SILVER OAK UNIVERSITY

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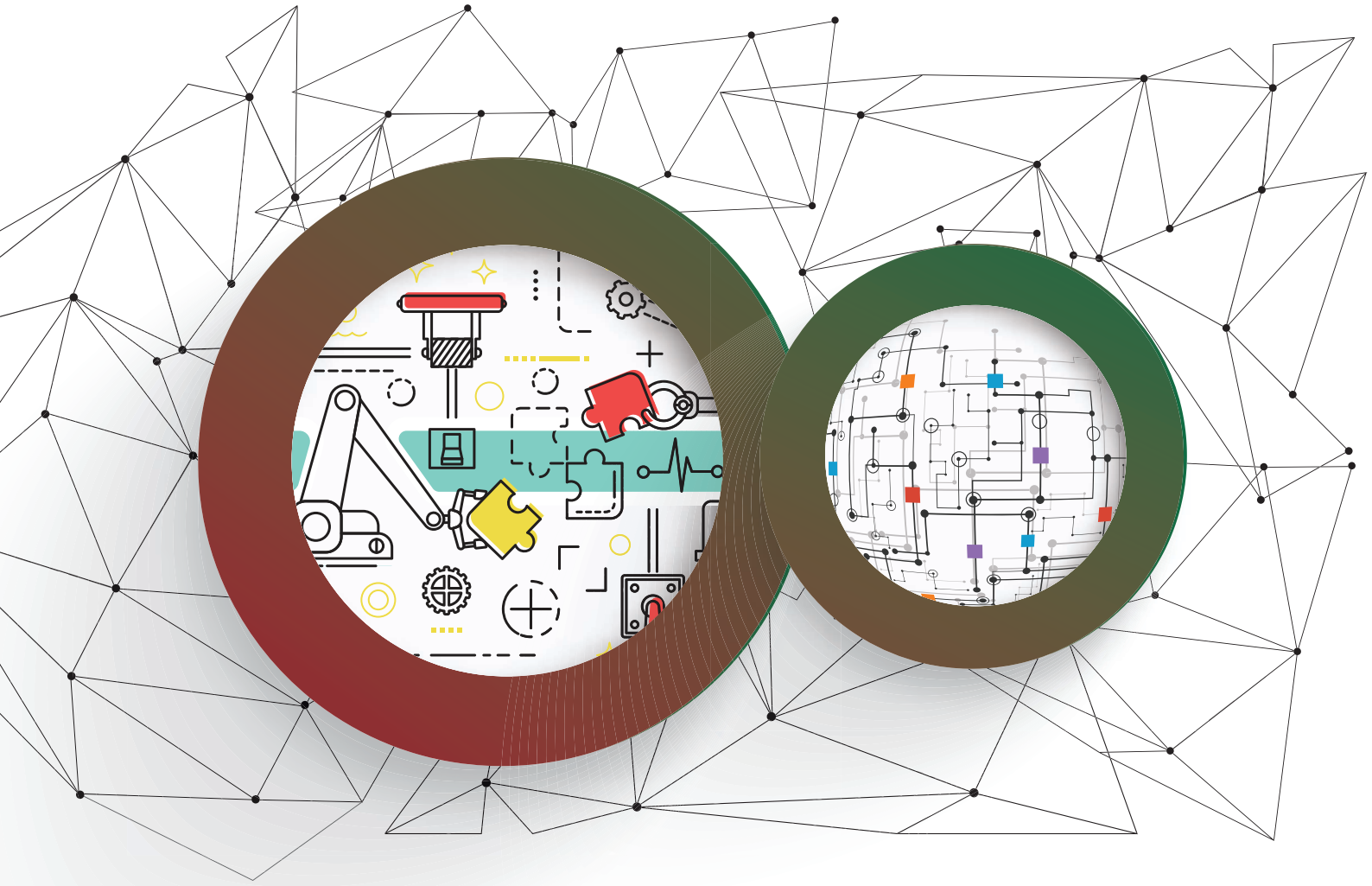
SILVER OAK UNIVERSITY

EDUCATION TO INNOVATION

SRJN



RESEARCHING FOR A BRIGHTER TOMORROW

POWER OF INNOVATION

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About the Journal

SARJAN is a peer reviewed research journal yearly published by Silver Oak University. The present issue is the seventh edition of SARJAN. It publishes thoughtful contributions from researchers that offers insight and perspective, knowledge and understanding of multi-disciplinary research in areas of various disciplines of Engineering; Computer Engineering, Information and technology, Electronics and Communications, Mechanical Engineering, Aeronautical Engineering, Civil Engineering, Chemical Engineering, Electrical Engineering, Basic Sciences Humanities and allied areas. The mission of the journal is to foster research culture and increase research productivity of the faculty members in the institute by promoting and publishing their research skills. The institute believes that research in academics can only solve the problem of employability in the country and cater to the needs of the industry. SARJAN has consistently since 2012 provided a platform to researchers and academicians to contribute in this pool of knowledge that helps the aspiring researchers and students to update themselves with latest innovations and knowledge and thus enhance their technical skills

FORWARD

Dear friends,

I am happy to note that as a regular feature Silver Oak University is publishing its yearly research journal SARJAN. Being a young institution and doing such motivating research activities, it's really excellent and indeed a proactive step as it is the need of a day and especially for an University. We know that 21st century is the era of knowledge application and such initiatives will empower the faculty members to exhibit their research skills which will also benefit the student community in present competitive times.

Dr. Saurin Shah
Provost
Silver Oak University

INDEX

A Review: Bioactive Secondary Metabolites - Alkaloids

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ABSTRACT

Plants have always been a basis for the traditional medicine systems as they have therapeutic applications like anti-tumor, anti-viral, anti-inflammatory, anti-malarial, anti-oxidative, anti-mutagenic and anti-carcinogenic. Plants are considered as a rich source of wide variety of ingredients which can be used for the development of drugs. Among these, alkaloids are important class of secondary metabolites.On the basis of their biosynthetic precursor and heterocyclic ring system , the compounds have been classified into various categories which include indole , piperidine , tropane , purine , pyrrolizidine , imidazole , quinolozidine , isoquinoline and pyrrolidine alkaloids. These are important therapeutic molecules due to their efficacy to prevent the onset of different degenerative diseases by scavenging the free radicals or binding with catalysts of the oxidative reactions, such as some metal ions. Several studies have been done in evaluation of alkaloids from various plants for its wide range of pharmaceutical activities. This review provides an overview of alkaloid drugs that are derived from the various plants and potential against various diseases.

Keywords: Plant alkaloids, secondary metabolites, Biosynthetic pathway, therapeutic compounds

1. INTRODUCTION

Plants produce a high diversity of different secondarymetabolites (Schafer et al., 2009; Wink M., 1988). Recently they encompassed about 1, 00,000 chemically identified, low molecular weight compounds. These molecules are commonly synthesized in a plant-, organ- and even cell-specific manner (Gregory et al., 2010). A prominent function is the protection against herbivores and/or microbial pathogens. Many of the secondary metabolites have interesting biological properties and quite a number are of medicinal importance (Schafer et al., 2009;Wink M., 1988).

Alkaloids constitute a very chemically diverse group of secondary metabolites with an estimated 12,000 different molecules sharing as a unique common feature- the presence of a nitrogen atom

within a heterocyclic ring and are derived from the amino acids (Gregory et al., 2010;Kaur et al., 2015).It is a cyclic compound containing nitrogen in a negative oxidation state which is of limited distribution in living organisms (Roy et al., 2017). These compounds are low molecular weight structures and form about 20% of plant based secondary metabolites (Kaur et al., 2015). They rank among the most diverse, efficient and therapeutically significant plant substances (Roy et al., 2017). The term 'alkaloids' was coined by the German chemist Carl F. W. Meissner in 1819 and the word is derived from the Arabic name 'al-qali' that is related to the plant from which Soda was first isolated (Kaur et al., 2015).Previous record shows that people across Asia , Europe and Africa used alkaloid-containing plants as early as 2000 BCE. In 19th century Friedrich Serturner isolated morphine. This led to the successful isolations and discoveries of isolated compounds by several European scientists including isolation of Xanthine (1817) , Strychnine (1818) ,Atropine (1819) , Quinine (1820) and Caffeine (1820) (Roy et al., 2017).

Alkaloids are produced by a large variety of organisms which includes bacteria,fungi, plants and animals (Roy et al., 2017;Cushnie et al., 2014). Alkaloids have a wide distribution in plant kingdom and mainly exist in higher plants, such as those belonging to Angiosperms like Ranunculaceae, Leguminosae , Papaveraceae , Menispermaceae and Loganiaceae (Roy et al., 2017; Lu et al., 2012). Alkaloids can occur in any part of the plant, though specific compounds may be limited to a certain part (e.g. quinine in cinchona tree bark). In terrestrial animals, alkaloids have been reported in insects, amphibians, reptiles, birds and mammals. Marine animals producing alkaloids include sponges, asteroids, tunicates, scleractinians and the dogfish shark. To date, more than 18,000 alkaloids have been discovered(Cushnie et al., 2014).

Alkaloids are mainly involved in plant defence against herbivores like feeding deterrence, microorganisms (antibacterial and antifungal activities), insects and also against other plants by means of allelopathically active chemicals (Roy et al., 2017).That is why they are used for medicinal purpose. They also have a wide range of physiological effects on animals (Kaur et al., 2015). Many alkaloids from plant extract have been used for several hundreds of years in medicine and even today it's a still prominent drug (Roy et al., 2017). They have influenced the human history profoundly due to their pharmacological properties such as antibiotic, anticancer along with their potential exploitation as narcotics, poisons and stimulants (Kaur et al., 2015). Ancient people used plant extracts containing alkaloids for treating a large number of ailments including snakebite, fever and insanity. Pure, isolated plant alkaloids and their synthetic derivatives are used as basic medicinal agents all over the world for that analgesic, antispasmodic and bactericidal effects. In humans, most of the alkaloids affects the nervoussystem, particularly action of chemical transmitters like acetylcholine,epinephrine,norepinephrine, gamma aminobutyric acid, dopamine and serotonin. Some alkaloids are used as antiseptic due to its antibiotic activity e.g. berberine in opthamics and sanguinarine in toothpastes (Roy et al., 2017). Moreover, several alkaloids exhibit significant biological activities, such as relieving action of ephedrine for asthma, the analgesic action of morphine and the anticancer effect of vinblastine. In fact alkaloids are among the most important

active components in natural herbs and some of these compounds have already been successfully developed into chemotherapeutic drugs , such as Camptothecin(CPT) , a famous topoisomerase I(TOP I) inhibitor and vinblastine which interacts with tubulin (Lu et al., 2012).

