dyspepsia and non steroidal anti inflammatory drug consumption.

1 to 2 week course of *H. Pylori* eradication therapy is an effective treatment for *H. Pylori* positive peptic ulcer disease. *H. Pylori* status has no effect on the speed or degree of GERD symptom relief. The management of dyspepsia in primary care is by antisecretory therapy. *H. Pylori* eradication therapy has a significant effect on H.Pylori-positive non-ulcer dyspepsia and NSAID induced gastric damage in subjects without *H. Pylori* infection.

1. Peptic ulcer disease (PUD) [41, 42, 43]

Gastric and duodenal ulcer, which occur in upper and lower part of GIT respectively, occurs as an imbalance between aggressive (Acid, Pepsin, H.Pylori) and defensive mechanisms (Gastric mucus, HCO_3-secretion, prostaglandins, Nitric acid and innate resistance of mucosal cell). *H. Pylori* infection is the major cause of PUD. Up to 95% of duodenal ulcers and gastric ulcers are associated with *H. Pylori* infection complications of acute and sub-acute peptic ulcers usually heal without leaving any visible scar. However, healing of chronic, visible and deeper ulcers may result in complication such as obstruction, haemorrhage and perforation. Eradication of *H. Pylori* reduces the relapse rate of Peptic Ulcer Disease.

2. Gastroesophageal reflux disease (GERD) [40]

When *H. Pylori* infection is associated with Gastroesophageal Reflux Disease, Patient has infrequent heartburn or dyspepsia (fewer than 3 times per week). Reflux is a very common problem presenting as heat burn, acid eructation. And sensation of stomach contents coming back in the food pipe. Motility disorder, acidity of gastric contents are the most important aggressive factors in causing symptoms and oesophageal lesions and increases the risk of esophageal carcinoma. Two strategies are followed for the management of this condition are to decrease the acid production (by PPI) or to increase the forward movement of GIT (so that the contents do not reflex upwards); the drugs used for increasing the GI motility are known as prokinetic drugs.

3. NSAID associated gastritis and gastric mucosal damage:

NSAIDs, which are weakly acidic, remain undissociated in the strongly acidic environment of the stomach and can easily penetrate the gastric lining and inhibit prostaglandin synthesis there. This inhibition leads to decrease in mucus production, bicarbonate secretion by the gastrointestinal mucosa and mucosal blood flow. Ulcers that are thought to be due to NSAIDs cause gastric mucosal damage. NSAIDs associated gastritis cause acute focal erosions, capillary damage, chronic inflammatory cells and epithelial damage. Patients with a previous history of peptic ulcer and those who have gastritis secondary to infection with *H. Pylori* are more prone than other people to develop peptic ulcer if they take NSAIDs. *H. Pylori* infection has been identified by some studies in more

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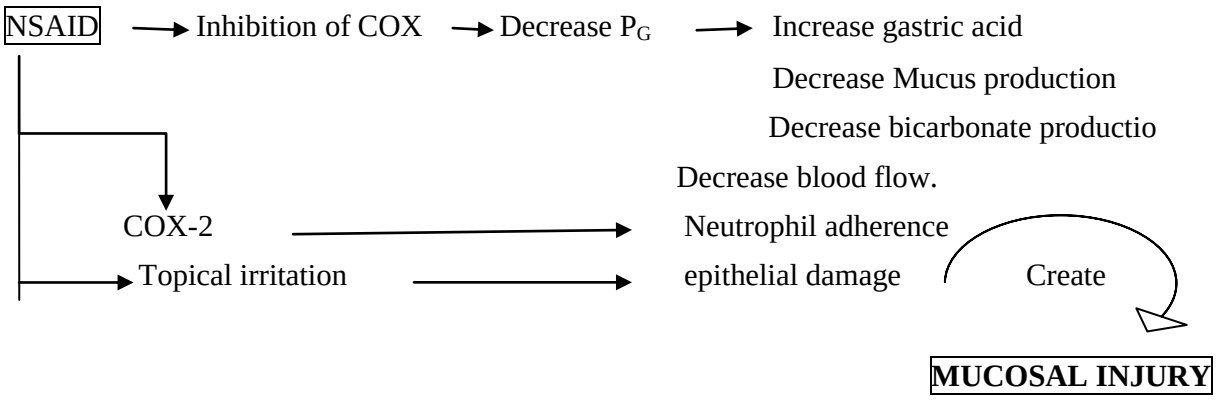
than 95% of people with peptic ulcers. the higher rates of complications and death secondary to peptic ulcer in the elderly may be in a part a reflection of the increased rates of H.Pyroli with age.[44, 45]

Although such estimates may be overstated, the recent studies suggest that it is associated with 80% of duodenal ulcers and 60% of gastric ulcers[46, 47]. Treatment profile shown in Figure: 6, 7.

Figure: 6



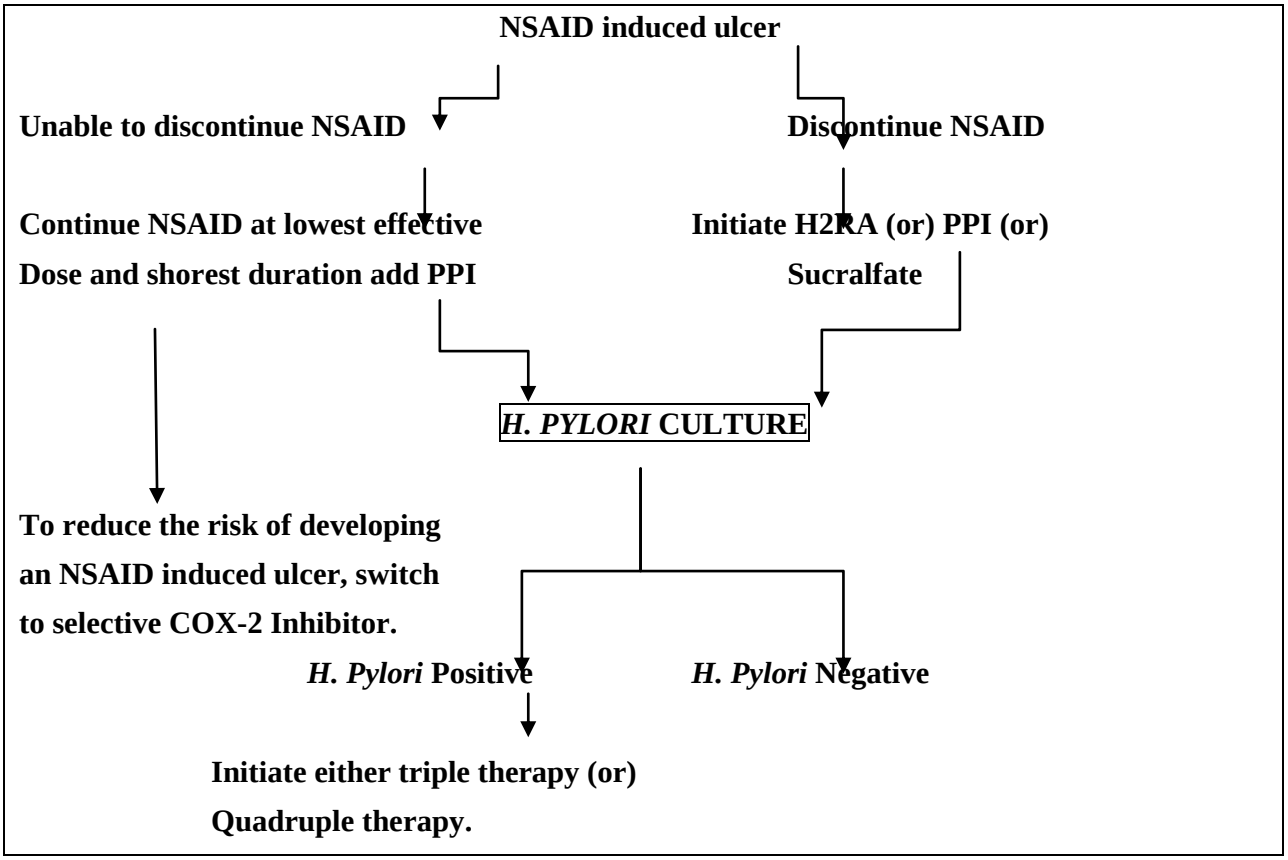


Figure 7: NSAID Induced ulcer treatment

b. Malt lymphoma and Gastric Malignancies[48, 49, 50]

H. pylori eradication is a definitive cure of low-grade gastric mucosa-assiociated lymphoid tissue (MALT) lymphoma which has found worldwide recognition and appreciation. Tumours

located in the distal stomach have a more favourable response than those in the proximal stomach. *H. Pylori* was identified as the most important risk factor for recurrence, and therefore an adequate eradication regimen and accurate regular evaluation for *H. Pylori* status are needed during follow up of primary low-grade MALT lymphoma. *H. Pylori* eradication is a definitive cure of low grade gastric mucosa associated lymphoid tissue. Long-term follow up of patients with MALT lymphoma is recommended as it is also possible that a metachromous gastric cancer can be detected.

Furthermore, chronic *H. pylori* infection is also associated with increased gastric cell turnover, probably of importance in malignant transformation. Gastric carcinogenesis, more intense bacterial infection and more severe polymorph nuclear cell infiltration may contribute to DNA damage and promote carcinogenesis in patients with gastric cancer. *H. pylori* infection leads to increased risks, in order to 4 to 9 folds, of developing precancerous gastric conditions especially when the infection occurs in childhood.

In 1994, the international agency for research on cancer (IAEC) Monograph committee classified *H. Pylori* as a class 1 carcinogen to humans.

Screening methods of H. *pylori* [52, 53]

1. Invasive tests

Screening by magnification endoscopy by histology, culture or urease tests. All these are biopsy based methods for detecting *H. Pylori* . The magnified endoscopic findings in the gastric body are classified into four patterns and then correlated with histology results.

- ✓ Type1-Histology of gastric mucosa: Normal antral mucosa with sparse infiltrate of lymphocytes in lamina propria.
- ✓ Type 2 & 3- *H. Pylori* infected mucosa: Active gastritis with neutrophils infiltrating epithelium and marked infiltrate of lymphocytes in lamina propria.
- ✓ Type 4 -Atropy: Atropy of antral mucosa with loss of specialized glands near muscularis mucosa.

Disadvantages:

After partially effective eradication treatment, low level of infection can easily be missed by endoscopic biopsy.

a) Histology

H-Pylori may be recognised in sections stained with haemaoxylin and eosin alone. Figure.8 indicates characteristic morphology of H-Pylori, can be seen in sections from biopsies the gastritis, atropy (or) intestinal metaplasia can also be assessed.

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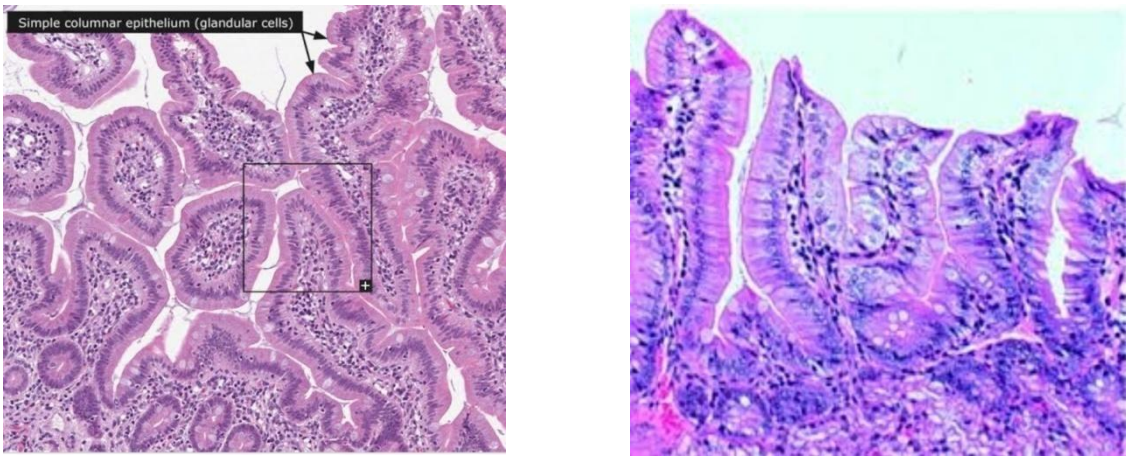

(a) Histology of normal Gut Mucosa **(b) Histology of *H. Pylori* infected gut mucosa**

Figure: 8 Histology of normal and infected gut mucosa

b) Culture

The Culture of *H. Pylori* can be unreliable. Risk of over growth (or) contamination makes it the least sensitive method of detection and it's the least readily available rest for use with endoscopy.

c) Urease test

Urease test are quick and simple tests for detecting *H. Pylori* infection. But indicate only the presence (or) absence of infection. Urease test based on an immunological detection of urease. This test based on pH change.

Eg: - CLO Test(Amphylobactor like organism test)

2. Non-invasive tests:

2. Non-invasive tests:

a) Urea breath test *(C^{13} - UBT)*

C –Urea breath test is based on the principle that a solution of urea labelled with carbon -13 will be rapidly hydrolysed by the urease enzyme of H-Pylori.

C^{13} – urea breath test detects current infection and is not radioactive. It can be used as a screening test for *H. Pylori*.

b) serology

1. ELISA: The colonisation of the gastric and duodenal mucus membranes with *Helicobacter pylori* can also be detected serologically using an enzyme Immino assay (ELISA) or by performing a western blot. Patients with confirmed exposure to *H. Pylori* often show a positive serological result. Since antibodies persist for a longer time after a *H. Pylori* infection, seropositive is also found in symptom free patients. The number of seropositive values rises with age. Using the ELISA and the detection of IgA and IgG antibodies against specific proteins of

H. Pylori by western blot, it is possible to diagnose an acute infection with *H. Pylori* , even if no germs can be found.

The sample with an unknown amount of antigen is immobilized on a plate. After the antigen is immobilized, the detection antibody (IgA, IgG) is added, forming a complex with the antigen. The detection antibody can be covalently linked to an enzyme. The plate is typically washed with a mild detergent solution to remove any proteins or antibodies that are non-specifically bound. Finally, the plate is developed by adding an enzymatic substrate to produce a visible signal, which indicates the quantity of antigen in the sample.

2. NPT (Near Patient Test)

H. Pylori infection elicits a local mucosal and a systemic antibody response. Circulating-IgG antibodies to *H. Pylori* can be detected by enzyme linked imminosorbent assay (ELISA) antibody (or) latex agglutination tests. These tests are simple, reproducible and inexpensive. They have been used widely in epidemiological studies.

Disadvantage:-

- ✓ In elderly people with lifelong infection, underlying atrophic gastritis has been associated with false negative results. Consumption of NSAID drugs has also been reported to affect the accuracy of ELISA.
- ✓ Serology cannot be used to determine *H. Pylori* eradication (or) measure the infection rate.

NPT-(Near Patient Tests) NPT can be performed in primary care and are much simpler than the C-Urea breath test; this is simple finger prick tests.

c) Stool antigen test

The test is based on a sandwich EIA with antigen detection. This is a qualitative test with a polyclonal rabbit anti *H. Pylori* antibody adsorbed to microwells as capture antibody. First, 100 micro litre of a diluted stool sample (10 micro litre, Stool in 0.5 ml sample diluents) and thereafter, peroxidise-conjugated polyclonal antibody solution is added to the wells and incubated for 1 hour at room temperature. Unbound material is removed by washing. After addition of a substrate solution. *H. Pylori* antigen could be detected by a colour change. A stock solution is added and the absorbance is read at 450 nm by spectrophotometer. The results are interpreted as follows: OD_{450} <0.140 was negative. OD_{450}>0.160 was positive.

3. In-vitro screening methods[54]

Methods to colonies *H. Pylori* infection and inducing gastric ulceration for in-vitro anti-ulcer studies are as follows,

Rats were ulcerated by using acetic acid. (according to the method described by Takagi et al., (1969) with modification) under anesthesia, laparotomy was performed in rats. Through a midline epigastric incision the stomach are exposed and 20% acetic acid(0.03 ml) was injected into the subserosal layer of the glandular position, using a microsiringe(0.05 ml), After closing the abdominal incision, the animals were maintained in individual cages with daily access to commercial food

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restricted to the term periods of 9-10 am and 5-6 pm. Thus allowing adequate fasting for administration of *H. Pylori* , and standard drugs (Amoxicillin 50 mg per kg + clarithromycin 25 mg per kg + omeprazole 20 mg per kg) and sample.24 hours after ulcer induction by acetic acid the animals were inoculated intragastrically with *H. Pylori* ATCC 43504($9x10^8$) suspended in Muller-Hinton broth, by using a cannula appropriate for orogastric lavages for the animals in the control. (Acetic acid induced ulcer groups without *H. Pylori* infection)

Only Muller-Hinton broth was orally administrated. The Orogastric inoculation of *H. Pylori* was maintained twice a day for 7 days, were as administration of sample and of the standard drugs, tools place twice a day, for 14 consecutive days. Starting from the 3rd day after ulcer induction by acetic acid. After treatment, the animals were sacrificed by cervical dislocation. Blood was collected from the inferior vena cava the stomach was removed for evaluation of gastric lesions. The Ulcerated area (mm2) was measured and the healing rate % was determined. Prostaglandin E2 (PGE_2) levels were measured from gastric mucosal scrapings and a fragment from each stomach was used for the histopathological examinations and for the urease determination.

Treatment of *H. Pylori* infection[55]

Management of *H. Pylori* infection including eradication regimens against *H. Pylori* infection is not distinguished as first line, second line or third line (except levofloxacin- containing therapy). *H. Pylori* kit consists of combination of proton pump inhibitors (PPI), antibiotics and its choice depends on degree of severity of disease. The selection of antibiotic classes for *H. Pylori* eradication in children is extremely limited. Standard triple therapy (PPI, Amoxicillin and clarithromycin) only achieved an eradication rate. Amoxicillin, clarithromycin and metronidazole are recommended to eradicate *H. Pylori* in paediatric patients. Gastric coat forming suspension or Probiotic supplementation during eradication therapy could significantly decrease the incidence of adverse events and increase the eradication rate during therapy to protect gastric bacterial flora.

First line treatment

Proton Pump Inhibitors (PPI) based triple therapy [57] has first line treatment. A combination of PPI, amoxicillin and Clarithromycin (PAC) or Metronidazole (PAM) is given twice a day. The European guidelines acknowledged that a 14 day treatment course may be more effective than a 7 day treatment course.

Second line treatment

Second-line treatment helps to overcome bacterial resistance. Recently levofloxacin-based therapy has been studied as second line therapy. Using levofloxacin has proven effective in the treatment of *H. Pylori* infection. The eradication rate is achieved with levofloxacin- based triple therapy. *H. Pylori* cure rates are higher with a 10-day treatment course. Clarithromycin should be avoided in second line treatment in most areas.

Quadruple therapy[58, 59, 60]

Bismuth-based quadruple therapy is the second line treatment of choice in many countries. For 14 days, Quadruple therapies comprising PPI, metronidazole, tetracycline and bismuth are effective alternative first line treatment which may be advocated in areas of high antibiotic resistance

Bismuth Salts [56]

Colloidal bismuth sub citrate and bismuth subsalicylate- on oral administration chelate proteins in the ulcer base and form a protective coating over the gastric mucosa. They also inhibit the growth of *H. Pylori* on gastric mucus. By these actions they promote ulcer healing in 4-8 weeks. Combination regimens including bismuth may be used in case of metronidazole and clarithromycin double resistance. This bismuth salts are not universally available due to toxicity.

Gastric acid suppression agent

Adequate acid suppression improves the efficiency of *H. Pylori* eradication regimens. Which includes oesomeprazole, rabeprazole and lansoprazole, are taken twice per day for 14 days treatment course. Increased duration of therapy has been recommended to overcome the falling eradication rates.

Potassium –competitive acid blockers (P-CABs) are a new class of gastric acid suppressing agents. P-CABs inhibit H^+/ K^+ ATPase mediated gastric acid secretion in a reversible and potassium- competitive manner[61]. Furthermore; P-CABs are predicted to be more effective than PPI in H. Pylori eradication therapies. Eg:-Vonoprazan. 7 day P-CAB based triple therapy Vonoprazan+ amoxicillin+clarithromycin twice a day is more effective than 7 day PPI based therapy. Alternatives include sequential therapy and Quinolone –based therapy,

Sequential therapy

Sequential therapy in which PPI plus amoxicillin are given for 5 days followed by PPI plus clarithromycin and tinidazole for next 5 days has eradication rate greater. This sequential therapy has proved superior to standard triple therapy. This treatment regimen appears to overcome clarithromycin resistance.

Adjuvant therapy

Eradication failure is usually associated with several factors, such as inappropriate treatment regimen, poor compliance and antibiotic resistance. The occurrence of side effects can reduce the compliance of patients on treatment regimens and lead to the development of bacterial resistance. This has lead to the development of alternative treatment options for *H. Pylori* . Adjuvant therapy with probiotics, bovine lactoferrin and curcumin have been studied in recent years.

A probiotic is defined as a living microbial species that, on administration. The most studied probiotics are lactic acid-producing bacteria, particularly lactobacillus species. Probiotics play a role in the stabilization of the gastric barrier function and decrease of mucosal inflammation. Some probiotic species such as Lactobacilli and bifodobacteria release bacteriocins that may inhibit *H.*

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Pylori growth and its adherence to gastric epithelial cell. *H. Pylori* treatment has shown a significant reduction in side effects with adjuvant therapy.

The progression of gastric precancerous lesions, glandular atrophy, and intestinal metaplasia in superficial gastritis, gastric erosion and gastric ulcer is strongly related to *H. Pylori* infection. Prospective studies are needed to evaluate whether eradication of *H. Pylori* infection will really diminish the risk of gastric cancer.

Conclusion:

This article focuses on *H. Pylori,* a gram negative spiral bacilli with flagella. This, is a waterborne infection, and is colonized in the mucosa of the stomach. Prevalence was found to greater in male 66.5% than in female 63.2%. *H. Pylori* gastric inflammation process (Gastritis) is associated with the development of ulcers, atrophy, intestinal metaplasia and gastric carcinoma; gastric inflammation is mediated by specific and nonspecific immune responses by release of cytokines. Both invasive and non invasive methods, by histological, serological techniques, and culture methods are used to detect *H. Pylori* infection. Finally from review of studies, it was concluded that the schedule of *H. Pylori* kit consists of proton pump inhibitors, mucus coat forming suspensions and antibiotics. Choice depends upon the inflammation condition and recovery. In- vitro colonisation of *H. Pylori* has been utilized to induce gastric ulceration, which is used as a resource for screening antiulcer activity of natural and synthetic drugs.

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CROP RESIDUE MANAGEMENT: NEED OF THE HOUR FOR REDUCING ENVIRONMENTAL POLLUTION AND MAINTAINING SOIL HEALTH

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Abstract:

India is an agrarian economy. A vast majority of land is used for agricultural purpose and a wide range of crops are cultivated in its different agro-ecological regions. More production of crops has led to generation of large amount of residues, creating problem for the farmer to prepare land in short time for the succeeding crop. Large amount of crop residue after harvest of the crop remain in the field and its management is a major challenge to the farmer. Farmers burn residue as it is cheap, easy, and economical. Burning of residue leads to deterioration of soil health, reduction in population of beneficial microorganisms resulting in reduced soil fertility. Burning agriculture residue is responsible for emission of different greenhouse gases. To deal with these issues, crop residue management methods should be adopted for achieving optimum production with less environmental pollution. Crop residues are of great economic values as livestock feed, fuel and industrial raw material as well as in conservation agriculture for which it is a pre-requisite. It contains considerable amount of plant nutrients and their proper application will have positive effect on nutrient status in rice wheat system. Long-term studies of the residue recycling have showed improvements in soil health.

Keywords: Crop residue management, Environmental pollution, Greenhouse gases, Soil health

Introduction:

In India, about 500-550 Mt of crop residues are generated every year out of which 93.51 Mt of wheat, 105.24 Mt of rice, 22.26 Mt of maize, 16.03 Mt of millets (jowar, bajra, ragi and small millet), 341.20 Mt of sugarcane, 7.79 Mt of fibre crops (jute, mesta, cotton), 18.34 Mt of pulses and 30.94 Mt are of oilseed crops. Among different crops, cereals generate maximum residues (352 Mt), followed by fibers (66 Mt), oilseeds (29 Mt), pulses (13 Mt) and sugarcane

(12 Mt). The cereal crops (rice, wheat, maize, millets) contribute 70% while rice crop alone contributes 34% and wheat ranks second with 22% of the crop residues (Fig.1). These crop residues are used for the purpose of animal feeding, soil mulching, manure, for thatching purpose and fuel for domestic and industrial use. Hence, crop residues possess tremendous value to the farmers. However, farmers burn the residues on farm to clear the field for sowing the next crop and this problem is intensifying in recent years. The residues of rice, wheat, sugarcane, cotton, maize, millet, jute, rapeseed-mustard and groundnut are typically burnt on-farm in different parts of the country. The problem is more serious in the irrigated agriculture, mainly in the northwest India where rice-wheat cropping system is dominant.The reason behind this is less availability of labor, increased cost of removing the crop residues by traditional methods and use of combine harvester for harvesting of crops. Farmers think burning as the most suitable and cost-effective method of disposing of rice straw andit also acts as an effective pest control procedure (Dobermann and Fairhurst, 2002). Gadde *et al.* (2009) estimated that the contribution of burning of rice straw to the total amount of greenhouse gas (GHGs) emissions in India is 0.05%, which not only result into huge loss of biomass in the form of organic carbon, plant nutrients, but also have negative impacts on soil properties as well as soil flora and fauna. Crop residue management is defined as the management of the amount and distribution of crop and other plant residues on a soil surface and its utilization as raw material in production of other useful product. There is a need to adopt crop residue management options for maintaining agricultural as well as environmental sustainability.

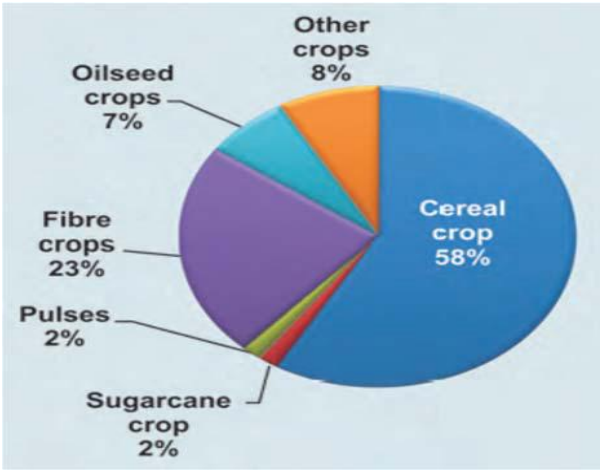

Figure 1: The share of unutilized residues in total residues generated bydifferent crops in India (calculated from MNRE, 2009)

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Crop residue management:

In India, rice wheat cropping system generates large amount of crop residues. Farmers generally harvest rice and wheat by combine harvester leaving residues in the field. Generally, the residues of cereal crops are utilized as cattle feed. Rice straw and husk are used as domestic fuel or in boilers for parboiling rice. Management of rice straw is more serious problem as compared to wheat straw, because there is very little turn-around time between harvesting of rice and wheat sowing. Different management options are available to farmers for the efficient management of crop residues such as livestock feed, mushroom cultivation, incorporation, surface retention and mulching, biochar and baling and removing the straw. Farmers use different straw management practices depending upon the situation.

Challenges in crop residue incorporation:

Residue incorporation can be an alternative to burning in rice-based cropping systems across Asia. But there are many problems associated with it. Some of them are-

a) Problem associated with soil incorporation: During land preparation of low land rice in Asia, the topsoil surface is typically inverted thereby incorporating crop residues remaining on the soil surface as standing stubble or loose straw. A mouldboard plough or disk plough is generally used for residue incorporation often with a shallow layer of floodwater. The rate of residue incorporation differs among tillage systems depending upon implement, intensity and mechanization level that are manual, animal traction or mechanized. There are difficulties during incorporation of residues like clogging of field implements with long straw. In rice-rice system, a strategy for rapid incorporation of residue before instant establishment of next crop is cutting the rice residue into 20-25 cm length followed by shallow mechanical incorporation (Zeng *et al*., 2001).

b) Labour intensive: Incorporation of large amounts of fresh residue in absence of suitable equipment will require more number of labourers (Dobermann and Fairhust, 2002).

c) N immobilization: The major drawback of straw incorporation is the immobilization of inorganic N and its negative impact on crop growth due to deficiency of nitrogen. Incorporation of rice straw into the soil after its harvest results into slow decomposition rate and soil nitrate is immobilized, reducing the N uptake and yield of successive crops. Farmers often feel hesitation to opt for incorporation of the rice residues because of the reason that fertilizer N is temporarily immobilized for the following crop leading to reduced productivity.

d) Fallow period: The time gap between incorporation of crop residue and land preparation, flooding and transplanting the next rice crop is crucial factor affecting residue management. This

time interval affects the extent of residue decomposition before transplanting, depending upon soil and climatic condition, and thereby affecting the beneficial or negative effects of residue incorporation on young rice seedlings. In intensive irrigated production system, two or three short duration rice crops are taken per year. As a result, the fallow between two crops can be only few days.