2. STRUCTURAL DIVERSITY AND CLASSIFICATION OF ALKALOIDS

Alkaloids are characterized by great structural diversity, the presence of a basic nitrogen atom being the only unifying feature. Most alkaloids possess just one nitrogen atom, but some have up to five. This nitrogen may occur in the form of a primary amine (RNH_2), a secondary amine (R_2NH) or a tertiary amine (R_3N) (Cushnie et al., 2014). In addition to carbon, hydrogen and nitrogen, alkaloids may also contain oxygen,sculpture and more rarely, other elements such as chlorine, bromine and phosphorous (Cushnie et al., 2014; Babbar N. 2015). Alkaloids can occur as monomers or they may form dimers (also known as bisalkaloids),trimers or tetramers. Such oligomers are typically homooligomers, but heterooligomers also occur (Cushnie et al., 2014).

There is no single taxonomic principle that would allow consistent classification of all alkaloids. Many researchers have proposed different classification for alkaloids. Alkaloids can be classified in the terms of their (1) biological and ecological activity, (2) Relation to chemical and technological innovations, (3) Chemical structure and (4) Biosynthetic pathway (Kakhia T).

2.1 BIOECOLOGICAL CLASSIFICATION OF ALKALOIDS

From the point of view of biological activity , it is possible to divide alkaloids into (1) Neutral or weakly basic molecules (e.g., lactams such as ricinine , certain N-oxides such as indicine) , (2) Animal-derived alkaloids (e.g., anuran , mammalian and arthropod alkaloids) , (3) Marine alkaloids ,(4) Moss alkaloids , (5) Fungal and Bacterial alkaloids , and (6) Non-natural alkaloids (structurally modified or analogues).

Nowadays, the group of compounds mentioned as non-natural alkaloids is growing especially rapidly as a result of bio-organic and stereochemistry research. Pharmacological research and the drug industry rapidly advance and promote the most promising new molecules for possible production applications. This is necessary, since the sources of infections (microorganisms) are constantly changing their species and infection ability, becoming resistant to medicines and antibiotics (Aniszewski T.2015).

2.2 CHEMOTECHNOLOGICAL CLASSIFICATION OF ALKALOIDS

Alkaloids can be divided into three large groups on the basis of their relation to the innovations in the fields of both chemistry and technology: (1) Natural alkaloids, (2) Biomimic and bionic alkaloids, and (3) Synthetic alkaloids. This divisions serves the scientific and practical needs well.

Natural alkaloids are all alkaloids existing in nature, presently known or still unknown by the science. They are molecules naturally synthesized and novelized in time by living organisms as a result of the evolution of life on Earth.

Biomimic alkaloids are natural alkaloids copied artificially by chemists in the laboratories. They are identical in structure to natural alkaloids. Bionic alkaloids are those biomimic molecules being novelized by the chemists and engineers using natural models and high-level technology. Bionic alkaloids are not identical analogues to natural alkaloids.

Synthetic alkaloids are molecules totally modelled by chemists and engineers using high-level technology, planned models and artificial synthesis. Synthetic alkaloids are not produced naturally by the living organisms (Aniszewski T.2015).

2.3 CHEMO-MOLECULAR CLASSIFICATION OF ALKALOIDS

Alkaloids can be divided into different types according to their pure chemical structures pointing first at the alkaloid base, a basic chemical nucleus. The following are basic types of alkaloids: acridones, aromatics, carbolines, ephedras, ergots, imidazoles, indoles, bisindoles, indolizidines, manzamines, oxindoles, quinolones, quinozolines, quinolizidines, phenylisoquinolines, phenylethylamines, piperidines, purines, pyrolidines, pyrrolizidines, pyrroloindoles, pyrydines, sesquiterpenes, simple tetrahydroisoquinolines, steroids, tropanes, terpenoids, diterpenes and triterpenes (Aniszewski T.2015).Fig.1

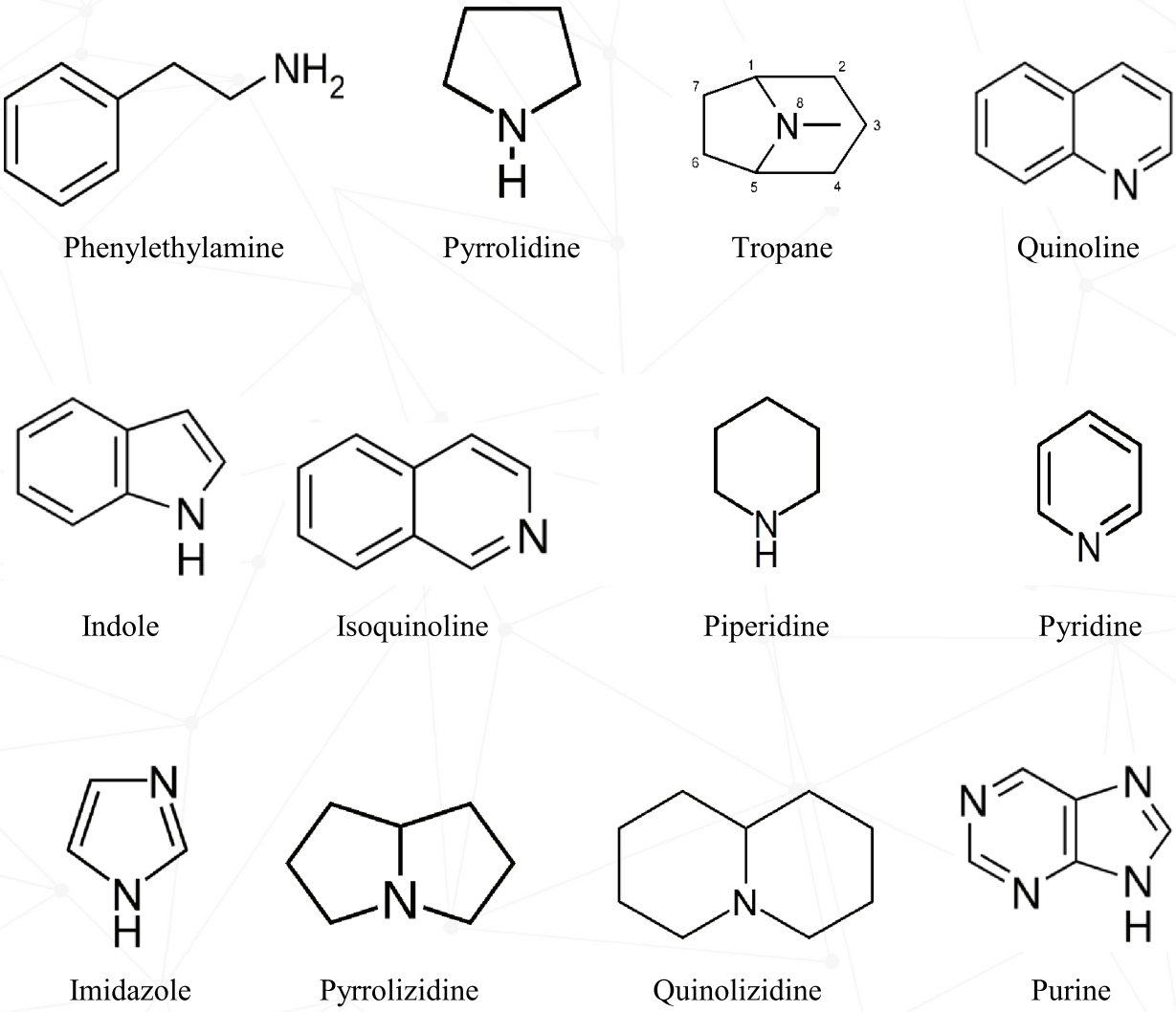


Fig.1: Structures of major groups of alkaloids

2.4 BIOSYNTHETIC SHAPE-CLASSIFICATION OF ALKALOIDS

Alkaloids are generally classified by their common molecular precursors, based on the biological pathway used to construct the molecule. From a structural point of view, alkaloids are divided according to shape, structure, and precursors (Aniszewski T.2015).One of the popular classifications that divide whole class of compounds into 3 categories are as follow: (1) True alkaloids, (2) Proto alkaloids, and (3) Pseudo alkaloids (Roy et al., 2017;Aniszewski T.2015). True alkaloids and proto alkaloids are derived from amino acids, whereas pseudo alkaloids are not derived from these compounds (Aniszewski T.2015).

(1) True-Alkaloids - These are the compounds which are derived from amino acids and a heterocyclic ring with nitrogen. These alkaloidsare highly reactive substances with biological activity even in low doses. All true alkaloids have a bitter taste and appear as a white solid, with the exception of nicotine, which is a brown liquid. True alkaloids form water-soluble salts. Moreover, most of them are well-defined crystalline substances that unite with acids to form salts. True alkaloids may occur in plants (1) in the free state, (2) as salts and (3) as N-oxides. These alkaloids occur in a limited number of species and families and are those compounds in which decarboxylated amino acids are condensed with a non nitrogenous structural moiety. The primary precursors of true alkaloids are such amino acids as L-ornithine, L-lysine, L-phenylalanine / L-tyrosine, L-tryptophan and L-histidine. Examples of true alkaloids include such biologically active alkaloids as cocaine, quinine, dopamine, morphine, usambarensine, nicotine,atropine, etc. True alkaloids can be as natural,biomimic, bionic and synthetic alkaloids (Roy et al., 2017;Aniszewski T.2015). Fig.2

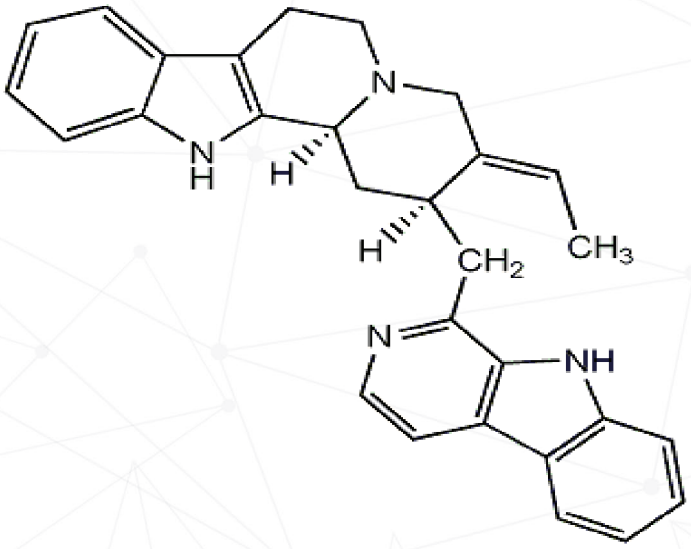


Fig.2: Usambarensine ($C_{29}H_{28}N_4$)

(2) Proto-Alkaloids – These are the compounds which contain nitrogen atom derived from an amino acid which is not a part of the heterocyclic ring. Such alkaloid include compounds derived from L-tyrosine and L-tryptophan. Proto alkaloids are those with a closed ring, being perfect but structurally simple alkaloids. They form a minority of all alkaloids. Hordenine, mescaline and yohimbine are good examples of these kinds of alkaloid. Chini et al. found new alkaloids, stachydrine and 4-hydroxy stachydrine, derived from *Boscia angustifolia*, a plant belonging to the Capparidacea family. These alkaloids have a pyroline nucleus and are basic alkaloids in the genus *Boscia*. The species from these genus have been used in folk medicine in East and South Africa. *Boscia angustifolia* is used for the treatment of mental illness and occasionally to combat pain and neuralgia. Examples of Proto alkaloids include adrenaline,ephedrine,mescaline, etc. Proto alkaloids can be as natural,biomimic, bionic and synthetic alkaloids (Roy et al., 2017;Aniszewski T.2015). Fig.3

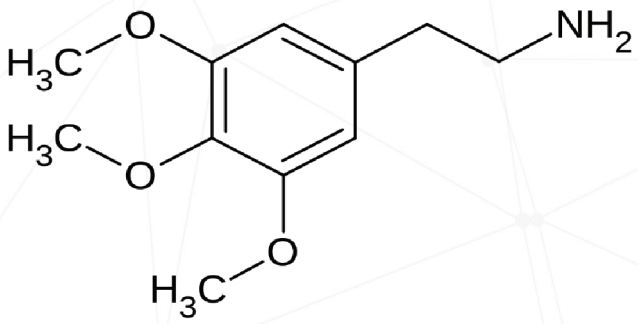


Fig.3: Mescaline ($C_{11}H_{17}NO_3$)

(3) Pseudo-Alkaloids – These are the compounds, the basic carbon skeletons that do not originate from amino acids. In reality, pseudo alkaloids are connected with amino acid pathways. They are derived from the precursors or postcursors (derivatives of the indegradation process) of amino acids. They can also result from the amination and transamination reaction of the different pathways connected with precursors or postcursors of amino acids.

These alkaloids can also be derived from non amino acid precursors. The nitrogen atom is inserted into the molecule at a relatively late stage, for example, in the case of steroidal or terpenoid skeletons. Certainly the nitrogen atom can also be donated by an amino acid source across a transamination reaction, if there is a suitable aldehyde or ketone. Pseudo alkaloids can be acetate and phenylalanine-derived or terpenoid, as well as steroidal alkaloids. Examples of pseudo alkaloids include compounds such as coniine , capsaicin , ephedrine , solanidine , caffeine , theobromine , pinidine , etc. pseudo alkaloids can be as natural , biomimic , bionic and synthetic alkaloid (Roy et al., 2017; Aniszewski T.2015). Fig.4

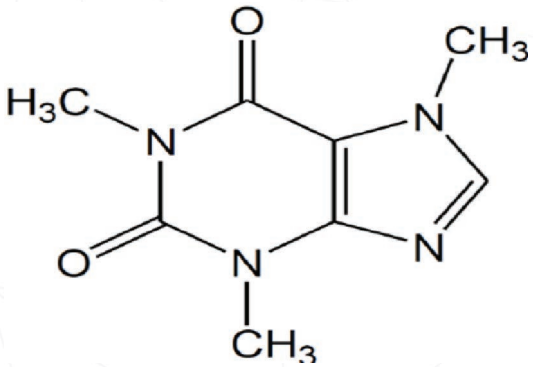


Fig.4: Caffeine ($C_8H_{10}N_4O_2$)

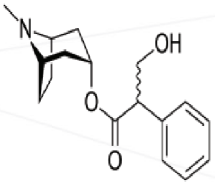
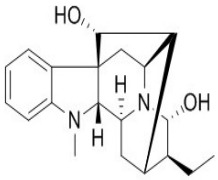
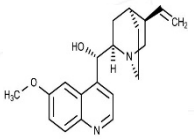
3.ALKALOID DRUGS FROM PLANTS SOURCE

In plants,alkaloids are not produced in large amount in normal circumstances. An alternative for the large production of alkaloids has been employed as in-vitro cell tissue or organ culture. Tissue culture provides a continuous, reliable and renewable source for larger production of alkaloids. There are several numbers of factors which can affect production of plant alkaloids. For optimization of culture condition for the maximum production, changes in physical aspects and nutritional elements were done. One study reported that the effect of salicylic acid (elicitor) enhance the production of Stemona alkaloids in *Stemona sp*. Another study reported that the production of alkaloid in *Atropa belladonna is*enhanced by the use of biotic stress that is *Aspergillus niger* extract (0.5 mg/ml) along with MS medium containing 1ml/l of NAA and BA.

It was also reported that the reverse tobacco liquid medium supplemented with tyrosine (12.5 ml/100 ml) gives maximum alkaloid production in *Papaver rhoeas* Linn. plant. Rajkumar et al., 2010 reported the production of nitidine from callus culture of *Taddalia asiatica.* They incubated

leaves in MS media along with different combination of growth hormones and the highest yield obtained at MS containing NAA (2 mg/l) and Kinetin (1 mg/l) (Table 1)

Table-1 Production of Bioactive molecules from different Medicinal plants

Alkaloid medicine	Plant	Plant picture	Structure	Activity	Product
Atropine	*Atropa belladonna*			Antidote to nerve gas poisoning	Abdominol, Espasmo, Protecor,
Ajmaline	*Rauwolfia serpentine*			Antiarrhythmic	Aritmina, Gilurytmal, Rauwopur,
Quinidine	*Cinchona officinalis*			Antirrhythmic	Cardioquin, Duraquin, Quindex,

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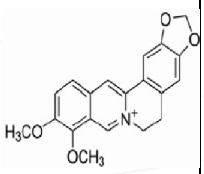
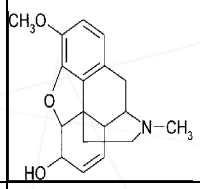
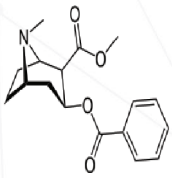
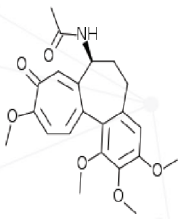
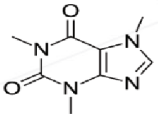
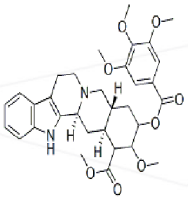
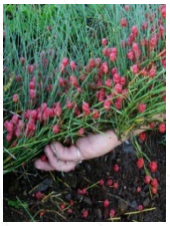
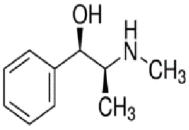
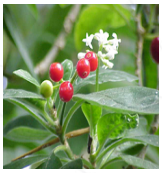
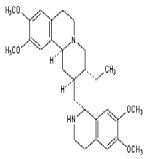
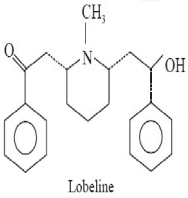
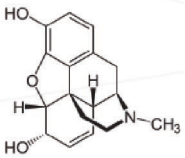
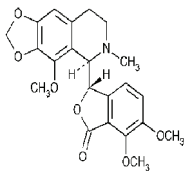
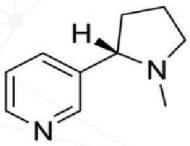
Berberine	*Berberis vulgaris*			Bacillary dysentery	Kollyr, Murine, Sedacollyre
Codeine	*Papaver somniferum*			Analgesic (Painkiller)	Antituss, Codicaps, Tussipax
Cocaine	*Erythroxylum coca*			Local anesthetic	Used in highly regulated clinical environments
Colchicine	*Colchicum autumnale*			Gout remedy	ColBenemid, Colgout, Verban
Caffeine	*Coffea arabica*			Central nerve system stimulant	Agevis, Thomapyrine, Vomex A

Deserpidine	*Rauvolfia canescens*			Antihypertensive, tranquilizer	Enduronyl
Ephedrine	*Ephedra sinica*			Antiasthmatics	Amidoyna, Bronchicum, Peripherin,
Emetine	*Carapichea ipecacuanha*			Antiprotozoal	Cophylac, Ipecac, Rectopyrine
Lobeline	*Lobelia inflate*			Expectorants	Citotal, Lobatox, Stopsmoke

Morphine	*Papaver somniferum*			Pain relief, diarrhea	Duromorph, Oramprph, Spasmofen
Noscapine	*Papaver somniferum*			Antitussive	Bequitusin, Degoran, Tussisedal
Nicotine	*Nicotiana tobacum*			Stimulant, nicotinic acetylcholine receptor agonist	Nicabate, Nicorette, Stubit

4. NOMENCLATURE OF ALKALOIDS

Individual alkaloids are assigned names in different ways, butalmost all names end with the letters ‘-ine’. Most alkaloidsare named after the organism or part of the organism from whichthey were isolated for example atropine from *Atropa belladonna*.When multiple alkaloids are obtained from the same source, a pre-fix or more complicated suffix is used like quinine, hydroquinine,quinidineor alternatively a series of letters like epicoccarine A, epicoccarine B.