Figure 2: A glimpse for post harvested paddy crop residues management alternatives (Singh *et al*., 2018)

Challenges in feeding rice straw:

a) Palatability: Cattle are more likely to eat rice straw if the time between rice harvest and straw baling is short. In addition, rice straw has small hairs or pubescence and cows will take some time to get adapted to it.

b) Low digestibility: Rice straw has very high silica content ranging from 8 to 14% as compared to alfalfa hay that has silica content of 1 to 2%. Silica is indigestible and leads to reduced digestibility of the feed. This is generally true in the rice leaves, which contain the highest levels of silica. This high silica level in combination with other mineral compounds produces an average ash content of 17% for rice straw in comparison to 7 to 8% for alfalfa.

c) Low protein: The crude protein of 2 to 7% on a dry matter basis in rice straw requires protein supplementation in order to meet the nutritional requirements of most cattle.

d) High in oxalates: Oxalates in rice straw reduces the absorption of calcium. This is not an issue in the intermountain region of California because their produce is supplemented with calcium rich alfalfa hay.

e) Safe storage: It is essential; to avoid leaching of soluble nutrients by rain and damage caused by mould. Damp feed is associated with mycotoxin contaminated by organism such as

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Aspergillus, Penicillium, Fusarium and *Alternaria* (Phillips *et al*., 1996). Feeding of mouldy straw has negative effect on health of animals as well as human beings that will consume that milk.

Different crop residue management strategies:

Livestock's Feed:

If the crop residues from the field are transported to dairy farms as a feed to cattle, then the major problem faced by farmers to manage can be reduced to some extent.It can be fed to livestock solely or in combination with certain supplements. However, crop residues, being unpalatable and low in digestibility, cannot form a soleration for livestock. Crop residues are low-density fibrous materials less amount of nitrogen, soluble carbohydrates, minerals and vitamins with varying amount of lignin which acts as a physical barrier and hinders the process of microbial breakdown. In overcome these issues residues should be processed, enriched with urea and molasses. It should be supplemented with green fodders such as sunhemp, horse gram, cowpea and gram. So, crop residues if used as feed then it will lead to improved feed efficiency of dairy cattle, farm profitability. This will benefit dairy farmers as well as reduce the air pollution caused by burning of crop residue.

Residue incorporation:

One of the practices followed by the farmers is the retention of residues to the field itself, which will promote the OM present in the soil resulting with the improvement of different physical conditions of soil (Hiel *et al*., 2018). Incorporation of straw is one of the best options for utilizing ricestraw left in fields. It enhances soil organic matter and N, P and K contents of soil but it also results in temporary immobilization of nitrogen and further extra nitrogenous fertilizer should be added to correct the increased C:N ratio at the time of residue incorporation. Crop residues incorporation increases crop yield from 15% - 35% in comparison to control treatment. Residue incorporation has been found to increase available phosphorus, exchangeable cations that are K, Ca and Mg as well as base saturation (Geiger *et al*., 1992; Steven *et al*., 1993). The rate of decomposition of residue can be increased by the application of an innovative and efficient integrated machine, Yanmar India. It is a machine with multiple functions such as combine harvesting, chopping rice straw, and spraying *Trichoderma* into chopped straw. Incorporation of rice straw into paddy soil is an effective method for managing rice straw, but it may result in a decline in production efficiency if done inadequately and ineffectively (Dobermann and Fairhurst, 2002; Mandal *et al*., 2004; Singh *et al*., 2005) and an increase in the emission of greenhouse gas (Sander *et al*., 2014). Most of the farmers do not follow the method

of incorporation due to slow decomposition rate of rice straw. A report at International Rice Research Institute (IRRI) showed that about 3,500kg carbon dioxide equivalent (CO_2-eq) per ha emitted and converted from CH_4 and N_2O in a rice crop season with straw incorporation. This amount of GHGs was almost 1.5 times more as compared to the amount emitted from the practice of rice straw removal. This has led scholars to conduct research on increased decomposition rate of rice stubbles (Goyal and Sindhu, 2011; Ngo *et al*., 2012; Singh *et al*., 2018a,b).

Crop residues as bio-fuel:

Biofuel is an alternative to reduce the dependency on fossil fuel. Different agricultural crop residues are lignocellulosic substances, which is the main source of renewable energy and an alternative to the fossil fuels (Leng *et al*., 2018). Conversion of ligno-cellulosic biomass into alcohol or ethanol after blending with gasoline as a fuel extender and octane and can be used in neat fuel in internal combustion engines. Estimates of ethanol production from different feedstock such as corn grain, rice straw, wheat straw, bagasse and saw dust varies from 382 to 471 L t^{-1} of dry matter. The process of ethanol production from crop residues is, however, evolving in India but still the process of conversion of crop residues into alcohol needs some technological improvements.

Crop residues as biochar:

In the recent year, biochar and crop residues have gained lots of attention as a viable strategy for maintaining soil health. Biochar is a fine-grained charcoal having high carbon material produced through slow pyrolysis that is heating of biomass in the absence of oxygen. It can potentially play a major role in the long-term storage of carbon in soil. Biochar obtained from plant biomass contains a unique recalcitrant form of carbon that is resistant to degradation by microbes, therefore it is involved in the process of carbon sequestration, when applied to soil (Lemann and Joseph, 2009). In addition, biochar has been shown to reduce emission of greenhouse gases from agricultural fields and also enhances quality of water through its strong absorption nature of contaminants (Spokas *et al*., 2009, Zhang *et al*., 2012). However, with the current availability of technology, it is not economically viable and cannot be popularized among the farmers. However, once all the valuable products as well as co-products such as heat energy, gas like H_2 and bio-oil are captured and used in the biochar generation process, it can become economically viable.

Crop residue as animal bedding and compost:

For the preparation of compost, crop residues are used as animal bedding and are then heaped in dung pits. One kilogram of straw absorbs approximately 2-3 kg of urine, which

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enriches it with nitrogen. The residues obtained after harvesting rice from one hectare field gives about 3 tons of manure as rich in nutrients as farmyard manure (FYM). The rice straw compost can be fortified with phosphorous using indigenous source of low graderock phosphate to make it value added compost with 1.5 % N, 2.3 % P_2O_5 and 2.5 % K_2O (Sidhu and Beri, 2005). Different types of composting methods such as aerobic, anaerobic and vermicomposting can be used to decrease the bulk amount of crop residue in the field itself.

Roof thatch:

Resource poor rice farmer's uses rice straw that are generally 90 to 130 cm long for covering the roofs of their hut. The life of roof thatch is generally two years depending upon the amount of rainfall, slope of roof, type of straw used that is cultivar and its silica content. Rice straws of slender grain variety used for roof thatch have longer life. Methods of harvesting and threshing play an important role in determining the quality of rice straw for its thatch use. Hand cutting rice straw is ideal for roof thatch if it is dried, bundled and stacked properly. Roof thatch is generally made at every alternate 2 to 3 years before onset of monsoon. Roof thatch made of straw becomes cool in summer and warm in winter. This is very economic to the farmers. One problem associated with using straw as roof thatch is that it can catch fire easily.

Crop residues for mushroom cultivation:

Mushroom cultivation using crop residues represents valuable conversion of inedible crop residues into valuable food. Mushroom contains two to three times as much protein as compared to vegetables and also an amino acid composition which is similar to that of milk or meat. Wheat and rice straws are suitable substrates for the cultivation of *Agaricus bisporus* called as white button mushroom and *Volvariella volvacea* that is straw mushroom. These two are most commonly grown fungi. Straw for the cultivation of *Agaricus bisporus* is usually mixed with hay and conversion of crop residue substrates into fungal bodies is possible. Cultivation of tropical mushroom is a way of utilizing agro-waste in the shortest possible time with additional advantage of quality food production containing high amount of essential amino acids. Straw is a suitable substrate for mushroom. There are several species of edible mushroom cultivated on rice straw throughout East and South-East Asia. Some of them are *Volvariella volvacea, Agaricus volvaceus, Amanita virgata* and *Vaginata virgate*. In India, mushroom is cultivated in coastal states such as Kerala, Tamil Nadu, Odisha, West Bengal and Assam. Tropical mushroom has the ability to grow at a higher temperature ranging from 28 to 35°C. It is fast growing thus has very short cropping cycle of 30 days and mushroom yield is approximately 10 to 15% of dry substrate. Straw mushroom is a good source of amino acids, which could supplement proteins

that are lacking in Asian diet. Rice-straw mushrooms are easy and cheap to produce and require little space and investment. They are nutritious food and has the ability to fetch a good price in the market.

Adverse Effects of Crop Residue Burning:

Burning of crop residues emits certain pollutants along with different GHGs in the atmosphere (Ravindra *et al*., 2019). such as methane (CH_4), carbon monoxide (CO), nitrous oxide (N_2O), oxides of nitrogen (NO_X) and sulphur (SO_X) and other hydrocarbons to the atmosphere. These gases lead to an increase in the levels of aerosols, acid deposition, tropospheric ozone and depletion of the stratospheric ozone layer. Many pollutants released from burning of crop residues can be a major causeof concern resulting into various air-borne/lung diseases. It is estimated that the burning of one tonnes of rice straw leads to loss of 5.5 kg Nitrogen, 2.3 kg phosphorus, 2.5 kg potassium and 1.2 kg sulphur in addition to organic carbon. The burning of straw increases the temperature of the top soil that results into rapid changes in carbon- nitrogen equilibrium. This leads to a loss of large amount of NPK from the soil. Crop residues can be a source of carbon, bio-active compounds and energy forrural households and small industries. Burning of agricultural crop residues increases the suspended particulate matter (SPM), aerosol, concentration of black carbon, SO_2 and NO_2 in the atmosphere (Mittal *et al*., 2009; Kharol*et al*., 2012). Heat from burning residues increases soil temperature causing the death of beneficial soil microbes. Therefore, it is important to find an alternative management strategy to utilize and dispose the crop residue in such a way that there is no effect on the environment as well as the fertility status of soil (Setter *et al*., 2020).

Crop Residue Management and Soil Health:

Crops mainly rice and wheat are exhaustive feeders of nutrient and due to this there is deteoriation of soil health under the rice-wheat cropping system. The amount of nutrients removed by rice and wheat are more than the amount added through fertilizers and recycled. Residues retention improves soil physical characteristics such as structure, infiltration rate, plant available water capacity, chemical characteristics like nutrient cycling, cation exchange capacity, soil reaction and biological properties that are soil organic carbon sequestration, microbial biomass carbon, activity and species diversity of soil microbes, soil quality (Singh *et al*., 2008).

Effect on soil physical health:

Crop residue management practices influence or affect soil physical properties such as soil moisture content, aggregate formation, bulk density and soil porosity. Incorporation or retention of crop residues in to the soils decreases bulk density and compaction of soil (Bellakki *et al*., 1998). The application of 16 t ha^{-1} of rice straw for 3 years reduced bulk density from 1.20 to 0.98 g cm^{-3} in the 0-5 cm layer on asandy loam soil. Breakdown of aggregates and formation of surface seal by the impact of raindrop resulted in an increase incompaction and reduction in

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porosity of soil leading to lower infiltration. Residue retention on thesurface can be the potential solution for this problem. Incorporation of crop residues decreased bulk density and increased infiltration rate, water holding capacity, microbial population, soil fertility in comparison to no residue treatment. Incorporation of residue along with NPK fertilizer has resulted in achieving highest yield, nutrient uptake, soil fertility statusand population of soil microbes (Singh *et al*., 2010).

Effect on soil biological health:

Soil microbial biomass (SMB) and microbial activity, which depends on the supply of organic substrates in soil is responsible for the availability of the nutrients such as N, P, S. The population of soil flora and fauna is depended on the phyto-biomass present in soil. Beri *et al*. (1992) and Sindu *et al*. (1995) observed that soil treated with crop residues held 5-10 times more population of aerobic bacteria and 1.5-11 times more fungi than soil. These were either burnt or removed. Verhulst *et al*. (2009) observed that, soil microbial biomass decreased with reducing amount of residue retained on the soil surface in the zero till treatments in long term trials of both rainfed as well as irrigated conditions. The soil microbial biomass shows the ability of soil tostore and cycle nutrients such as C, N, P and S and organic matterand plays a major role in physical stabilization of aggregates. Crop residues are also known to enhance nitrogen fixation in soil by asymbiotic bacteria like *Azotobacter chrococcum* and *A. agilis*. Increase in soil microbial population the leads to an increase in the activity of soil enzymes responsible for conversion of nutrient from unavailable to available form.

Effect on soil chemical health:

The pH of the soil is an important factor in deciding the availability of the nutrients and is greatly influenced by the crop residues incorporated in thesoil. Straw application for long term helps building soil organic matter level and N reserves. It also enhances the availability of macro- and micro-nutrients. Beri *et al*. (1995) conducted a 11 year study and found that incorporation of residues inrice wheat system as increased the availability of P and K content, in the soil as compared to removal of residues. Gupta *et al*. (2007) observed from the 3 year study that the inorganic and organic P, reduced P sorption, and enhanced release of P increases with the incorporation of crop residue in comparison to straw burned. About 50-80% of micronutrient (Zn, Fe, Cu and Mn) taken up by rice and wheat crops can be recycled through residue incorporation. Crop residue has significant effect on the availability of micronutrients such as zinc and iron in rice (Singh *et al*., 2005 and Gupta *et al*., 2007). Residue characteristics and soil and crop management practices also affect the rate of residue decomposition. Many researchers have reported areduction in microbial biomass when residues are burnt. Residue incorporation results in more microbial activity than residue removal or burning.

Conclusion:

Burning of crop residues is a major concern and to solve issues related to its management strategies should be adopted. Crop residue plays an important role in improving physical, chemical and biological properties of the soil. It also plays an important role in ensuring the country's food security by achieving agricultural sustainability. Burning of residues not only affect the soil health but also pollutes air leading to global warming. Since, burning crop residues produce certain carbonaceous materials, gases like NO_2, SO_2, and many greenhouse gases. So, it is crucial to manage the residues in a sustainable manner to avoid negative effect on the soil as well as to atmosphere Farmers should be made aware about the management practices such as livestock feed, residue retention (both conservation and conventional tillage), composting, biofuels (biochar preparation and bio oils) and mulching. All stakeholders that are farmers, supply and value chain service providers, researchers, extension agents, policy makers, civil servants and consumers need to be involved in utilizing the full potential of these valuable resources for sustainability and resilience of Indian agriculture.