Alkaloids may also be named after the geo-graphic location of their source for example tasmanine was isolated from a Tasmanian plant, their pharmacological activity like eme-tine induces vomiting or, in some cases, after their discoverer for example pelletierine after Prof. Pelletier (Cushnie et al., 2014; Evans W.2009).

5. PHYSICO-CHEMICAL CHARACTERISTICS

Despite their structural diversity, alkaloids share many physical and chemical properties. Because they possess a nitrogen atom with an unshared pair of electrons, alkaloids are basic hence their name, which literally means alkali-like. The degree of this basicity varies depending on the structure of the molecule and the location of other functional groups (Cushnie et al., 2014). Alkaloids also include some related compounds with neutral and even weakly acidic properties. They are bitter in taste. The alkaloid quinine for example, is one of the bitterest tasting substances known and is already significantly bitter at a 1×10^{-5}M. (Roy et al., 2017). Most alkaloids contain oxygen in their molecular structure, those compounds are usually solid, colorless crystals at ambient conditions (Cushnie et al., 2014;Babbar N. 2015). Oxygen-free alkaloids, such as nicotine or coniine, typically volatile, colorless and oily liquid. Some alkaloids are colored, like berberine: yellow and sanguinarine:orange. Most alkaloids are weak basic, but some such as the bromine and theophylline are amphoteric. Many alkaloids dissolve poorly in water but readily dissolve in organic solvents, such as diethyl ether, chloroform or 1, 2-dichloroethane. Caffeine,cocaine, codeine and nicotine are water-soluble (with a solubility of ≥1 g / L), whereas others, including morphine are highly water-soluble (0.1-1 g/L) (Cushnie et al., 2014). As organic bases, alkaloids form salts with mineral acids such as HCL and H_2SO_4 and organic acids such as tartaric acid or maleic acid (Kakhia T). These salts are usually soluble in water and ethanol and poorly soluble in most organic solvents (Cushnie et al., 2014; Babbar N. 2015). Possessing a proton-accepting nitrogen atom and one or more proton-donating amine H-atoms, alkaloids readily form hydrogen bonds with proteins, enzymes and receptors. This, coupled with the frequent presence of proton-accepting and proton–donating functional groupssuch as phenolic hydroxyl and polycyclic moieties, explains the exceptional bioactivity of the alkaloids (Cushnie et al., 2014;Kittakoop et al., 2014).

6. PROPERTIES– PHARMACOLOGICAL AND TOXICOLOGICAL

6.1 PHARMACOLOGICAL PROPERTIES

It includes analgesic (e.g. codeine), central nervous stimulant (e.g. brucine), central nervous depressant (e.g. morphine), antithypotensive (e.g. ephedrine), antihypertensive (e.g. reserpine), antipyretic (e.g. quinine), anticholinergic (e.g. atropine), antiemetic (e.g. scopolamine), oxytocic and vasoconstrictor (e.g. ergometrine), antitumor (e.g. vinblastine) and antimalarial (e.g. quinine) activities. These activities are exploited both in traditional medicine (e.g. quinine-rich cinchona bark in the treatment of malaria) and modern medicine (e.g. vinblastine in the treatment of

cancer). Other alkaloids have been incorporated in human culture as recreational drugs and drugs of abuse (e.g. caffeine, nicotine, psilocybin, cocaine) (Roy et al., 2017; Cushnie et al., 2014)

6.2 TOXICOLOGICAL PROPERTIES

Some alkaloids are highly toxic and there have been many incidents of human poisoning. In studies of 350 plant – derived pyrrolizidine alkaloids, approximately one-half were hepatotoxic and several were carcinogenic. This is due to mammalian liver oxidases transforming the compounds into reactive pyrrole structures that alkylate nucleic acids and proteins. Also, the furoquinolines are phototoxic and photomutagenic due to the furan double bond reacting covalently with DNA. Other examples include aconitine (cardio toxic), lycorine (enterotoxic), nicotine (teratogenic) and strychnine (neurotoxic).Some of the alkaloids used in medicine, at supratherapeutic doses, can also be toxic. Well-known historical examples include belladonna (containing atropine) used as poison in Roman times, and ergot (containing ergometrine) responsible for thousands of the deaths in the middle ages (Cushnie et al., 2014;Matulkova et al., 2012; Green et al., 2012; Dasari et al., 2011).

7. BIOLOGICAL ACTIVITIES OF ALKALOIDS

The role of alkaloids for living organisms that produce them is still unclear. It was initiallyassumed that the alkaloids are the final products of nitrogen metabolism in plants, as urea inmammals. It was later shown that alkaloid concentrations varies over time, and this hypothesiswas refuted. Most of the known functions of alkaloids are related to protection.However, some animals are adapted to alkaloids and even use them in their ownmetabolism. Such alkaloid-related substances as serotonin, dopamine and histamine areimportant neurotransmitters in animals. Alkaloids are also known to regulate plantgrowth. Another example of an organism that uses alkaloids for protection is the *Utetheisaornatrix*, more commonly known as the ornate moth. Pyrrolizidine alkaloids render theselarvae and adult moths unpalatable to many of their natural enemies like coccinelid beetles,green lacewings, insectivorous hemiptera and insectivorous bats (Babbar N. 2015).

Alkaloids are known for a variety of biological activities and each having its own specific mechanism of action. Most of these mechanisms have been proved, but some have been hypothesised. Here we discuss the important biological activities of alkaloids (Kaur et al., 2015).

7.1 Muscle relaxant

Alkaloids are known to have muscle relaxant property. D-tubocurarine is one such example that possesses the antiparalytic activity due to its ability to obstruct the acetycholine receptor spots which enable the muscles to unwind at neuromuscular intersections. The aporphine alkaloids including corstubenne, magnoflorine, isothebaine and isocorydine, isolated from *Mahonia aquifolium* were reported to relax the contractions induced by nor-adernaline as compared to those induced by KCl in isolated rat aorta (Kaur et al., 2015).

7.2 Antioxidant property

The alkaloids are known to possess antioxidant activities due to their ability to act as scavenger of free radicals, metal chelating activity or electron or hydrogen donation ability. A quinoline alkaloid, obtained from the aleurone layer of *Oryza sativa* cv. *Heugjinmi*, was reported to exhibit moderate antioxidative characteristics using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals as substrate (Kaur et al., 2015; Correche et al., 2008).

7.3 Anticancer activity

The vinblastine and vincristine alkaloids obtained from *Catharanthus roseus* (Apocynaceae) are popularly used for the treatment of patients suffering from leukemia and hodgkin's disease. These alkaloids exert chemopreventive effect by terminating or causing depolymerisation of protein microtubules that forms the mitotic spindle in cell division. This results in hindrance in division and separation of tumour cells and reduces the incidences of cancer. Divalent calcium cation (Ca^{2+}) is known to regulate energy output and cellular metabolism by acting as a major signalling molecule during cell signal transduction. *Vinca* alkaloids are found to decrease calcium uptake rate and its amount into mitochondria and thus might lead to a change in cytoplasmic Ca^{2+}concentration that appears to enhance the cytotoxicity by selective release of cytochrome c or increasing the production of ROS.DNA damaging effect of deoxyamphimedine–a pyridoacridine alkaloid, isolated from *Xestospongia* sponges found in Philipines, is related to reactive oxygen species related phenomenon. Neoamphimedine–another member of this class, is reported to effect cytotoxicity via topoisomerase-2-dependent DNA aggregation/catenation. Deoxiamphimedine resulted in the production of ROS that attack DNA as a result of single strand breaks (SSB) and double strand breaks (DSB) (Kaur et al., 2015).

TNF-α–a cytokine, is known to play a pivotal role in the regulation of inflammation. Conophylline was reported to down regulate the expression of the TNF-α receptors on the cell surface and in this way inhibit the TNF-α induced NF-κB activation. Lycorine and its synthetic derivative are known to induce cell cycle arrest, up regulate the expression of pro-apoptotic proteins (caspase 3, 7 and 9) and down regulate the antiapoptotic proteins (Mcl-1, Bcl-2) (Kaur et al., 2015; Lamoral-Theys et al., 2010).

Ceramide accumulation is another remarkable process associated with cancer chemoprevention. The increased availability of ceramide within the cell either by activating sphingomylinase or by blocking degradation of ceramide under the cytotoxic effect of chemotherapeutic agent resulted in modulation of associated cell signalling pathways. The resultant effect of this alteration is cell cycle arrest, terminal cell differentiation and apotosis . Ceramide induced cell death is reported to be of two types depending on the dependency of transcription factors. The component alters the activity of apoptosis related proteins of BCl-2 family and its relation with preapoptotic proteins (Bax and Bad). The over expression of antiapoptotic proteins might block the ceramide mediated cell death without having an effect on its generation. The much exploited role of *Vinca* alkaloids including vincristine and vinblastine in treatment of leukemia might be due to increased accumulation of ceramide in cells. Vinblastine has been reported to elevate the levels of cellular ceramide even at 1.5nM concentration that furthermore induced cell death in KB-3-1 human epidermoid carcinoma cells (Kaur et al., 2015).

Nuclear factors such as NF-κB have an important role in the process of inflammation. Its presence is reported in cells that expresses cytokines, chemokines, growth factors, cells adhesion molecules and some acute phase proteins. The activation of NF-κB involves the phosphorylation of IκBs by serine residues (Ser32, Ser36) via the IκB kinase (IKK) signal-o-some complex. The phosphorylated IκB are ubiquitous and their degradation by 26s proteosome liberated free NF-κB that is translocated to the nucleus where it binds to κB binding sites in the promoter regions of target genes and induces the transcription of pro-inflammatory mediators e. g. iNOS, COX-2, TNF-α and IL-1B,-6 and-8 (Kaur et al., 2015). Another important factor iNOS (inducibleNitric Oxide Synthase) is expressed in response to interferon-γ, lipopolysaccharide (LPS) and various pro-inflammatory cytokines. No modulates acute and chronic inflammatory responseby acting as potent vasodilator and thus maintain vascular homeostasis. The expression of COX-2 is induced in immune cells such as macrophages in various stress conditions that led to increase in prostaglandins (PGs) level. The elevated PGs level resulted in tumor growth due to angiogenesis and inhibition of apoptosis. Poncirin isolated from the fruits of *Poncirus trifoliata* is a potent inhibitor of LPS-induced NO, PGE2, TNF-α and IL-6 production in macrophages cells and it acts at transcription level. Inhibitory effect of Poncirin was found to be associated with NF-κB inactivation via the blockage of IκB-α phosphorylation. In addition to this, poncirin significantly declined the TNF-α and IL-6 release and their mRNA expression alongwith reduction of COX-2 and iNOS expression in macrophages cells in dose dependent manner. Quinoline alkaloids isolated from *Evodiarutaecarpa* showed inhibitory effects against NF-κB activity (Kaur et al., 2015).