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EXTINCTION OF ORGANISM FROM PLANET EARTH

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Introduction:

The extinction is a death or loss of an organism from any ecosystem. The loss may be due to natural calamities or human effect. The effect may be destruction of habitat, which causes removal of organism forever from the earth. The extinction of organism may be at generic or species level. The loss of last member of a group is treated as extinction of a species. In extinction breeding capacity of organism get lost with increase in the age. The evolution in an organism promotes development of new varieties or species. The species of plant or animal can become vulnerable and extinct if they are unable to cope with changing environmental conditions and strong competition for food, water and shelter. The species become functionally extinct if there are only few individuals of a group, survive under adverse habitat condition. The organism cannot reproduce if starvation prevails and weak health. The species cannot reproduce if there is absence of male or female organism or sparse distribution. The disappearance of species is taking place at a faster rate due to physical or chemical or biological factors. The terrestrial and aquatic ecosystems are harmed due to habitat destruction and leading to extinction. The natural catastrophic events and introduction of exotic species has altered the ecological niche of species. The successful establishment of invasive species may become detrimental to native species. The pollution is an important aspect of degradation or deterioration of healthy environment. The pollution takes place in water, air and in soil due to human activities. The toxic chemicals and effluents are added into the ecosystems from time to time which affecting and causing death or loss of large number of organism.

The extinction of an organism is an irreversible loss. The extinction of organism may takes place due to various reasons such as flood, fire, landslide, wind erosion, chronic environmental stress, severe competition among species, disease epidemic, potential predation, physical infrastructure construction,mining activities, establishment of industrial plants etc. The stagnation of oceanic circulation and post-glacial temperature, global warming are also promoting to the extinction of species. The natural calamities such as volcanic eruption, assault

of comet and meteoric eruption are pose the extinction of organism. The loss of organism may be due to epidemic diseases, population collapse and food chain disturbances. The loss of species may take place abruptly or gradually. The species extinction affects on species richness and genetic diversity. The species richness enhances productivity and stability of ecosystem. The loss of even a single species affects on the food chain at different trophic levels and affect on the entire ecosystem.

The International Union for Conservation of Nature (IUCN) keeps the information regarding extinction of organisms or species at global level. The information constitutes of the data regarding the status of organism and threatened and extinct species. The data of threatened species is known as Red data. The whole data regarding threatened species is called as Red Data Book. The IUCN is the world's most comprehensive inventory regarding conservation data of organism all over the world. The evaluation of organism is made on the basis of certain criteria pertaining to threat. The criteria's of evaluation remains uniform for all categories organism globally. The IUCN works co-ordinately with different countries and organizations to assess the threatened and extinct species. The mandate of IUCN is to convey the urgency of conservation of threatened species.It provides information to the researchers, scientist, policy makers and even to the public.It also provides information regarding the present status of species in view to draw attention of people towards the conservation of biodiversity. The threatened species are characterised on the basis of population dynamics and measures of mathematical models. The quantitative metrics is one of the methods of evaluating the degree of endangerment of organism. The threatened species are divided into three categories on the basis of degree to which they are threatened. The three categories are vulnerable species, endangered species and critically endangered species. The species which have been evaluated and have sufficient data,are only considered as threatened species.According to Environment Protection and Biodiversity Conservation (EPBC) Act 1999 and Flora and Fauna Guarantee (FFG) Act 1988, the threatened species are categorised as below.

i) **Extinct species:** It includes the plant or animal species that have not been seen in the wild state or wild condition since last 50 years and the last member of the species has died.

ii) **Extinct species in the wild state:** It includes the species only exists in captivity and no longer found in the wild state or condition and the last member of the species has died.

iii) **Critically endangered species:** It includes the species which is facing an extremely high risk of extinction and will extinct if proper care is not taken.

iv) **Endangered species:** It includes the species which will face danger of extinction in near future of about 10 to 20 years. The species may be extinct but have been seen in

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the wild state less than 50 years ago. The species is at high risk of extinction in the wild state. The extinction may be local or regional or worldwide The endangered species can be rehabilitated with captive breeding and habitat restoration. The status of global conservation of species includes Data Deficient (DD) species, Near Threatened (NT) species and Least Concern (LC) species.

v) **Vulnerable species:** It includes the species which may likely to move into the endangered category very soon, if conditions or situation is not changed and made favourable for survival within 20 to 50 years. The species is at high risk of extinction in the wild state in coming few years. The top predators play a critical role in ecosystems by means of food chain at different trophic levels. The top predators are a vulnerable for extinction. The species with small geographic range or niche are more vulnerable due to introduction of invasive species and human activities.

vi) **Near Threatened species:** It includes the species whose numbers are decreasing and will be vulnerable or endangered in near future.

vii) **Conservation dependent species:** It includes the species which may move to any of the above category of threatened species, if conservation strategies are not applied.

Causes of extinction of species:

The extinction of plant and animal species may be caused due to natural and anthropological reason. The extinction affects on the species richness in an ecosystem. The reasons of extinction are described as below.

1) **Exploitation of natural resources**

The exploitation of natural resources is increasing day by day due to ever increasing rate of human population. The human greed towards utilization and commercialization posed a great stress on the extinction of plants and animals species. The development in science and technology has enabled exploitation and utilization of natural resources. The plants and animals are harmed by man to get domestic and commercial benefits. The exploitation of resources has increased to earn more money and enrich the status of life. The over exploitation has affected on the extinction of species and in turn loss of biodiversity. The loss of biodiversity affected on the genetic diversity of wild plants and animals. The extinction of a species affected on food chain of ecosystem. The harvesting of organism to fulfil the greed, many species of organism are threatened and are on the verge of extinction.

2) Habitat disturbance

The survival of organism depends upon the interactions with abiotic and biotic factors of environment. The habitat of organism may be aquatic, terrestrial, mangroves, coral reefs,

wetlands etc. The habitat constitutes of unique species of plants and animals. The habitat is the residence or dwelling place of organism. Every organism has its specific ecological niche. The disturbance in habitat affect on the ecological niche of an organism. The effect of habitat degradation will be on food, water and shelter. The effect leads to suffering of organism. The low productivity makes many species starved and destroyed. The deforestation has caused death of large number of plants and animals species. The forest fire, floods, soil erosion, volcanic eruption has destructed the habitat of many organisms on earth. The human activities such as mining, construction, establishment of industrial plants, agricultural activities have destroyed large number of species in an ecosystem.

3) Pollution

The introduction of pollutants in ecosystems causes pollution. The pollution is caused by man directly or indirectly through different domestic and commercial activities. The Air is made polluted due to addition of carbon monoxide, carbon dioxide, nitrogen oxide etc. These gases are released in air due to burning of fossil fuel and organic matter. The water is polluted due to release of toxic chemicals and industrial effluents. The land is polluted due to excessive use of chemical fertilizers, insecticides and pesticides to get higher yield of crops. The pollution has affected death of many plants and animal's. The pollution has made environment impure and hazardous for organism. The addition of chemicals in ecosystem enters through food chain and become toxic and detrimental to organism. The degradation of ecosystems due to pollution has compelled organism to extinct all over the world.

4) **Global warming**

The global warming is one of the reasons of extinction of species. The warming effect is enhanced due to release of carbon monoxide, CFCs, Methane, Nitrous oxides in atmosphere.Due to increase of concentration of these gases; there is increase in the temperature. The high temperature cannot tolerate by many species of plants and animals. The temperature affects on the life of many organism that lead to death. The increasing of green house gases in atmosphere depletes the vital ozone layer. The depletion of ozone permits the entry of ultraviolet radiations, red radiations, far red radiations, X-ray radiations on the earth. These radiations affect on the organism and causes death and extinction.

5) Introduction of exotic Species

The introduction of exotic species in an ecosystem may be due to human interference or natural events. The introduction of plant and animal species is going on continuously for domestic purpose or for commercial utilization. The introduction of a species may takes place region to region or state to state or nation to nation. The attempts are being made to establish the exotic species to native or local place. The introduced species always compete with native species for food, water and shelter. The native weak species get affected and starved due to

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invasive species. The successful establishment of introduced species lead to death and extinction of native species. The exotic species may carry disease with them and spread it to native locality. The weak and susceptible species of organism succumb to the disease and get lost or extinct forever. The death due to disease affects on the species richness of ecosystem.

6) Disease development

The spread of epidemic diseases may become harmful to plant or animal species. The disease spread affect on the life and cause death and extinction. The weak and susceptible species becomes extinct easily. The species diversity reduces the effect of disease development and in turn extinction of less number of species.

7) Lack of genetic diversity

The limited geographic range or ecological niche is the main cause of small size of population. The small population has less genetic diversity. The less genetic diversity makes organism vulnerable to extinction. The environmental catastrophic events and exotic species make them weak. The resistance capacity in small population remains weak. The genetic diversity shows variation in survival potential of species. The genetic diversity enables the species to save life and danger of extinction. If the rare species are coming in contact with the abundant species, then the chances of extinction of rare species are enhanced. The large and variable gene pool makes population robust and less prone to extinction.

8) Mutual extinction or co-extinction

In nature, there are many organisms that are depended on each other for food and shelter. There exist a food chain and food web in any ecosystem.In the trophic level of food chain, one organism is eaten by the other. The symbiotic association is one of the way of leading life among organisms.In symbiosis one organism helps to other to provide food and in turn get shelter.If any one organism fail to survive, then the other gets suffered from food and shelter, Thus both the organisms become vulnerable and extinct. If one species is affected, then the dependent species also gets affected.If the host is dead then the parasites also get dead due to starvation. The co extinction happens in those species who are symbiotically associated and dependent with each other. The co-extinction affects on the loss of biodiversity.

The extinct species of Plants and Animal:

According to IUCN following species are declared as extinct

Plants

Acalypha dikuluwensis, Acalypha wilder, Amaranthus brownie, Angraecopsis dolabriformis, Angraecum astroarche, Basananthe cupricola, Centaurea pseudoleucolepis, Cyanea eleeleensis, Cyanea linearifolia, Cyanea mauiensis, Cyanea minutiflora, Cyanea parvifolia, Cyanea sessilifolia, Cyperus rockii, Cyrtandra olona, Delissea sub cordata, Delissea undulate Eulophia

stenopetala, Euphrasia mendoncae, Fissidens microstictus, Habenaria petromedusa, Heliotropium pannifolium, Hibiscadelphus woodii, Lepidium amissum., Lepidium obtusatum, Logania depressa, Melicope macropus, Melicope nealae, Miconia abscondita, Myosotis laingii, Nobregaea latinervis, Ornithogalum visianicum, Sanicula kauaiensis, Schiedea amplexicaulis, Stachytarpheta fallax, Stellaria elatinoides, Trilepidea adamsii, Viola cryana, Wikstroemia Hanalei etc.

Mammals

Bettongia anhydra, Conilurus capricornensis, Dusicyon avus, Leporillus apicalis, Melomys rubicola

Rodents

Notomys robustus, Pennatomys nivalis, Pipistrellus murrayi, Pseudomys auritus etc.

Birds

*Acrocephalus luscinius, Acrocephalus musae, Acrocephalus nijoi, Acrocephalus yamashinae, Acrocephalus yamashinae, Aegolius gradyi , Akialoa ellisiana, Akialoa lanaiensis, Akialoa stejnegeri, Alectroenas payandeei, Aplonis ulietensis, Bermuteo avivorus, Chenonetta finschi, Coenocorypha barrierensis, Coenocorypha iredalei, Colaptes oceanicus, Columba thiriouxi, Dryolimnas augusti, Eclectus infectus, Foudia delloni, Hemignathus lucidus, Himatione fraithii, Loxops wolstenholmei, Nesoenas Cicur, Nyctanassa carcinocatactes, Pipilo naufragus, Porphyrio paepae, Prosobonia cancellata, Pyrocephalus dubius**,** Tachybaptus rufolavatus, Tribonyx hodgenorum, Zosterops conspicillatus, Zosterops semiflavus etc.*

Reptiles

*Alinea luciae, Chelonoidis abingdonii, Clelia errabunda, Contomastix charrua, Copeoglossum redondae, Cyclura onchiopsis, Emoia nativitatis, Erythrolamprus perfuscus**,** Leiocephalus cuneus, Leiolopisma ceciliae, Nactus soniae, Scelotes guentheri etc.*

Fish

Alburnus nicaeensis, Anabarilius macrolepis, Aphanius splendens, Atherinella callida, Cyprinodon arcuatus, Labeo worthingtoni, Megupsilon aporus, Noturus trautmani, Platytropius siamensis, Pseudophoxinus handlirschi, Tristramella sacra etc.

Invertebrates

Bradycellus chavesi, Bythinella gibbosa, Bythinella limnopsis, Bythinella mauri tanica, Bythinella microcochlia, Bythinella punica, Calathus extensicollis, Calathus vicenteorum, Cambarellus alvarezi, Cambarellus chihuahuae, Centrobunus braueri, Chambardia letourneuxi, Dicrogonatus gardineri, Eucarlia alluaudi, Galba vancouverensis, Geonemertes rodericana, Germainaia geayi, Heleobia spinellii, Hirstienus nanus, Islamia ateni, Labidura herculeana, Leiorhagium solemi, Macrobrachium leptodactylus, Margatteoidea amoena, Melanoplus spretus, Mercuria letourneuxiana, Metazalmoxis ferruginea, Neocnemis occidentalis,

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Neoplanorbis tantillus, Orthomorpha crinite, Pacifastacus nigrescens, Peromona erinacea, Plectostoma sciaphilum, Pleorotus braueri, Pleurobema perovatum, Procambarus angustatus, Sitalcicus gardineri, Spirobolellus praslinus, Stagnicola pilsbryi, Stipax triangulifer, Thomasettia seychellana, Tokea orthostichon, Unio madagascariensis, Unio malgachensis, Vitrea storchi, Zonites santoriniensis, Zonites siphnicus etc.

Conclusion:

The conservation of plant and animal species is a major task before biologist. There is many reasons of extinction of biological species all over the world. The extinction of organism may takes place due to natural catastrophic events as well as human cause. The humans are causing destruction of plant and animal species directly and indirectly. The plants and animals are exploited to fulfil the domestic needs and for commercial marketing. The exploitation of organism leads to extinction of genetic diversity in an ecosystem. The extinction of species reduces the biodiversity on earth planet. The rate of extinction is increasing day by day due to human greed for selfish utilization. The extinction of one species may becomes the cause of other species. The loss or extinction of species can be avoided through different measures taken for conservation of biodiversity. The species can be conserved through establishment of protected areas, habitat restoration, and reduction of pollution, in-vitro multiplication of endangered species and management of species. The prevention of species from extinction is a need of time. The decline of wild species requires more attention to conserve it. The efforts towards the sustainable development would be helpful to conserve biodiversity.

Future Perspective:

The balance of nature is depleting continuously due to natural calamities and anthropological activities. The ecosystems are degrading due to pollution. The human activities like mining, construction work, habitat disturbance and exploitation of plants and animal resources are the causes of extinction of organism and loss of biodiversity. The introduction of invasive or exotic species is harmful to native species and population. The global warming has affected displacement of animals in search of food, water and shelter. The terrestrial and aquatic environment is deteriorated significantly by human beings. The land degradation has reduced the primary productivity of an ecosystem. The rate of human population is ever increasing globally. The increase in human population posed a pressure on extinction of organism. The heavy metals, solvents, toxic sludge and chemicals has destroyed the ecosystems on the earth. There is urgent need to reduce different types of pollution. The technological development towards reduction of pollution and sustainable development is a need of time for conservation of biodiversity and

prevention of extinction of species. The highest priority should be given to sustainable use of natural resources to avoid danger of extinction of organism. There is a need of continuous efforts to conserve biodiversity and nature through applying the principles of In-situ and Ex-situ conservation.

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NATUROPATHY: AN EXOTIC APPROACH FOR RESTORING HEALTH

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Introduction:

As the name suggests, naturopathy is an alternative therapeutic systemIn this system nature's healing force is used to treat illness. It works on the belief of vitalism. This chapter mainly emphasizes on the history of naturopathy, basic principles of naturopathy, treatment methods used in naturopathy and different systems of naturopathy. Since 1980s naturopathic physicians have been showing increased awareness to establish schools, standardize education and accreditation. The main aim of naturopathic system is to impart the knowledge of patient regarding healthy life style. In naturapathic system symptoms of disease are not suppressed rather whole person is treated. Diet, herbs, exercises etc. are used in treatment of any disease without using standard drug or surgery. According to American Cancer Society, 2008, the word "naturopathy" originates from a Latin term which means "nature disease".

Principal of naturopathy:

1. **The self interested energy of nature (*Vis Medicatrix Naturae*):** The task of naturaluropathic doctor is to help, promote and improve this cycle by identifying and removing health hazards and encouraging the development of a safe internal and external environment.
2. **Monitor and treat the causative factors (*Tolle Causam*):** Instead of merely removing or reducing signs, the naturopathic practitioner tries to address the symptoms of the disease.
3. **Doctor as Maestro (Docere):** The word "doctor" originally means teacher. A primary goal of naturopathic medicines to educate the patient, and to demonstrate health self-responsibility. Naturopathic physicians often understand and harness the healing value of the interaction between doctor and patient.
4. **Treat the Complete personality:** In Naturopathy recognize the harmonious fuctions and focus on it. Health and sickness arise from a variety of causes such as physical, behavioral, cognitive, genetic, economic, social and other. Since overall wellness also requires mental wellbeing, naturopathic physicists empower individuals to make their personal spiritual growth a reality (Atwood, 2003).

Methods used of treatment in naturopathy

1. **Physical Methods:** Naturopaths often use hydrotherapy; exercise; massage; chiropractic-like naturopathic relaxation techniques; immobilization by bracelets and splints; ultrasound, diathermy, or heat therapy; electrical stimulation; and light treatment. (Coulter and Willis, 2004).

I. Bath Therapy

a. Hip-bath: Hip-bath is the frequently used bath in naturopathy. Baths energize the internal system by revitalizing the circulation of blood by its contact with cold water, applied scientifically within the body. Hip-bath is performed in a tub, specially made for this bath. It is a raised tub, with back seat.

b. Steam-bath: Steam-baths are given in a small wooden cabin with a chair to seat the patient. The cabin has a hole at the top, to keep the head out of it. The cabin is airtight, with a cloth wound around the neck to let no steam escape from the cabin. The patient has no clothes on except underwear.

c. Hot-foot bath: The person is made to sit on the chair with his Sloths on and a blanket covering his whole body except the head, which is covered with a wet towel. A glass of water is served to the person before the bath. His legs are dipped in a tub, covered by the blanket, containing bearable hot water. As the water loses heat, some water is taken off and equal amount of hot water is added to keep the temperature of water constant.

Hip-bath

Steam-bath

Hot-foot bath

d. Hot-and-cold bath: These baths are generally taken in a closed chamber. There are two buckets - one containing bearable hot water and other normal cold water. The bath is preceded by dry massage, i.e. rubbing the parts of body in direction of the heart. First the hot water is poured over the body, about two mugs, followed by four mugs of cold water. The process is repeated, concluding with cold water.

e. Sun-bath: The best time for sun-bath is in the morning, usually within 2 hours after sunrise. Sun-bath should be taken without any clothes or with just underwear for about 15-20 minutes. One needs to lie down on soft ground or a carpet, keep eyes closed and take the

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sun-rays fully on the whole body. The sun-bath that is taken to cure various disorders is usually taken when the sun is quite hot, to allow the body to get complete perspiration. The head is covered with a towel and the garments are taken on to the water. One needs to have a glass of cold water before having such a bath.

Hot-and-cold bath

Sun-bath

II. Therapeutic packs

a. Mud packs: To get the mud, the earth is dug up at a clean place, and the mud is picked up from 4 to 6 inches beneath the surface. The mud is then sieved and made wet with water, making it as thick as dough. The prepared mud is then spread over a thick cloth placed on a wooden plate. The cloth is then placed over the affected part, thus covering it fully with mud. A piece of woolen cloth is then wrapped over the mud pack, enabling the cold effect of the mud to last longer.

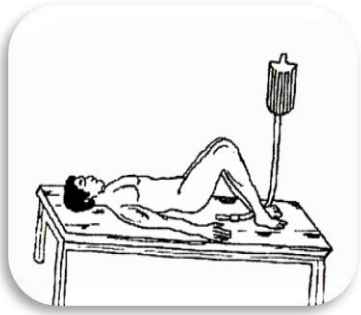
b. Wet-sheet pack: Wet-sheet pack is very useful in bringing down the fever. For this, a bed sheet is drenched in cold water and squeezed. The wet bed sheet is then spread on the patient's empty bed. Without any clothes, the patient is forced to lie on the bed sheet and the bed sheet is fully wrapped around his body, leaving only the face out and the head is wrapped by with a wet towel. Now the wrapped up body is completely covered with one or two blankets.

III. Other physical methods

a. Enema: Usually enema is given before going on a fast or for treating constipation and related problems. Cold water enema and herbal enema are two types of enema.

b. Massage: Massage aids in improving blood circulation in the body, which helps in effective elimination of toxic waste from our body. Massage may usually be oil massage or dry massage. In both types of massage, the direction is always towards the heart, i.e. downwards

from the head and upwards from the feet.



Enema

Massage

c. **Fasting:** Fasting is considered one of nature-cure's most effective methods. In Naturopathy, fasting is practiced for allowing the nature to perform its cleaning process in the body, without any obstruction and any additional load of food to digest. Types of fast include: complete fast, partial fast, fasting on milk, fast on all-fruit diet, fast on restricted diet, etc.
d. **Regulation of diet:** According to Naturopathy, we can treat many ailments simply by changing our daily diet plan. Eating natural foods has a cleansing and purifying effect on our body and also helps us in keeping away from diseases. It should be noted that for a balanced diet, our diet should be alkaline rich. Acidic food leads to production of greater amount of toxic waste in our body, making our body more susceptible to various ailments. Therefore to make the pH of our blood more alkaline, we need to increase intake of vegetables and fruits, especially leafy vegetables like spinach, fenugreek, etc. Dairy products like milk, cheese, yoghurt are allowed; but ghee is prohibited. Naturally extracted oils like olive oil, mustard oil may be used in little quantity (Trudeau, 2004).

Different systems of naturopathy:

All the systems are based on the principle of treating whole body without much focusing on the symptoms of disease in contrast to allopath where symptoms of disease are mainly focused:

1. Homeopathic system
2. Ayurveda system
3. Unani system
4. Siddha system
5. Color therapy
6. Hydrotherapy
7. Acupuncture Therapy
8. Acupressure Therapy
9. Yoga Therapy and Meditation
10. Aromatherapy
11. Massage Therapy

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Homeopathic system:

Homeopathic method is based on the principle of treating the body's gross disharmony instead of getting treatment for any specific disease. Most importantly; homeopathic medicines do not cause side effects. In homeopathic treatment one medicine can be prescribed for several types of diseases and symptoms. For example, all asthmatics get the same treatment by conventional physians, since they already have full knowledge needed to detect and treat the disease based on symptoms of asthma. But, in fact any asthmatic patient has many symptoms beyond the disease symptoms. In any individual, shortness of breath can be found in the presence of the open air but this may relieve the shortness of breath in another. One may feel worse after midnight, another in the morning. One may be having craving for salt, another for sweets. One may be irritable, another weepy. Hence, it is evident that people respond as a whole, as an organism, to disease influences. This response is visible throughout the person as various signs and symptoms. In homeopathy, all these symptoms taken together represent the patient's symptoms.

Treatment: Homeopathy tends to relieve the effects of a wide variety of acute and chronic diseases including: Allergies, asthma, eczema, hay fever, headaches, respiratory infections and Stress (Becker-Witt, 2004).

Ayurvedic system:

Ayurvedic medicine system is the world's oldest organized system of natural medicine. It was originated in ancient India. According to Ayurveda our body is told to be organized in accordance with three major characteristics, namely, Vata, and Kapha and Pitta. All the diseases are specified with their relation with any of these characteristics or 'doshas'. Ayurvedic medicines are prepared from natural herbs and have no side effect on the body (Launsø, 1981).

Ayurvedic system first provided philosophical framework that determined the therapeutic practice with good effects. Its philosophical base is somewhat determined from 'Samkhya' and 'Nyaya vaisheshika' streams of Indian logic (Ramachandra Rao, 1987). The diagnosis is always done by considering the patient as a whole object to be examined. The physician performs a deep study of the patient's internal physiological characteristics and mental disposition. Certain factors, such as the body tissues affected, humors, disease location site, vitality of patient, daily routine of patient, dietary habits, digestion condition And descriptions of a patient's medical, social, cultural, and environmental status.

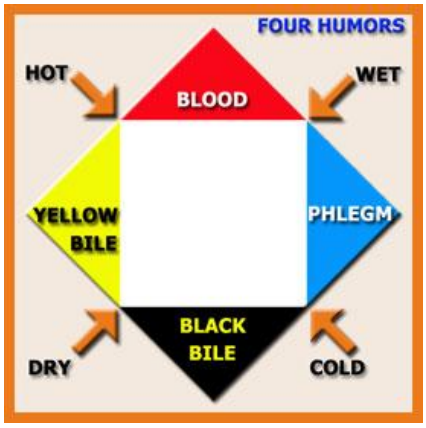
The general examination is known as the 10-fold test by which a doctor tests the following factors in the patient.

1. Psychosomatic constitution,
2. Susceptibility disease,
3. Tissue quality
4. Body buildup
5. Anthropometry
6. Adaptability
7. Mental health
8. Digestive power
9. Exercise endurance
10. Age.

Additionally examination of pulse, urine, stool, tongue, voice and speech, skin, eyes and overall appearance is also accomplished (Kurup, 2004).

Treatment: Balancing of disturbed humors (doshas) through diet regulation, life-routine and behavior correction, drugs administration and resorting to preventive non-drug therapies known as 'Panchkarma' (Five process) and 'Rasayana' (rejuvenation) therapy are different Ways with which to treat disease in ayurvedic system (Chopra, A. and Doiphode, V.V. 2002).

Unani system:



Unani Medicine is just one form of traditional medicine practiced in middle-east & South-Asian countries (Rahman, 2001). Earth, Air, Fire, Water are four basic components of body. By unani system having different temperaments i.e. cold, hot, and wet, and dry. The definition of the four humors is based on Unani medicine; Phlegm, Saliva, Yellow Bile and Black Bile.

There is equilibrium among the humors and the body functions in healthy, state of body. Disturbance in the balance of humors may cause illness. It is believed that six essentials are needed for maintenance of a healthy state. They are:

a. Air,
b. Food and drink,
c. Bodily movements and response,
d. Psychic movement and repose,
e. Sleep and wakefulness and
f. Evacuation and retention (Rahman, 1994; 1996).

Pulse, urine, and stool monitoring plays very important role in the diagnosis of disease in Unani system.

Treatment: Four kinds of treatments are used to treat disease conditions.

Regimental therapy: It is medication-less therapy, such as exercise, massage, turkish shower,

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douches etc.

Dietotherapy: Dietotherapy is based upon patient-specific dietary regimen advice. **Pharmacotherapy:** Drugs are used for treating the disease. Generally drugs obtained from plant, animal or mineral sources (Khaleefathullah, 2002).

Siddha medicine system:

Siddha medicine system is practiced in a few parts of South India particularly within the state of Tamilnadu. The term 'Siddha' is derived from 'Siddhi'- which suggests achievement. Siddha's materia medica system of medication is primarily based on metals and minerals drugs root. Matter and vitality are the two overwhelming substances concurring to the Siddha concepts which have major impact in forming the Universe's nature. They are known as Siva and Sakthi in Siddha system. Matter cannot exist without vitality and vice-versa. 'Ashtasthana pareeksha' is utilized for conclusion in Siddha medicine system (examination of eight locales) that envelops consider of nadi (beat), kan (eyes), swara (voice), sparisam (touch), varna (colour), na (tongue), mala (dung) and neer (pee). Siddha medicine system moreover takes after ashtanga concept with respects to treatment methods (Narayanaswamy, 1975).

Color therapy:

Color therapy is also known as cromotherapy. It is a gentle non invasive complementary therapy that can be employed to help a variety of health issues.

- **Red:** It activates the processes that have been stagnating. It also strengthens the senses.
- **Blue:** It soothes, inhibits, focuses, cools down, re-regulates hyperactive, inflammatory processes. It brings serenity and clarity.
- **Yellow:** Yellow cause's stimulation without excitation, extends energy, reinforces weak processes, and strengthens the nerves.
- **Green:** It is relaxes, Keeps the mental and physical strength, releases tension.
- **Orange:** Restores, Warm, it stimulates, and it works better than red. Unlocks deadlocked

processes, cheers, encourages, mitigates.