In addition to this, Benzyisoquinoline alkaloids due to the presence of phenolic hydroxyls or similar reactive groups are reported to act as inhibitors of lipid peroxidation stimulated by Fe2+/cysteine in rat liver microsomal fractions. Martefragin A-an indole alkaloid, isolated from red alga *Martensia fragilis* has been reported to show inhibitory activity on NADPH-dependent lipid peroxidation in rat liver microsomes (Kaur et al., 2015).

7.4 Antimicrobial and amoebicidal activity

The alkaloids of phenanthridine nature, isolated from *Chelidoniummajus* Linn. were reported to exhibit antifungal activity against the clinical drug-resistant yeast isolates. Diterpenoid alkaloids isolated from *Delphinium* spp. were known to possess moderate antifungal activity, along with antifeedent activity against insect species *Spodoptera littoralis* and *Leptinotarsa decemlineata.* The quinoline alkaloids including skimmianine, kokisaginine and masculine isolated from *Raulinoa echinata* were reported to exhibit antifungal activity against *Leucoagaricus gongylophorus*, the symbiotic fungus of leaf cuttings ants (*Attasexdens*) and in vitro against trypomastigote forms of *Trypanosoma cruzi*(Kaur et al., 2015; Meng et al., 2009).

Other activities

Berberine, an alkaloid isolated from *Berberis vulgaris* L., has been found to ameliorate type 1 diabetes due to the reduction in Th17 and Th1 cytokine secretion. The decreased secretion is achieved with the suppression of Th17 and Th1 differentiation by activating ERK1/2, and by inhibiting p38 MAPK and JNK activation (it down-regulated the activity of STAT1 and STAT4) respectively. The powdered leaves and roots of *Mallotus oppositifolium* were reported to be rich

in alkaloids and have been demonstrated to exhibit antioxidant and anti-inflammatory activities in beta-carotene linoleate model system and carrageenin induced rat paw oedema animal model . The phenoxazone alkaloids isolated from red-orange bracket fungus *Pycnoporus cinnabarinus* was also reported to exhibit anti-inflammatory activity as well as antiviral and antimicrobial activities (Kaur et al., 2015; Cui et al., 2009; Dias et al., 2009)

8. BIOSYNTHESIS AND REGULATION OF ALKALOIDS

Alkaloids are classified in several families that present totally different biosynthetic pathways. Four major families , for which the biosynthesis and the regulation are more particularly studied , are Monoterpene indole alkaloid(MIA) , Benzyl Isoquinoline alkaloids (BIA), Tropane and Nicotine alkaloids (TNA) and Purine alkaloids(PA)(Fig.5, Table-2).Despite their chemical diversity, alkaloids share the fact that they originate commonly from primary metabolites such as amino acids or bases (Fig.5). Except for *Nicotiana tabacum*, no genome sequencing project exists for the major alkaloids producing plants. Therefore, most of the enzymatic steps have been identified using classical biochemical and molecular biology studies. The ongoing elucidation of some biosynthetic pathways illustrates the recruitment of enzymes belonging to recurrent multigene families such as cytochrome P450 monooxygenases, acetyl transferases or methyltransferases. Recent expressed sequence tags (EST) transcriptomic projects focusing on MIA-producing species, BIA-producing species and TNA-producing species have been helpful for the identification of some missing enzymatic stepsand should accelerate this process in the near future (Table-3). Metabolic profiling studies of the major alkaloid-producing species have also been performed recently, sometimes in association with transcriptomic studies. Together, these approaches now allow tremendous progress in understanding these complex alkaloids biosynthetic pathways (Gregory et al., 2010).

8.1 BIOSYNTHESIS OF MONOTERPENE INDOLE ALKALOIDS (MIA)

The approximately 2,000 MIA chemical structures described so far are widespreadin a large number of plant species. Some of thesemolecules are of interest to human health, such as the anticancer drugs vinblastineand vincristine and the antihypertensive drug ajmalicine specifically produced in*Catharanthus roseus*, the anti-arrythmic ajmaline produced in *Rauvolfia serpentina*,or the anticancer compound camptothecin produced mostly in *Camptotheca acuminate*(Fig.5, Table-2). These molecules are part of the large array of MIAthat a single plant species is able to produce. For example, there are more than 130 MIA in *C. roseus*. The elucidation of MIA biosyntheticpathways in the three mentioned species has undergone major recent progresswith the identification of 42 clones corresponding to 31 enzymatic steps (Table-3).In *C. roseus* alone,
27 enzymatic steps have been studied (25 cDNA clones and twoadditional enzymatic activities with no assigned clone). In these species, the pathwaysshare a common origin with strictosidine synthase (STR), catalyzing thecondensation of the indole precursor tryptamine with the terpenoid precursorsecologanin to form the first MIA, strictosidine. The upstream biosynthesis of theindole precursor derived from the shikimate pathway via tryptophan, and of theterpenoid precursor originating from the methyl erythritol phosphate (MEP) pathway,is also shared within these plant species. Strictosidine b-glucosidase (SGD), catalyzing the deglucosylation of strictosidine, is the last common enzyme for thebiosynthesis of 2,000 MIA, since the resulting aglycon is the starting point for manydifferent species-specific lateral MIA pathways, with the observed possibility for

agiven species to harbour more than one of these pathways (e.g. *C. roseus*). Many enzymatic steps are yet to be discovered (Gregory et al., 2010). Fig.5

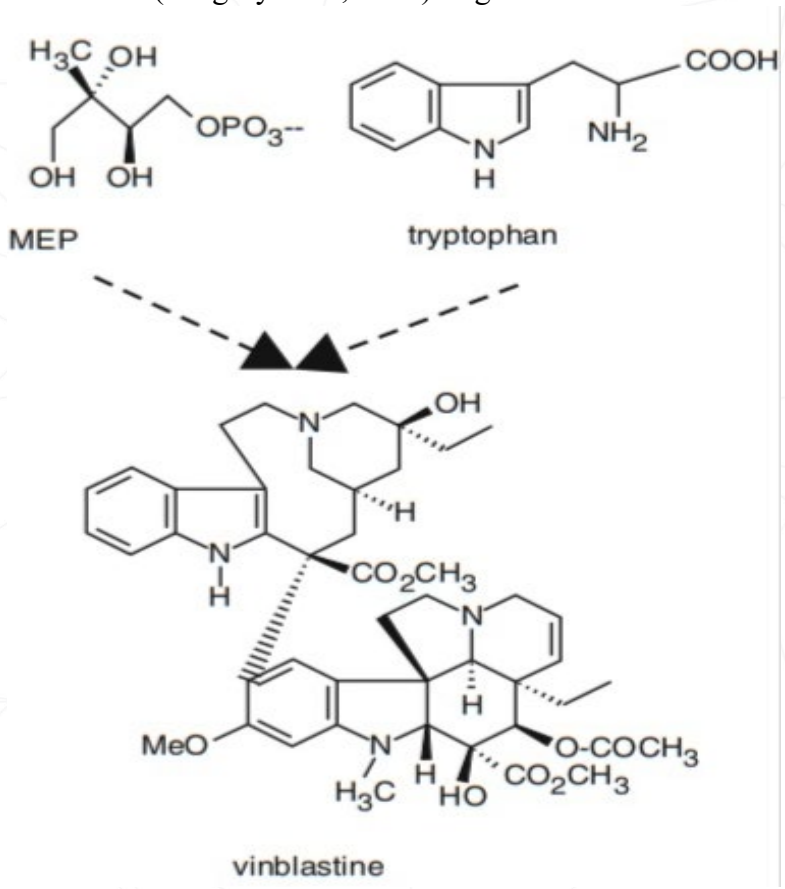


Fig.5 Biosynthesis of MIA

Table-2 Sources, chemical diversity and biological activity of four major families of alkaloids

Alkaloid family	Family (plant species)	Active compounds	Pharmacological activity	Target
Monoterpene indole alkaloids (MIA)	Apocynaceae Catharanthus roseus	Vinblastine, Vincristine	Anticancer	Tubulin
	Rauvolfia serpentina Nyssaceae	Ajmalicine Ajmaline	Antihypertensive Anti-arrhythmic	an Adrenergic receptor Na^+ channels

	Camptotheca acuminata	Camptothecin	Anticancer	DNA topoisomerase I
Benzylisoquinoline alkaloids (BIA)	Papaveraceae Papaver somniferum	Codeine Morphine Papaverine	Antitussive, analgesic Analgesic, narcotic Spasmolytic, vasodilators	Nicotinic acetylcholine (nACh) receptor m3 Opioid receptor c-AMP phosphodiesterase
	Eschscholzia Californica Ranunculaceae	Sanguinarine	Antibacterial, proapoptotic	FtsZ (bacterial cytokinesis), mitoch. pathway
	Thalictrum flavum Coptis japonica	Berberine Berberine, sanguinarine	Antibacterial, antimicrobial	DNA
Tropane and nicotine alkaloids (TNA)	Solanaceae Hyoscyamus niger	Hyoscyamine	Anticholinergic, narcotic, myorelaxant	Muscarinic receptor
	Datura stramonium	Scopolamine	Anticholinergic, narcotic, myorelaxant	Muscarinic receptor
	Atropa belladonna	Hyoscyamine	Anticholinergic, narcotic, myorelaxant	Muscarinic receptor
	Nicotiana tabacum	Nicotine	Neurostimulant, insecticide	nACh receptor
Purine alkaloids (PA)	Coffeeae Coffea arabica	Caffeine	Central nervous system stimulant	Adenosine A_1& A_{2A} receptors; phosphodiesterase