- **Purple:** It is converts energy at a high level, helps mental processes, relieves anxiety, and reduces nervous irritations (Bioptron, 1998).

Different Ways of Using Colors by Therapists:

The way specialists utilize color to treat their patients can shift or maybe significantly. Most common:

I. **Colored filter:** The patient is uncovered to being passed through colored filters.

II. **Solarised water:** Spring water is poured into a recolored glass bottle and kept in coordinate daylight for a few hours. It is accepted that the water will get the vibrations of the bottle's color.

III. **Solarised cream:** A holder of non-perfumed and non-colored cream is put beneath or in a glass channel and uncovered to daylight. As with the spring water, the cream at that point assimilates that color's vibrations. These creams are at that point utilized remotely for treating skin conditions.

IV. **Color channeling:** The specialist filters the body's atmosphere and after that acts as a channel, transmitting color onto the quiet.

V. **Ingesting color:** It is accepted that the properties of color can be gotten to a certain degree through nourishment stuff, particularly in the event that the food's color is characteristic and it is created in daylight (Theo Gimbel, 1980).

Hydrotherapy:

Hydrotherapy or hydrotherapeutics is sometimes referred to as hydropathy (Beamon, 2004). Hydrotherapy is a therapeutic method that uses water at different temperatures and in different states of aggregation to treat certain disorders (Dumitrascu *et al.*, 2012). Many practitioners of 'hydrotherapy' feel water has adequate curative properties and that, in contrast to other medicinal agents, is safe (Beamon and Falkenbach, 2004). In this therapy the water employed may be natural, mineral or aquatic, pure or combined with other substances or herbals, with medicinal infusions or decoctions like Chamomile, marshmallow, walnut leaf, etc. and different natural mixtures, including oil, iodine, sulphur, water, gas and so on (Dumitrascu *et al.*, 2012).

The pool classes have been developed to maximize these advantages of exercising in water with close attention to the specific needs of these patients.

a. **Impedance of water:** Impedance increases the workload of muscles, increasing strength and inhibiting chorea form movements.

b. **Turbulence:** Turbulence is made when any protest moves through water shaping a wake and vortexes behind the question. This causes resistance to the development. When

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standing still, keeping up adjust against turbulence gets to be a more troublesome work out.

c. **Buoyancy:** Buoyancy gives bolster for a body in water, in this way giving flexibility of development not conceivable on dry arrive.
d. **Hydrostatic weight:** Weight of water is felt over the body when a individual enters the pool. Weight is most apparent on the chest divider, standing up to extension. Individuals with HD more often than not have diminished crucial capacity (and are exceptionally habitually smokers) and they will have to be work harder to realize a great respiratory work within the pool. Respiratory and beat rates increment with work.
e. **Warmth:** The warmth of the water causes unwinding of tense muscles, with a few diminishments of chorea shape movements (Batcheler, 2001).

There are many are the specific treatments that can be accomplished by hydrotherapy.

Pelvic and stomach issues: They are cured basically by sitz shower that can be made with hot or cold water. Among them we have for case, the treatment of hemorrhoids, stoppage, kidney illnesses, infections of the liver.

Problems of the circulatory framework: Such as destitute circulation, varices. It is more often than not connected with cold water. Be that as it may it isn't satisfactory to rheumatic patients which respond adversely to cold.

Problems of body pain: Such as rheumatic torment, migraine, arthrosis etc. These are primarily persistent torments which are treated with hot water (Dumitrascu *et al.*, 2012).

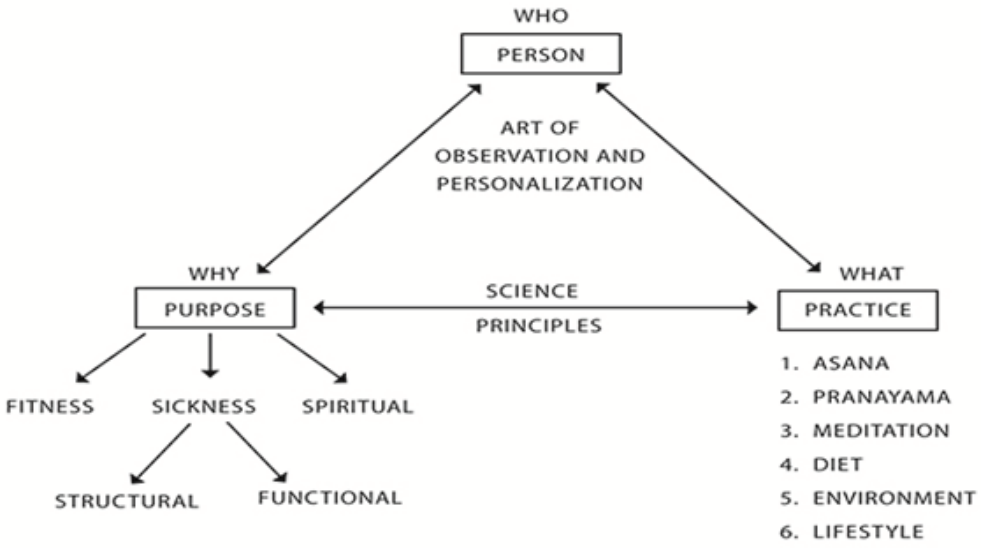
Yoga therapy and meditation:

The word “yoga” comes from a Sanskrit which means union, joining, or to link together as one whole (Paul, 2007). Yoga is the technology and research overcoming the intrinsic resistance to creating a union of brain, soul and whole body in all life. Meditation is an integral component and the essence of yoga (West, 1979). The concept is to create a healthy body, an alert mind, an emotionally stable state & a spiritually enlightened life. If we interpret "Yoga" therapeutically, then obviously under the physical, psychological, mental, emotional & spiritual sub-headings we will need to discuss the various aspects of Yoga.

The following is pronounced as Yoga Therapy Limbs.

1. Yama,
2. Niyama, Mitaahara-Act codes & Diet regulations
3. Kriya – Cleaning Process
4. Asana – Physical Postures
5. Pranayama – Breathing techniques

6. Mudra/ bandha – Neuro-muscular locks
7. Relaxation
8. Dhyana – Meditation



Principle of Yoga Therapy:

- **Yama (The five "abstentions"):** Ahimsa (non-violence), Satya (Truth, non-lying), Asteya (non-stealing), Brahmacharya (non-sensuality, celibacy), and Aparigraha (non-possessiveness).
- **Niyama:** Shaucha (purity), Santosha (contentment), Tapas (austerity), Svadhyaya. (Vedic scriptural research to learn For God and spirit, and Ishvara-Pranidhana (God-given surrender).
- **Asana:** In Patanjali's Sutras, literally means "seat," Which applies to the place of seat used for meditation.
- **Pranayama ("Suspending Breath"):** prana, breath, "ayama," to prevent or restrain. Often translated as power of the life force.
- **Pratyahara ("Abstraction"):** removal of the sense organs from the external objects.
- **Dharana ("Concentration"):** Focus focused on a single object.
- **Dhyana ("Meditation"):** Intense reflection of the essence of the meditation piece.
- **Samadhi ('liberation'):** integrating conscious with the meditative entity (Feuerstein, 2000).

Diagnosis in Yoga Therapy:

There are number of ways by which disease and stress in the body can be diagnosed includes:

- Pulse reading
- Demography, the reading of changes in skin
- Iridology, the reading of iris of the eye

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- Observations of symptoms relative to kapha, pitta, vatta
- Analysis of sputum, sweat. Urine, feces.
- Analysis of lakshana (traits), vasana (pre-deposition), dosha (humours), klesha (obstruction) and ritti (manner).

Application of Yoga Therapy:

- Increasing flexibility
- Increasing lubrication of the joints, ligaments and tendons
- Complete detoxification
- Excellent toning of the muscles
- Relief in chronic diseases (Bera, T.K. and Rajapurkar, M.V.1993).

Aromatherapy:

Aromatherapy; fragrance based treatment is the treatment or avoidance of illness by utilization of essential oil. Other stated uses include pain and uneasiness diminishment, upgrade of vitality and short-term memory, hair loss avoidance, and decrease of eczema-induced tingling. Aromatherapy is the type of alternative, complimentary medicine, Often called Essential Oil therapy, Can be described as the science and art of utilizing aromatic essences extracted naturally from plants to stabilize, harmonize and encourage the health of body, soul and spirit.

It seeks to unify physiological, psychological and spiritual processes to enhance an individual's innate healing process Principal of Treatment and selection of Essential Oil in the holistic aromatherapy, an aroma therapist carefully makes the selection of essential oils for customized treatment typically by in-depth consultation through understanding individual's existing health problems, life styles, and emotional state.

Aromatherapy can be employed using following ways:

a. **Massage:** Aromatherapy massage may bring the advantage of the essential oil and the same is true of the massage. It is found that the effect of touch can be very valuable to relax the person and soothe the nerves. To make therapeutic massage oil need to blend up to 31% of essential oil into base/carrier oil.
b. **Bath:** The warmth of a shower not only calms an individual; it too empowers the skin to retain the essential oil way better. The mixed oil ought to be included once the shower has been run as the warm will empower dissipation. As it were the non aggravation essental oils like lavender & german chamomile can be included straightforwardly to a shower, all other oils ought to be to begin with mixed either within the required base oil or in a cupful of full fat milk.
c. **Burners and Vaporisers:** These can be utilized to deodorize, disinfect or essentially make a

extraordinary environment. Breathing in the vapors can moreover be therapeutically beneficial. Put small water within the best Brenner part and include 7-10 drops of basic oil to it sometime recently lighting the candle below.

d. **Inhalation:** A stream inhalation is a good way of treating coughs, colds, sore throats and for cleansing the skin. Put very hot water in a bowl and add 3-4 drops of essential oil. Then lean over the bowl, place a large towel over your head and inhale the vapors.
e. **Spitz Bath:** A spitz shower is an great way of treating hemorrhoids thrush pruritus etc. Half fill a level bucket of a little shower with warm water include 4-6 drops of mixed essential oils. Swirl the water and sit in it for 10 minutes. Comparative treatment can be utilized as hand showers & foot showers.
f. **Compresses:** Depending on the sickness a hot or cold compress is a successful way of treating numerous nearby complaints. To create a hot compress pour warm water into a bowl and after that include the essential oil.

Massage

Bath

Burners and Vaporisers

Inhalation

Spitz Bath

Compresses

Application of Aromatherapy:

- ❖ Pain
- ❖ Depression
- ❖ Insomnia
- ❖ Agitation
- ❖ Quality of life
- ❖ Tumors
- ❖ Anxiety
- ❖ Viral infections
- ❖ Fungal infections
- ❖ Bacterial infections
- ❖ Nausea
- ❖ Burns
- ❖ Fatigue
- ❖ Stress
- ❖ Inflammation

(Schnaubelt and Kurt, 1995)

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Massage therapy:

Massage (muh-SAHZH) can be characterized as the systematic manual stimulation of the body's soft tissues by motions such as pressing, kneading, squeezing, rolling, tapping and clicking for therapeutic purposes such as improving blood and lymph circulation, muscle relaxation, pain relief, physiological equilibrium restoration and other physical and mental benefits.

The massage techniques commonly used today come specifically from the systems of Sweden, Germany, French, English, Chinese and Japan.

Kinds of Massage Systems:

The Swedish system: is based on Western anatomy and physiology principles and uses conventional eurage, petrissage, vibration, friction, and tapotement manipulation techniques. The Swedish method either uses gradual and gentle motions, or vigorous and braced, as per the effects that the practitioner needs to thrive.

1. **The German method:** German method integrates all of them Swedish practices and promotes Usage of the various therapeutic bath forms.
2. **French and English systems:** Most of the movements in Swedish massage are also used for massaging your body.
3. **Chinese medicinal practice of acupuncture acupressure systems:** It's based on conventional Oriental medicine concepts. It employs various methods to stimulate acupuncture points to control Qi (the strength of life force). The aim of this approach is to induce psychological improvements in the person being treated and also to relieve pain, discomfort or other physiological imbalances.
4. **The Japanese system:** Shiatsu, a form of finger pressure, is also named, based on the oriental idea that the body does have a set of energy points (tsubo). Accordingly, when pressure is exerted towards these points, circulations improve and nerves are stimulated. This method is known for improving body metabolism and alleviating a variety of physical conditions.
5. **Sports massage:** refers to a method of massage especially planned to prepare an athlete for an upcoming event and to support in the body's regenerative and restorative capacities following a painstaking work out or competition. These results are induced through complex manipulations, which enhance blood and lymph circulation. Some gestures in sports massage are intended to break down lesions and adhesions, or reduce fatigue. Polarity therapy: This is a technique Randolph Stone (1890–1971) built using both Eastern and Western-derived massage manipulations. Exercises and patterns of thinking are used to stabilize the body physically as well as energetically.

6. **Rolfing:** Rolfing is a standardized method built out of Dr. Ida Rolf's technique of systemic integration. Rolfing aligns large parts of the body by fascia (FAH-shuh) or connective tissue manipulation.
7. **Touch for health:** is a simplified form of applied kinesiology (ki-nee-see-AHL-o-jee; principles of anatomy in relation to human movement) developed by Dr. John Thie. This method involves techniques with the East and the West origins. Its target is to relieve stress on muscles and internal organs. There are also several styles of bodywork and alternative health-related practices that use specialized kinesiology (a form of muscle testing) to derive information about the conditions of the body or how Relevant material, or type of treatment might affect it.
8. **Neuromuscular techniques:** The study of osteopaths Dr. Stanley Lief and Boris Chaitow began in Europe around 1940. Paul St. John, Bonnie Prudden, Janet Travell, Lawrence Jones, Judith DeLany and Dr. Leon Chaitow have evolved Western methods, among others. Varieties Involve myotherapy, neuromuscular therapy;point trigger therapy, muscle energy training, orthobionomy, and Neuromuscular therapies use similar manipulations to Swedish massage tosystematically Trigger or sedate neuroreceptors which are normally in the contractile tissue. Reflex action helps to standardise contractile tissue and brings the bodymore toward balance.
9. **Craniosacral therapy:** has been developed by Dr. John Upledger andresearchers at the Upledger Institute in Palm Beach Gardens, Florida. Craniosacraltherapy is a gentle, advanced method of evaluating and enhancing thefunctioning of a physiologic body system called the craniosacral system. Duringcraniosacral therapy, trained practitioners use a light touch (i.e., equivalent to anickel's weight) to feel the rhythmic motion theoretically created by the movementof the cerebrospinal fluid within the craniosacral system. Craniosacraltherapy treatment techniques are noninvasive and usually indirect approaches,intended to resolve restrictive barriers and restore symmetric, smooth craniosacralmotion. Craniosacral therapy is effective for a broad range of physiologicconditions associated with pain and dysfunction and is used as a preventivehealth practice because of its ability enhancing function of the central nervous system and bolster the body's resistance to disease.