8.2 BIOSYNTHESIS OF BENZYLISOQUINOLINE ALKALOIDS (BIA)

BIA constitute a diverse class of more than 2,500 compounds with, for some of them, potent pharmacological properties and socio-economic importance. In opiumpoppy (*Papaver somniferum*) alone, more than 80 alkaloids have been identified.BIA have also been widely studied in other members of the Papaveraceae, such as*Eschscholzia californica*, or members of the Ranunculaceae such as *Thalictrum flavum* and *Coptis japonica* (Table-2). The biosynthesis of BIA starts with thecondensation of two tyrosine derivatives to produce (S)-norcoclaurine. Four characterized enzymatic steps are necessary to produce (S)-reticuline, the centralprecursor of the five major BIA sub pathways, leading to palmatine, berberine,sanguinarine, laudanine and codeine/morphine respectively. Overall, regardless ofthe plant model species, a total of 39 clones

and 19 enzymatic steps have beencharacterized (Table-3), making the BIA biosynthetic pathway one of the bestcharacterised plant natural product complex pathways (Gregory et al., 2010). Fig.6

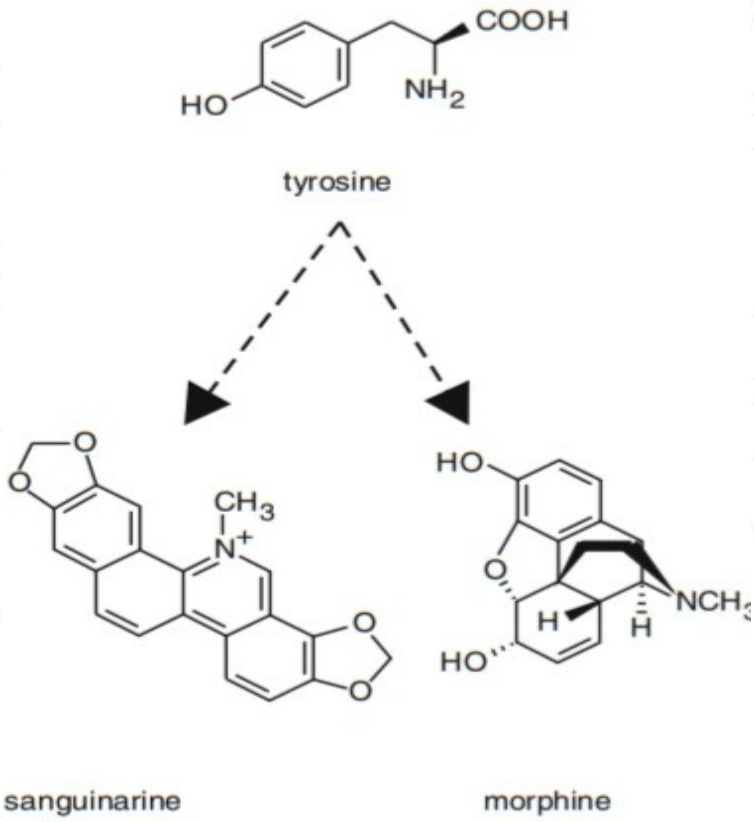


Fig.6 Biosynthesis of BIA

8.3 BIOSYNTHESIS OF TROPANE AND NICOTINE ALKALOIDS (TNA)

TNA are widely used in medicine as nonselective muscarinic antagonists affectingperipheral and central nervous systems (Table-2). This alkaloid family is foundmainly in *Solanaceae* species, such as *Hyoscyamus Niger, Datura stramonium, Atropa belladonna* or *Nicotiana tabacum*, and accounts for more than 200 differentcompounds. TNA are amongst themost studied alkaloids and their pharmacological effects are well documented.However, their biosynthetic pathways are still only partially understood. TNA arederived from the amino acids ornithine and arginine (Fig.5), and the earlybiosynthetic steps leading to N-methylputrescine formation have been elucidated in several species (Table-3).The oxidative deamination of N-methylputrescineleads to N-methylpyrrolium cation, which constitutes a branching point towardstropane alkaloids and nicotine alkaloids respectively. The final steps of both pathwaysare partially characterized, but molecular information concerning the centralsteps is still missing. Overall, seven enzymatic steps of the tropane alkaloidbiosynthetic pathway and four enzymatic steps of the nicotinic alkaloid biosyntheticpathway have been characterized (Gregory et al., 2010). Fig.7

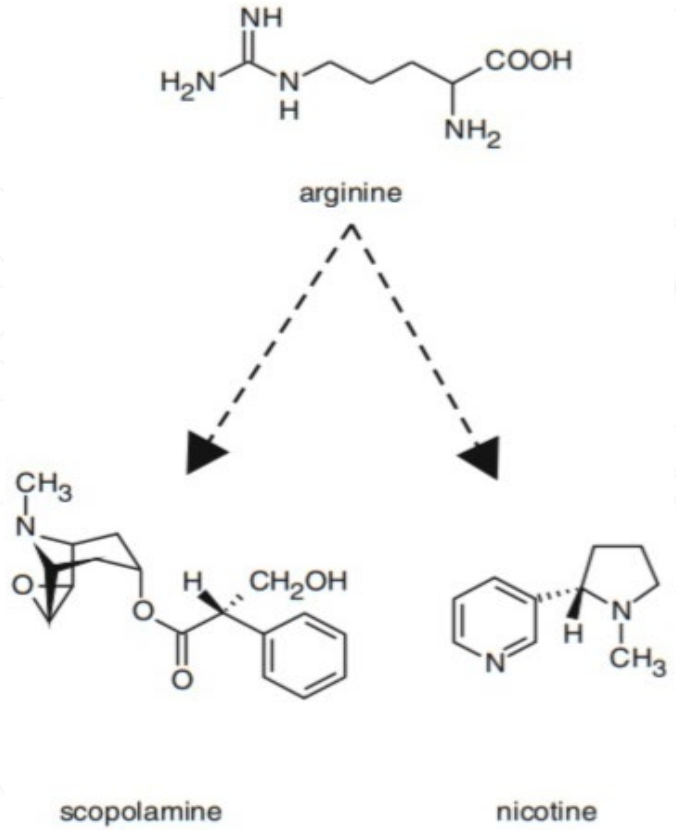


Fig.7 Biosynthesis of TNA

8.4 BIOSYNTHESIS OF PURINE ALKALOIDS (PA)

PA are natural products derived from purine nucleotides (Fig.8). The main PA are caffeine and theobromine that affect the central nervoussystem as neurostimulants and are synthesized by several plants, including *Coffeaarabica, Camellia sinensis* or *Theobroma cacao*(Table-2). The

Table-2 Sources, chemical diversity and biological activity of four major families of alkaloids

initial precursorof PA is xanthosine, which is supplied by at least four different pathways. The maincaffeine biosynthetic pathway has four enzymatic steps, comprising three characterized S-adenosylmethionine-dependent N-methylation reactions and one uncharacterized nucleosidase reaction (Table-3). However, according to structuralstudies of N-methyltransferases involved in caffeine biosynthesis, it has beensuggested that the ribose hydrolysis could be performed by xanthosine 7 N-methyltransferase (Gregory et al., 2010). Fig.8

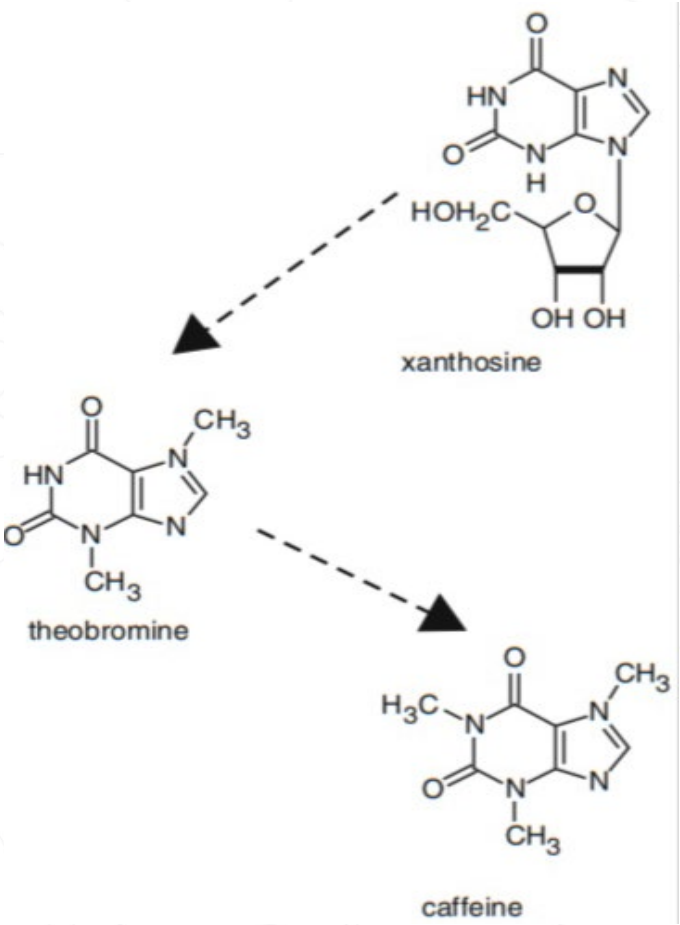


Fig.8 Biosynthesis of PA

Table-3 Molecular resources for alkaloid biosynthesis

Family (plant species)	NCBI hints (nt seq)	NCBI hints (EST)	Alkaloid biosynthetic enzymes (GenBank access numbers, full enzyme name and abbreviation)
Alkaloid family: monoterpene indole alkaloids (MIA)			25 [CAA09804 (1-deoxyxylulose 5-phosphate synthase, dxs), ABI35993 (2-deoxyxylulose-5-phosphate reductoisomerase, dxr), ACI16377 (4-diphosphocytidyl-methylerythritol 2-phosphate synthase, cms), ABI35992 (4- diphosphocytidyl-2-C-methyl-D-erythritol kinase (cmk), AAF65155 (mecs), AAO24774 (hds), ABI30631 (hdr), ABW98669 (idi), ACC77966 (gpps), CAC80883 (g10h), Q05001 (NADPH cytochrome P450 red),
Apocynaceae Catharanthus roseus	20,820	19,899	
Rauvolfia serpentina	17	-	7 [CAA44208 (strictosidine synthase: str1); CAC83098 (strictosidine b-D-glucosidase: sgd1); AAF22288 (polyneuridine aldehyde esterase: pnae); Q70PR7 (vinorine synthase: vs); AAF03675 (raucaffricine b-D-glucosidase: r
Nyssaceae Camptotheca acuminata	31	-	10 [ABC86579 (1-deoxy-D-xylulose-5-phosphate reductoisomerase: dxr), O48964

			(isopentenyl pyrophosphate: dimethylallyl pyrophosphate isomerase: idi1), and O48965 (epsps), AAV64030 (5-enolpyruvylshikimate 3-phosphate synthase), and (idi2) AAB97526; AAU84988; and AAU84989 (anthranilate synthase a subunit: asa1). (tryptophan decarboxylase: tdc1); AAB39708 (tryptophan synthase b subunit: tsb); [10-hydroxygeraniol oxidoreductase: 10hgo; AAB39709(tdc2)]
Alkaloid family: benzylisoquinoline alkaloids (BIA)			
Papaveraceae Papaver somniferum	20,458	20,340	16 [P54768 (tyrosine/DOPA decarboxylase: tydc1); P54770 (tydc3); AAX56303 (S-norcoclaurine synthase: ncs1); AAX56304. (ncs2); AAQ01669 (norcoclaurine 6-O-methyltransferase: 6omt); AAP45316 (coclaurine N-methyl
Eschscholzia californica	9,161	9,083	5 [O64899 (CYP80B1 renamed CYP80B3=N-methylcoclaurine 30 -hydroxylase); AAC39454 (CYP82B1=N-methylcoclaurine 30 -hydroxylase); BAE79723 (reticuline-7-O-methyltransferase: 7omt); CYP80B2=N-methylcoclaurine 30 -hydroxylase); O64900]
Ranunculaceae Thalictrum flavum	10	-	9 [AAG60665 (tyrosine/dopa decarboxylase: tydc1); AAR22502 (norcoclaurine synthase: ncs); AAU20765 (norcoclaurine 6-O-methyltransferase: 6omt); AAU20766 (coclaurine N-methyltransferase: cnmt); AAU20767 (CYP80B3=N
Coptis japonica	135	37	9 [BAF45337 (norcoclaurine synthase: ncs); Q9LEL6 (norcoclaurine 6-O-methyltransferase: 6omt); Q948P7 (coclaurine N-methyltransferase: cnmt); Q9LEL5 (30 hydroxy-N-methylcoclaurine 40 omt); Q39522 (scoulerine) BAB68769 (CYP719A=methylenedioxy bridge-enzyme: 9-O-methyltransferase); BAB12433 (CYP80B2=N-methylcoclaurine-30 -hydroxylase); BAF80448 (CYP80G2=corytuberine synthase); Q8H9A8 (columbamine O-methyltransferase: coomt);

Family (plant species)	NCBI hints (nt seq)	NCBI hints (EST)	Alkaloid biosynthetic enzymes (GenBank access numbers, full enzyme name and abbreviation)
Alkaloid family: tropane and nicotine alkaloids (TNA)			
Solanaceae Hyoscyamus niger	23	-	P50164 (tropinone reductase); ABD39696 (CYP80F1=littorine mutase/monooxygenase); and BAA82263 (putrescine N-methyltransferase: pmt); BAA85844 (tropinone reductase: tr1; tr2); P24397 (hyoscyamine 6-b-hydroxylase: h6h)]
Atropa belladonna	58	-	[BAA78340 (hyoscyamine 6-b-hydroxylase: h6h), BAA82264 (putrescine N-methyltransferase: pmt1), PMT2, and PMT2)]
Datura stramonium	31	-	P50134 (ornithine decarboxylase), CAB64599 (arginine decarboxylase: adc1), CAE47481 (putrescine N-methyltransferase: pmt), P50162 (tropinone reductase: tr1), P50163 (tr2), and P50165 (trh) are examples of enzymes.

Nicotiana tabacum	1,673,039	240,440	[Ornithine decarboxylase (AAQ14852), putrescine N-methyltransferase (AAF14881), methylputrescine oxidase (ABI93948), and nicotine N-demethylase (DQ131886)]
Coffeeae			
Coffea arabica	6,079	1,577	10 [BAC43756 (theobromine synthase: cts1); BAC43757(cts2); BAC43755 (7-methylxanthosine synthase: xrs1); BAC43760 (caffeine synthase: ccs1); BAC43761 (ctcs7)]
Camellia sinensis	3,732	3,288	[ABP98983] (tcs, caffeine synthase]
Byttnerioideae			
Theobroma cacao	7,469	6,790	[BAE79730] (bcs1: caffeine synthase]

9. EXTRACTION AND ESTIMATION

Due to high interest of this valuable compounds, researchers from all over the world have tried to find new and better techniques for the extraction and the estimation of alkaloids. Like all the other secondary metabolites, the extraction of alkaloids was also started with the paper chromatography (PC). It was the easiest method for extraction, which was rapid and cheap (Kaur et al., 2015; Roy et al., 2017). The whole sample could be analyzed in a few minutes with the help of ultraviolet light (Kaur et al., 2015). Chronologically, it was followed by the similar method of Thin layer chromatography (TLC) (Kaur et al., 2015; Roy et al., 2017). This method was also rapid and required the Rf values for the estimation of the alkaloids (Kaur et al., 2015). It was a reproducible method and has a low detection limit as compared to PC (Kaur et al., 2015; Roy et al., 2017). High performance liquid chromatography (HPLC) method for the isolation of alkaloids has always been a widely preferred method (Kaur et al., 2015). One of the earliest known methods for the isolation of alkaloids by HPLC was reported by Wu and Wittick (Kaur et al., 2015; Roy et al., 2017). It is highly accurate and has the ability to detect very small quantities of the compound (Kaur et al., 2015). Gas chromatography (GC) was another highly appreciated method for the isolation and estimation of alkaloids (Kaur et al., 2015). Brochmann – Hanssen and Svendsen (1962) were the first to report the successful estimation of alkaloids by GC (Kaur et al., 2015; Roy et al., 2017). Although, not as famous as HPLC, this method has been used for both the qualitative as well as quantitative analysis of alkaloids. Thewonderful results even at low efficiency and the simple procedure were the main reasons for its acceptance (Kaur et al., 2015). Other important methods used for the extraction of alkaloids are microwave assisted method, ultrasound assisted method, supercritical carbon dioxide extraction method and the combination of ultrasound and surfactants for the extraction of alkaloids (Kaur et al., 2015; Roy et al., 2017; Zhou et al., 2012). Many methods for the estimation of alkaloids have been formulated. Both the physical and chemical methods are widely used. Among these, chromatographic methods are always of high interest, as these can be employed for both the extraction and estimation processes (Kaur et al., 2015). The major chromatographic techniques

employed for the estimation of alkaloids are PC,TLC, HPLC and GC (Kaur et al., 2015; Roy et al., 2017). Along with these methods, some important chemical methods are also used viz. ELISA and Radio Immuno Assay (RIA). But the major method for the characterization of alkaloids are the spectroscopic methods involving Mass Spectroscopy (MS) and Nuclear Magnetic Resonance (NMR). These spectroscopic methods can be used either alone or in combination with the chromatographic techniques (Kaur et al., 2015; Rudzinska et al., 2009).

10. MEDICINAL APPLICATIONS

Medical use of alkaloid-containing plant has a long history, and thus, when the first alkaloids were isolated in the 19thcentury, they immediately found application in clinical practice. Many alkaloids are still used in medicine, usually in the form of salts, including the following:(Table-4)

Table - 4. Alkaloids and their therapeutic actions

Alkaloid	Action
Ajmaline	Antiarrhythmic
Atropine , Scopolamine , Hyoscyamine	Anticholinergic
Caffeine	Stimulant , Adenosine receptor antagonist
Codeine	Cough medicine , analgesic
Colchicine	Remedy for gout
Emetine	Antiprotozoal agent
Ergot alkaloids	Sympathomimetic, vasodilator, Antihypertensive
Morphine	Analgesic
Nicotine	Stimulant , Nicotinic acetyl choline receptor agonist
Physostigmine	Inhibitor of acetyl cholinesterase
Quinindine	Antiarrhythmic
Quinine	Antipyretics, Antimalarial
Reserpine	Antihypertensive
Tubocurarine	Muscle relaxant
Vinblastine , Vincristine	Antitumor
Vincamine	Vasodilating , Antihypertensive
Yohimbine	Stimulant , Aphrodisiac

Many synthetic and semi-synthetic drugs are structural modification of the alkaloids, which were designed to enhance or change the primary effect of the drug and reduce unwanted side-effects. For example,Naloxone, an opioid receptor antagonist, is a derivative of the baine that is present in opium (Babbar N. 2015).

11. FUTURE POTENTIALS OF ALKALOIDS

Natural alkaloids derived from plants may be lead compounds, and have begun to gain popularity worldwide for promoting health care as well as disease prevention (Shi et al., 2014;

Hui et al., 2012; Li et al., 2009). They offer a diverse range of structurally distinctive bioactive molecules, have been used as a major source of innovative and effective therapeutic agents. In-depth study on metabolic transformation, efficacy and safety of alkaloids, will accelerate their natural resource development and utilization of alkaloids. Furthermore, the biological screening of active alkaloids, using a wide variety of scientific tools and the interactive collaboration of experts in diverse scientific disciplines will become research hotspot, providing new and essential healthcare opportunities (Shi et al., 2014).

12. CONCLUSION

These important class of secondary metabolites have been found to exhibit many important biological properties such as muscle relaxant, analgesic, anticancer and antioxidant properties. These are used for the curative purposes and are helpful for the mankind. Interestingly, it has been found that alkaloids are not only beneficial to humans, but in certain cases may even be life threatening. Progress in metabolomic and transcriptomic analysis as well as system biology approaches are likely to yield more rational strategies to improve alkaloid biosynthesis. A large number of extraction and estimation methods of alkaloids have been formulated. These are developed to ease the researchers in the study of this metabolite and these methods are improvements to the previous methods. With the advancements in the field of science and technology, alkaloids are being exploited for various purposes. Alkaloids have also been utilized for the pharmaceutical and curative purposes. It is hoped that this valuable metabolite may be used to cure many lethal diseases like cancer. We would like to conclude that alkaloids are useful for plants, animals, as well as humans. They can be employed for pharmaceutical purposes, due to its presence in almost all the vegetables and medicinal plants. Attention is required in testing this compound for the curative purposes of the human diseases.