Applications of Massage therapy:

- **Circulation of Blood:** Perhaps the most basic principle in this field is that improved blood circulation is advantageous for almost all health conditions. Tension in the muscles and other soft tissues can reduce circulation, leading to inadequate food supply and inadequate removal of waste or toxins from body tissues
- **Lymphatic Fluid Movement:** The lymphatic system is just as extensive as the blood

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stream. Lymphatic fluid drainage plays a major role in cleaning the body of waste, toxins, and pathogens. Massage also helps the lymph system, particularly in cases where lymphatic Drainage is affected by injury or surgery (e.g. in women with postmastectomy).Elliot Greene explains the method as that of breaking apart the scarring that had developed between the bones, the vertebra and the ribs of her bones and connective tissue or fascia, all of which had been trapped together.

- **Blood:** The flow through the region was healed and depression returned in her spine, which had been visible, slowly started to diminish. The spine returned to full range of motion.
- **Release of Toxins:** Chronic stress or injury to the body's soft tissues may result in normal metabolism accumulation of toxic by-products. Hands-on methods help to drive the toxins into the natural pathway and removal processes of the body.
- **Release of Tension:** Chronic muscle tension can accumulate and affect the structure of the body and its work attributable to high stress habits, traumas, lifestyle or injury. It also affects psychological health. The loss of anxiety facilitates greater recovery, which has major physiological and psychological effects.
- Structure and feature depend on one another: the body's musculoskeletal structure affects function and structure is affected by function. Stress or trauma will adversely alter any of these. Massage therapy and bodywork may help to improve healthier structure and function, facilitating improved circulation, more ease of motion, greater flexibility, and chronic tension patterns to be released.
- **Stress reduction:** Stress is progressively assumed to cause illness and probably 80- 90% per cent of all illnesses are caused by stress. Massage therapy is an important, non-drug tool for stress management and relaxation promotion. Energy: In this tradition several modalities function through the movement of energy into the body as a way of facilitating healing. Energy may be regulated or stimulated to travel around and through the body in ways that influence the body's physical structure and work, as well as emotional well-being. Such work may require hands-on contact, or it can be performed with no physical body touch **(**Beck, 2010**)**

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EUTROPHICATION IN VELLAYANI LAKE – A REVIEW

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Abstract:

Lakes and rivers are the lifelines of our country. Anthropogenic activites had deteriorated the quality of these bodies. Same is with the vellayaniLake, one among the three rains fed freshwater lakes in kerala. Nutrients like phosphates and nitrates released during aquaculture and agricultural practices contribute to the eutrophication of the lake. Different limiting factors like temperature, pH, sunlight, and dissolved oxygen are known to affect thelife of aquatic animals in eutrophic Lakes. Surface runoff from agricultural fields nearby brings down fertilizers and other agro chemicals. Therefore, strict control and management strategies must be implemented for its management. The rapid increase in population and urbanization has resulted in the deterioration of water resources particularly lakes are deteriorating due to serious pollution stresses inflicting the scarceness of water resources. Several environmental factors other than nutrients also aggravate the problem of eutrophication.

Keywords: Vellayani Lake, eutrophication, nutrients, algal blooms

Introduction:

Eutrophication is a condition in which the waterbody is enriched with nutrients which then induce excess algal growth.This may result in algal blooms which are deleterious to the aquatic life. Eutrophication is thus the gradual process of nutrient enrichment of a lake, as it changes from an oligotrophic state (nutrient- poor) to a eutrophic state (nutrient-rich).

Vellayani Lake is located in the outskirts of Thiruvananthapuram district and is utilized for drinking needs of Kalliyoor – Venganoor – Vizhinjam - Kovalam Gramapanchayats of Thiruvananthapuram District. This Lacustrine wetland is the second largest freshwater Lake of Kerala. This lacustrine wetland is one of the three rain-fed freshwater lakes in Kerala. The lake is located between north latitude 8^0 24’90’’- 8 26”30’ and east longitude 76^0 59’08’’-76^0 59’47’’at

the capital city Thiruvananthapuram. The upper part of the lake is converted into a reservoir which is used for irrigational purpose.

This drinking water source is under threat from various practices like illegal sand mining, encroachment, land reclamation, eutrophication, leaching of pesticides, aquaculture practices etc. Moreover a large part of the lake is used for lotus cultivation Lotus (*Nelumbo nucifera),* a large perennial, aquatic herb with big round floating leaves, which again aggrevates the problem. The effects of these impacts not only affect the socio-economic functions of the waterbody but also lead to the loss of its structural biodiversity.

Nutrients enter into lakes through natural and man-made processes. Hence, for a better understanding on the biogeochemical process that control inputs, recycling and removal of nutrients in aquatic system is quite essential for understanding the productivity of the water bodies. Study on the physico-chemical parameters and phytoplanktons revealed that in certain sites the physico- chemical parameters were above the desirable limits and the pollution indicator phytoplanktons like Closterium, Nitzschia, and Oscillatoria were found and so these sites are in the verge of pollution. The water is getting polluted mainly by the domestic wastes and plastic bottles dumped in certain stations of the lake.

Deterioration of water quality is generally due to organic and inorganic contaminants entering the waterbody throughsurface runoff and leaching. The accumulation of excess nutrients in the water bodies result in excessive growth of weeds like Eichornia, Pistia etc. This may deprive the waters with our precious oxygen and make water unfit for drinking.

Review of literature:

The slow, but steady urbanization rate and subsequent pressure on the land resources has put Vellayani wetland system at risk by reclamation as well as drastic land use .Exotic species like water hyacinth, salviniaetc poses a major threat to the wetlands by causing heavier inputs of nutrients leading to eutrophication. Therefore management requires a proper assessment of functions and values of wetland, together with an understanding of traditional management practices and active participation of the stakeholders and participation of the local people. Plants are a major factor influencing methane emissions from wetlands, along with environmental parameters such as water table, temperature, pH, nutrients and soil carbon substrate. These may be one among the reasons for the changing rainfall patterns and altered environmental temperatures.

Krishnakumar *et al.* (2019) evaluated nutrient status and heavy metals in the sediments of Vellayani Lake and found out that the Lake is under stress consequent to urbanization and

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unscientific agricultural practices in the catchment areas.Studies conducted by Gopinath and Ajitkumar (2014) noticed that in certain sites the physico chemical parameters were above desirable limits and the pollution indicator phytoplanktons were also found. Dharmapalan (2014) pointed that the Lake is under serious threat due to several anthropogenic activities. Recent works carried out by Veena *et al.* (2014), reaffirmed that anthropogenic interventions have constantly changed the landscape characteristics of Vellayani catchment area Revathy *et al.* (2017) observed higher values for nutrients like phosphates and nitrates. This may be due to the higher phyto- planktonic production, decaying macrophytes and runoff from thagricultural catchment areas. Vijayan *et al.* (2017) indicated that the area of water body which was 558.93 ha during 1973 was reduced to 243.39 ha in 2011. The study revealed that the drastic reduction in the lake area is due to irrational human activities like unsustainable exploitation of the ecosystem services due to demographic pressures and urbanization.

According to Kumar (2018) there has been evidence of increasing eutrophication and presence of pesticides in the Lake. Besides the rich biodiversity the ecosystem was severely threatened by illegal sand mining and leaching from the nearby paddy fields.

Conclusion:

In Kerala, wetlands are facing tremendous pressure owing to rapid developmental activities and excessive utilization of various components of the environment especially, water and land. Even though, the qualitative degradation of the ecosystem is well understood, the quantitative estimation on the destruction rate of wetlands in Kerala is poorly identified.The major issues facing the wetlands of Kerala are related to pollution, reclamation, mining, encroachment, eutrophication, and biodiversity loss. The unscrupulous exploitation of the fragile wetland system and undesirable input of residues exceeding the assimilative capacity of wetlands are resulting in various kinds of pollutionResearch studies studied revealed that anthropogenic activities like agriculture in catchment area, sandmining causes nutrient enrichment in Vellayani Lake. Hence necessary judiciary measures must be implemented inorder to protect this precious freshwater ecosystem.

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THE AQUATIC RESPIRATION OF FRESH WATER CRAB *BARYTELPHUSA GUERINI* AFFECTED BY ORGANOPHOSPHATE (DIMETHOATE)

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Abstract:

Food plays a vital and predominant role for the survival. It is said that there has been no balance between the population explosion and the production of food grains. The Malthus law explains the growth of population increasing day–by–day in geometric progression which food production is in arithmetic progression. Food crops in the field and in storage are vulnerable to damage from pests. In man–made agriculture, losses from pests are very high. Respiration is a process during which the organisms obtain oxygen from external medium and use it for the purpose of energy release during oxidative metabolism. As such the process of respiration in animals is studied by determining the oxygen consumption. The total oxygen consumption of the animal reflects the basal metabolic statues which reflect the general effect of several intrinsic and extrinsic environmental stresses. This serves not only as a tool in evaluating the susceptibility or resistance potentiality of animal, but also useful to correlate the behaviour of the animal. This research article doing best to detect the effect of organophosphate i.e. Dimethoate on aquatic respiration about oxygen consumption by female crab *Barytelphusa guerini*. The papers represent the results with Statistical analysis and graphical representation.

Keywords: Aquatic Respiration, *Barytelphusa guerini*, Organophosphate (Dimethoate)

Introduction:

One of the most important classes of the present day synthetic pesticides is the organophosphorus insecticide of which more than 100 are widely used as agents to combat plant pests and ectoparasites and in part to combat endoparasites of domestic animals. Some of the important advantages of organophosphate compound, as the preferred insecticides of modem era, are the wide spectrum of action on plant pests, high insecticide activity, low persistence, rapid metabolism and poor accumulation in the body of animals.

The organophosphates, a class of compounds with many diverse members are noted for their high biological activity, Biological activity of insecticidal organophosphates is not limited

just to insects, they are toxic to mammals and other organisms also particularly to those in which cholinesterase plays a vital role. The organophosphates have come to be used extensively as insecticides replacing many of the older compounds.

The organophosphorus compounds are more toxic to mammals than the organochlorine compounds (Brown, 1978). They have the important advantage of being relatively not so persistent either in the physiological environment or with the organismdue to their biodegradability. They have less cumulative and ecologic effects and thus are valuable substitute for the more persistent organochlorine compounds. In the last three decades more than one hundred organophosphorus insecticides have been used to kill the insect pests (Hayes, 1982).

The usual symptoms of organophosphorus poisoning in mammals are defecation, urination, lacrimation, muscular twitching and muscular weakness. In severe cases, prostration and clonic, sometimes tonic, convulsions follow. Light symptoms are usually parasympathetic in nature (e.g. brady–cardia, salivation and meiosis); though a number of symptoms due to stimulation of organs.

The effects of organophosphorus include defecation, urination, lacrimation, contraction of the pupil, decreasing of the heart beat and a drop in blood pressure, in addition to paralysis are also observed. Some organophosphates produce paralysis of legs (in human beings and birds) or hind limbs which could be result of damage to myelin sheath surrounding the nerve fibers.

Material and Method:

Oxygen consumption was determined by Wrinkler method (Welsh and Smith, 1953). The freshwater female crab *Brytelphusa guerini*was used for experimentation. The animals were collected from their natural habitat and maintained in the laboratory before and during the experimentation. The selection of the crabs for the experimentation was made on the health of the crabs. The pesticide pollutant selected for the study was Dimethoate.

The apparatus used in this experiment was similar as described by Saroja. The apparatus mainly consisted of a reservoir (R) and respiratory chamber (RC). A 500ml wide mouthed bottle was used as respiratory chamber. The size of the bottle was such that it was not too big for the enclosed crab to give considerable difference in oxygen content between initial and final sample. The chamber was coated with black paint to avoid the activity due to light.

Before starting the experiment crab was left in running tap water for about 10 minute to facilitate them to reach a state of normality from a state of excitement; if any. After this equilibration period, one crab was kept in respiratory chamber without causing any damage to the animal and initial sample was collected immediately as described above. Then the crab was allowed to respire for one hour. Immediately after one hour final sample was collected. The amount of dissolved oxygen in these samples was determined by the stander Winkler’s method,

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as given.The total oxygen consumption and rate of oxygen consumption was calculated by considering the wet weight of the animals.

The values for total oxygen consumption are expressed as ml. (c.c.) of O2 animals / hr and for the rate of O2 consumption are expressed as ml. (c.c.) of 02 / gm / hr wet. wt. of animal.

Result and Observation:

Effect of Dimethoate on Total Oxygen Consumption and Rate of Oxygen Consumption in Freshwater Female Crab *Barytelphusa guerini*

Effect of Dimethoate causes changes in total oxygen consumption. Total oxygen consumption expressed in ml/lit., is the average of six observation ± S.D.

Table 1: Effect of Dimethoate on total oxygen consumption in female crab *Barytelphusa guerini*

Sr. No.	Duration of Exposure	Control	Experimental
1	24	1.184 ± 0.081	0.601 ± 0.058 ***
2	48	1.208 ± 0.064	1.791 ± 0.041***
3	72	1.241 ± 0.076	1.394 ± 0.051**
4	96	1.208 ± 0.083	1.171 ± 0.061**

Note: 1) Values expressed as Beats/min. of animals,

2) Each value is mean of six observations ± S.D.

3) Value are significant at * = P<0.05, ** = P < 0.01, ***=P < 0.001 and NS – Not significant

Effect of Dimethoate causes changes in rate of oxygen consumption. Rate of oxygen consumption expressed in ml/hr/gm wt. /lit., is the average of six observation ± S.D.

Table 2: Effect of Dimethoate on rate oxygen consumption in female crab *Barytelphusa guerini*

Sr. No.	Duration of Exposure	Control	Experimental
1	24	0.033 ± 0.002	0.018 ± 0.003*
2	48	0.034 ± 0.003	0.056 ± 0.002**
3	72	0.036 ± 0.002	0.044 ± 0.001***
4	96	0.029 ± 0.002	0.033 ± 0.002***

Note: 1) Values expressed as Beats/min. of animals.

2) Each value is mean of six observations ± S.D.

3) Value are significant at * = P<0.05, ** = P < 0.01, ***=P < 0.001 & NS – Not significant

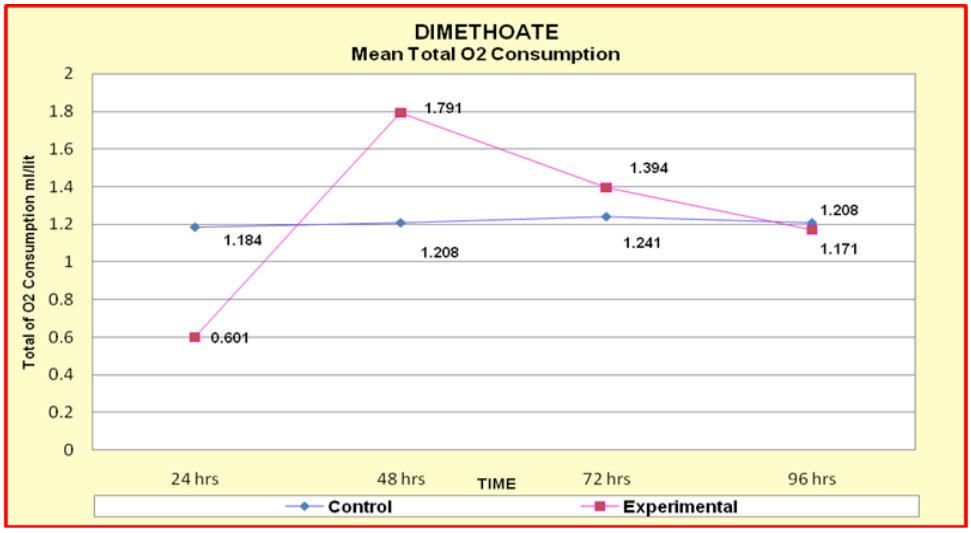

Figure 1: Effect of Dimethoate on Total oxygen consumption In *Barytelphusa guerini*

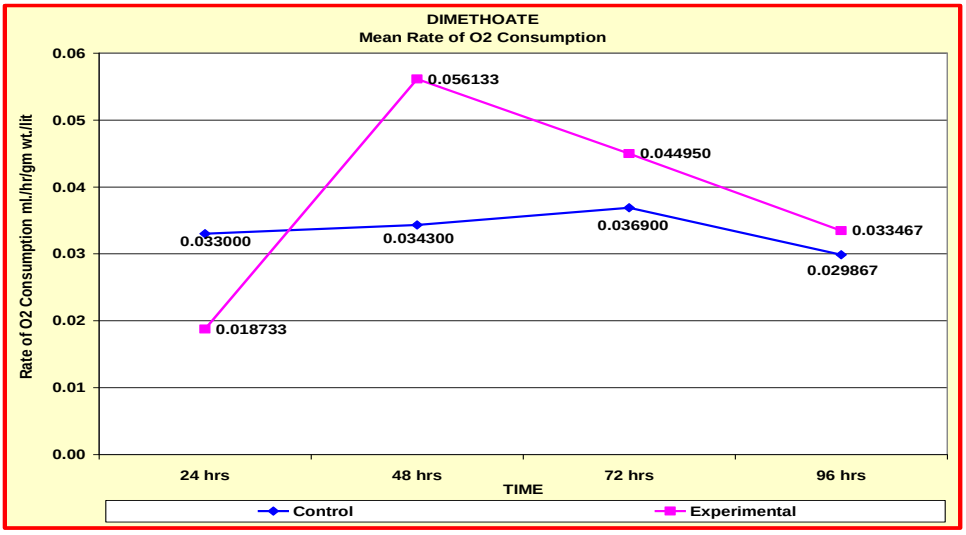

Figure 2: Effect of Dimethoate on Rate of Oxygen consumption in *Barytelphusaguerini*

Results:

- The freshwater female crab *Barytelphusa guerini* showed variation in the rate of oxygen consumption and total oxygen consumption when exposed in Dimethoate.
- In the present investigation it was showed that the oxygen consumption in the animal exposed to Dimethoate was decreased upto 96 hours. The Dimethoate exposed animals showed initially decreasing at 24 hours while increased rate of respiration at 48 hours and then gradually decreased in oxygen consumption upto 96 hours as compared with control.

Effect of Dimethoate:

The total oxygen and rate of oxygen was studied in the present study. The effect of dimethoate show increased trend at 24 hours and then slowly decline upto 96 hours. The oxygen rate and total oxygen consumption shown in Table (1 and 2) and Figure (1 and 2).

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Discussion:

The process of diffusion to occur a higher concentration of oxygen and lower concentration of carbon dioxide must be maintained outside the membrane than the blood of respiratory membrane. Therefore, the second requirement for respiratory system is a ventilation mechanism to move the respiratory medium (air or water) past the respiratory membrane (Webster and Webster, 1974). In crustaceans this is accomplished by sustained vibrating movements of the scaphognathite of mixillae and exopodites of maxillepedes, which create a constant water current passing over the gills. Apparently, vibrating movements of the Scaphognathite and exopodites are produced by a set of muscles which are under nervous control (Hyman, 1959). Derangement in the rhythmic movements of these organs as a result of AcheE inhibition could lead to a decrease in the rate of water flow over gill surface and hence in the amount of dissolved oxygen available for diffusion. Maintance of structural integrity of gills is essential for respiration to occur at a normal rate (Procosser, 1973). Damage to structural integrity of gill may adversely affected respiration. Most organochlorine insecticides, including endosulfan, were found to cause damage to gills of fishes and *Crustaceans* (Uthaman, 1977). These observations suggest that endosulfan cause gill damage resulting decrease in oxygen consumption.

Another factor that could be implicated in causing decrease in oxygen consumption of crabs exposed to sublethal concentrations of endosulfan is the formation of a thin 'mucus film' over gill surface which is likely to affect respiratory rate by reducing the effective surface area of diffusion and the rate at which diffusion occur. Formation of thin mucus film over gill has been observed in fishes (Kumar *et al.,* 1982) and crabs (Subhadra Devi, 1985) exposed to different pesticides such a phenomenon has also been observed in the present study which may account for the decrease in animal oxygen consumption. Further it has been observed that mucus deposition over gills surface was greater, is responsible for a greater decrease in oxygen consumption. The decline in the rate of oxygen consumption after an initial increase and after prolonged exposure periods might be the result of the unset of poisoning. The decline is depending upon the increasing concentrations of in the pollutants at the time of experiment. An initial increase and then gradual decrease in the rate of oxygen consumption during acute exposure to pesticide endosulfan & dimethoate in present study.

The decrease in oxygen consumption may also be due to failure of crab to compensate for the new steady state of metabolism to the stress of heavy toxicant pesticides endosulfan and dimethoate.

Conclusion:

The rate of oxygen consumption of an animal and its tissues reflects the respective metabolic rates and hence energy output. Size is one of the important factors influencing the metabolism of animals. It is an important variable which plays a pivotal role in the metabolism of organisms. While the influence of the environment is on metabolism, the effect of that influence is displayed through the activity of an organism whose metabolism has been affected.Growth rate increases as a result of increased energy intake and indirect proportion to respiratory output. This strategy has been recorded for many poikilothermic and homeothermic animals.

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BIOLOGY AND SIGNIFICANCE OF METHANOGENS

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Abstract:

Methanogens are very important and captivating microorganisms, both from a biological, as well as for a technological point of view. Methanogens belong to the domain archaea. They are able to use hydrogen as a sole source of reducing power for methane production. Swamps, oil fields, rice fields, peat bogs, marshland, digestive systems of animals, landfills, digesters, biogas plants, hydrothermal vents and hpersaline lakes are some most probable sites for the occurrence of methanogens. Methanogens exhibit different cell shapes such as sarcina, rods, spheres, and spirals. *Methanobacterium aarbophilicum*, *Methanobacterium athermoautotrophicum*, *Methanospirillum ahungatii*, *Methanobacterium formicium*, *Methanobacterium mobile*, *Methanococcus vannieli* and *Methano sarcinabarkeri* are some renowned species of methanogens. The field of methanogenic research is quite vital since these microbes are able to produce methane which is a storable and flammable biogas. Beside methane production, methanogens can be used in many applications such as sewage water treatment, degradation of pollutants, decolorizationof azo dyes, electricity production and decomposition of organic waste.In this chapter, wehave reviewed biology and significance of methanogens.

Keywords: Methanogens, archaea, biogas, methane, extremophile

Introduction:

The term methanogen was used for the first time by M. P. Bryant for a group of prokaryotic microorganisms. Methanogens are involved in biological formation of methane (CH_4) by utilizing CO_2, H_2 and/or acetate, formate, and methylamine in hypoxic or anoxic condition. Methanogens belong to the domain archaea which are different than eubacteria

(Bapteste et al. 2005; Pathak and Sardar, 2012).Nutrient requirements for various methanogenic bacteria have been thoroughly assessed by Zeikus in 1977. All methanogenic bacteria are able to use hydrogen as a sole source of reducing power for methane production. Some reactions for energy metabolism in methanogens are given below (Zinder, 1993; Zeikus, 1977; Balch et al. 1979).

$4H_2 + HCO_3^- + H^+ \longrightarrow CH_4 + 3H_2O \quad \Delta G^{0'} = -32.5$ kcal/mol.

$4HCO_2^- + 4H^+ \longrightarrow CH_4 + 3CO_2 + 2H_2O \quad \Delta G^{0'} = -34.7$ kcal/mol.

$4CH_3OH \longrightarrow 3CH_4 + CO_2 + 2H_2O \quad \Delta G^{0'} = -76.4$ kcal/mol.

$CH_3COO^- + H^+ \longrightarrow CH_4 + CO_2 \quad \Delta G^{0'} = -8.6$ kcal/mol.

Habitats of methanogens and Sources for isolation:

Naturally, biological methanation has been found at many places such as swamps, oil fields, rice fields, peat bogs, marshland and digestive systems of animals. Therefore, these sites are the most probable habitats of methanogens. Moreover, methanogens can be isolated from extreme environments such as hydrothermal vents and hpersaline lakes. Methanogens can be also found in some non-natural habitats, for example, landfills, digesters and biogas plants.Thus, isolation of methanogenic species has been reported from many diverse sources (DeLong 1992; Ahring, 1995; ZeppFalz 1999; Pathak and Rathod 2014). *Methanobacterium* species are abundantly found in lake sediments and sewage sludge digestors samples. Some sources of isolations are reported here. *Methanobacterium aarbophilicum* was isolated from wetwood of living trees. *Methanospirillum ahungatii* was isolated from sewage sludge. *M. ruminantium* strain M1was isolated from rumen.A list of some other methanogens and their isolation source has been given in Table 1. For the isolation of methanogens, the collected samples need to be inoculated in a selective enrichment medium, for example, LP basal medium is used for selective enrichment and growth of some methanogenic species. Incubation of sludge enrichments at 65^oC promotes the isolation of thermophilic species of methanogens. Zeikus (1977) had personally observed that *Methanospirillum* species were frequently associated in high numbers with *Thiopedia* blooms in the surface muds of shallow eutrophic ponds.

Morphological and microscopic features of methanogens:

Methanogenic bacteria have been observed in diverse forms i.e. sarcina, rods, spheres, and spirals. However a great variety in cell shape is found in many methanogenic species like short to straight rods and regular to ellipsoidal spheres. Cell wall construction of methanogenic bacteria and eubacteria is different. Hence methanogenic bacteria are not sensitive to penicillin

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and many other antibiotics. Many species of methanogenic bacteria have been reported as Gram positive, Gram negative and Gram variable (Zeikus, 1977; Megonigal, 2003).

Table 1: Methanogens and their isolation sources (Blasco-Gomez *et al.*, 2017)

Methanogen	Isolation Source
Methanocaldococcus jannaschii	White smoker chimney of the East Pacific Rise at a depth of 2600 m
Methanopyrus kandleri	Black smoker chimney from the Gulf of California in a depth of 2000 m
Methanohalophilus zhilinae (halophilic)	Saline lake in Egypt
Methanoregula boonei	Acidic peat bog
Methanobacterium sp. *Methanosarcina mazei TMA* *Methanobrevibacter arboriphilus*	Rice fields
Methanobrevibacter thaueri *Methanobrevibacter gottschalkii* *Methanobrevibacter wolinii* *Methanobrevibacter woesei*	Feces of cattle, horse, sheep and goose
Methanobrevibacter sp.	Intestinal tract of insects such as termites
Methanobrevibacter ruminantium	Intestinal tract of herbivorous mammals
Methanobrevibacter smithii *Methanosphaera stadtmanae* *Methanomassiliicoccus luminyensis*	Human faces
Methanosarcina sp. *Methanosphaera sp.* *Methano brevibacteroralis*	Human dental plaque

Some renowned species of methanogens:

Some taxonomically described methanogenic species include *Methanobacterium aarbophilicum, Methanobacterium athermoautotrophicum, Methanospirillum ahungatii, Methanobacterium formicium, Methanobacterium mobile, Methanococcus vannieli* and

Methanosarcina barkeri. Of these former three species are available in American type culture collection (ATCC) (Zeikus, 1977; Boone *et al.*, 1993).

Significance of methanogens:

Methanogens immensely used in anaerobic digestors to treat sewage water and aqueous organic pollutants (Appels *et al.*, 2008).Methane produced by methanogens is a storable energy carrier (Bauer *et al.*, 2008). Actually, methanogens act as biocatalysts and have the potential to contribute to a solution for future energy problems worldwide. The main technical application of methanogens is production of methane as biogas by digestinga variety of organic substrates such as chicken manure, pig manure, sugar beet, grass, maize, microalgae etc.This biogas can be used as fuel to drive automobiles and it has been practiced from a long time in kitchen for food cooking purpose Electricity production is also possible by using methane biogas (Enzmann *et al.*, 2018). Methanogens are commonly employed in biological methanation in many industries of developed and developing countries. Electromethanogenesis is a new application of methanogens undertaken for the study by many researchers (Blasco-Gomez *et al.*, 2017). Methanogens can also produce hydrogen, in limited hydrogen availability condition. In oil field, residual oil can be used for methane production by using consortia of methanogens. Methanogens in sludge form can be use for decolorization and partial degradation of azo dyes such as acid orange 6, acid orange 7, and acid orange 52 (Yemashova et al. 2004).Genetically modified methanogens can be used in many applications by virtue of recombinant DNA technology (Brune 2018; Chaudhary *et al.*, 2018; Beaver *et al.*, 2021).

Conclusion and future prospective:

Many anaerobic habitats, including marine and freshwater sediments, marshes and swamps, flooded soils, bogs, geothermal habitats, and animal gastrointestinal tracts exhibit their own significance and diversity. Methanogens play a vital role in these ecosystems. There is need for deep research on methanogenic archaea since their exploitation and industrial implementation could solve many problems of currently growing population. Methanogens are an important biological tool that can be used for the welfare of human beings.

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