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Studies on Properties of Microbial Amylase

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ABSTRACT

Amylase can be found in several fungi, yeast, bacteria and actinomycetes; however, an enzyme from mold sources and bacteria has dominated the use in industry. The use of amylase in industrial reaction depends on its different factors, such as its composition, substrate specification, large reaction products, optimal temperature, and optimal pH12. The bacterium α-amylase is preferred for use in the processing of starch and textile industries due to its action at high temperatures (75-105°C) and it's neutral to alkaline pH13. Generally, the production of this enzyme has been effected by submerged fermentation. Some bacterial sources are important species. Like *Bacillus subtilis, Bacillus staerothermophilus, Bacillus amylo liquefaciens, Bacillus licheniformis, Bacillus acidocaldarius, Bifidobacterium bifidum and Bifidobacterium acerans*. The current review focused on bacterial amylase and this review examines the following chapters: Amylase, Microorganisms and amylases, Physiology of amylases, maturity studies on bacterial amylase production and commercial use of amylases.

Key Words: Amylase, Enzyme, Bacteria, Fermentation, Physiology

INTRODUCTION

Microorganisms have been a major contributor to it production of various industrial enzymes. The global market for industrial enzymes is estimated at $ 2 billion in 2010 and is expected to

increase an annual growth rate of 3.3%. These starch degrading - amylolytic enzymes are greatly important for biotechnological applications from food, fermentation, textiles, paper, pharmaceutical to sugar industries (Kunamneni, Permaul, and Singh 2005). Conversion of starch into sugar, syrup and dextrins forms a large part of the starch processing industry. (Prasanna V. Aiyer 2005). Starch degrading enzymes such as amylase have been detected great attention due to their visibility technical significance and economic benefits (Gupta, A; Gupta, V.K.; Modi, D.R.; Yadava 2008). Nowadays, amylases such as α-amylases, β-amylases and glucoamylases represent one of the most important enzyme groups within the field of biotechnology (Bravo Rodríguez et al. 2006).

α-amylase (EC 3.2.1.1, 1,4-α-D-glucan glucanohydrolase, endoamylase) is an old calcium containing enzyme which catalyzes starch hydrolysis and related carbohydrates by cleavinginternal α-D- (1-4) glycosidicconnecting, producing glucose, maltose, maltotriose,and other oligosaccharides (Ryan, Fitzgerald, and Van Sinderen 2006). It is a part of familythirteen in the classification of glycosidehydrolases. This family is the most diversefamilies of glycoside hydrolase, which contains manyenzymes which are able to stimulate various reactions, such as hydrolysis, transglycosylation, condensation and cyclization (Ben Ali et al. 2006).

The use of enzymes in industrial processes is beginning to fulfil its promise. Enzymes contain their High levels of catalytic activity in aqueous solution. According to industrial standards, only medium temperatures and pressures are required. So, the exploitation of the enzyme industry makes the development of hygienic, environmental friendly procedures. Moreover to its beauty, its specification maintains unwanted reactions a minimum, to increase yield. Industrial processes, however, still require enzymes to perform under extreme and unfavourable conditions. Because of this, a major problem for industrially important enzymes, is the lack of stability. An example that shows both opportunities, and limitations of

industrial use of enzymes, is processing of starch, regarded as an improper achievement of modern industrial biotechnology. Amylase has a lot of function in starch saccharification as shown in Table 1. Amylolytic enzymes receive an extensive spectrum applications in the food industry for production of glucose syrups, high fructose corn syrups, maltose syrups, reducing the viscosity of sugar syrups, to reduce the formation of haze in juices, solubilization and starch saccharification fermentation of alcohol in the brewing industry, and get a variety of app on baking, paper, textile and cleaning industries (Pandey et al. 2000). In most cases the enzymatic process is inhibited because of high substrate and product concentration and enzyme instability under repetitive or long-term use.

AMYLASES

Enzymes which hydrolyze starch molecules to give diverse products including dextrin and progressively smaller polymers composed of glucose units are known as Amylases (Saranraj and Naidu 2013). These enzymes are very important in present day biotechnology with applications ranging from food, fermentation, textile to paper industries. To meet industrial demands, microbial enzymes are used although amylases can be derived from several sources, including plants, animals and microorganisms. Nowadays, microbial amylases are available commercially in a large number, replacing chemical hydrolysis of starch in starch processing industry (Pandey et al. 2000).

The history of amylase began in 1811 when the first enzyme to reduce starch was discovered by Kirchhoff. This was followed by several reports of digestion amylases and malt amylases. It was much later in 1930, that Ohlsson suggested this separation of enzymes that break down starch into malt such as α- and β-amylase according to anomeric the type of sugar produced by the enzyme reaction. α-Amylase (1,4-α-D-glucan-glucanhydrolase, EC. 3.2.1.1) is a widely distributed secret enzyme. A-Amylases of different origins have been learned more.

Amylases can be divided into two categories, endoamylases and exoamylases. Endoamylases catalyze hydrolysis randomly to inside of the starch molecule. This action creates line formation and branches of oligosaccharides which have different chain lengths. Exoamylases hydrolyze from non-reducing end, which leads in succession to shorter products. Today a large number of enzymes are known which hydrolyze the starch molecule into different products and integrated action of diverse enzymes are needed to hydrolyze starch completely. Many reviews are available for amylases and their applications, however, they are not mainly covering α-amylases at length.α-amylases are one of the most popular as well an important type of industrial amylase and the current review highlights various aspects of microbial α-amylases.

α - Amylases are the members of family 13 of the glycoside hydrolase group of enzymes which catalyze the hydrolysis of the internal α-1,4-glucosidic bond in starch (Kadziola et al. n.d.). These enzymes are mainly used in food and detergent industries (Aghajari et al. n.d.). The 3D structures of α-amylases from various sources such as *Aspergillus oryzae, Aspergillus niger, porcine pancreas, barley, human saliva, Bacillus licheniformis, Bacillus stearothermophilus* and *Alteromonas haloplanctis* have been observed (Suvd et al. 2001).

The α-amylases which have two additional calcium binding sitesare extracted from *Bacillus licheniformis* and *Bacillus stearothermophilus* (Tanaka and Hoshino 2002), while a fourth site which is also able to bind a calcium ion has been identified in the chimeric protein build from the genes encoding *Bacillus licheniformis* and *Bacillus amyloliquefaciens* α-amylases (Hashim et al. 2005).

α-amylases from *Bacillus licheniformis*, *Bacillus amyloliquefaciens* and *Bacillus stearothermophilus* are among the most common studied amylase and are very homologous respect to the primary and tertiary structure (Nielsen et al. 2003). Despite their similarities in

structure, however, these reported amylases vary greatly in strength and thermal stability. Thermal dysfunction of *Bacillus* α-amylases has been suggested that be involved in a two-step process, with the first step of reversible unfolding, followed by irreversible conformational changes (Fitter et al. 2001). Calcium ions also contribute to inclusive programs of thermal dysfunction of *Bacillus* α-amylases, where it has been proposed that the first step involves the regenerative breakdown of calciumions from a native enzyme, which is followed by irreversible denaturation at high temperatures (Simons et al. n.d.).

MICROORGANISMS AND AMYLASES

Amylases can be found in many sources, such as plants, animals, viruses and fungi. Due to the short growth time, biochemical diversity and enzyme release concentration may increase by genetic modification, enzymes from bacterial sources usually combine industrial requirements (Mishra and Behera 2008; Nascimento De Oliveira et al. 2007). Most of these the enzymes used to date have been found in mesophilic microorganisms. Applications for these enzymes are limited because of them relative stability to extreme temperatures, pH and ionic energy (Utong, James ; Al-Quadan, Farouk ; Akel 2006). Therefore, efforts were made thermophilic and halophilic enzymes bacteria, which can be used in many difficult areas such as industrial processes where concentrated salt solution and high temperatures used would inhibit many enzymatic conversions (Amoozegar, Malekzadeh, and Malik n.d.; Saxena et al. 2007).

α-amylase is distributed worldwide into animals, plants and the kingdom of bacteria. Over last few decades, much research has been done on extracellular amylase produced by many varieties of microorganisms (Lonsane and Ramesh n.d.). There are great benefits to use microorganisms in the production of amylases. Like, mass production capacity and microbes are easy to manipulate to obtain enzymes of desired characteristics. α-amylase can be taken

from several molds, yeast, viruses and actinomycetes, however, enzymes from fungal and bacterial sources has prevailed applications in the industrial sector (Saranraj and Naidu 2013).

Many species of *Bacillus* and thermostable Actinomycetes including *Thermonospora* as well *Thermoactinomyces*are a variety of manufacturers enzymes (Messaoud et al. 2004). The genus Bacillus produces large amount of their extracellular enzymes amylases and proteases which are essential for industrial significance. A highly thermostable and alkaline α-amylases is found in mesophile *Bacillus* sp. (Saxena et al. 2007). Thermophilic bacterium *Bacillus stearothermophylus* provides another form of commercial production of thermostable α-amylases.

Alkaline and thermotolerant amylases are produced by *Bacillus* spp. Eg, *Bacillus licheniformis*, and *Bacillus halodurans* (Setyorini et al. 2006). These enzymes are purified and characterized. Some of them are reported to be malto-oligosaccharide producing enzymes (Hashim et al. 2005). The production of oligosaccharides containing thermostable amylase is desirable because it is more stable than normal amylase, showing high activity at 30-40°C. The enzyme was not suitable for the production of oligosaccharides because the main product produced by soluble starch was glucose, and maltose (G2) and maltotriose (G3) were considered subgroups. Therefore, we have recently attempted to differentiate the producers of thermal amylase, showing activity under alkaline conditions, and tested reaction products.

Benefits of using thermostable amylases in industrial processes include a reduction in the risk of pollution and costs of external cooling, a better melting of substrates, lower viscosity which allows for faster mixing and pumping (Lin, Chyau, and Hsu 1998). The development of saccharifying amylolytic enzymes operating at high temperatures (90°C), directly benefit the starch industries. However, using α-amylase production processes at higher temperatures

will require new process construction and advanced knowledge of thermophilic bacteria (Lévêque et al. 2000). Procedures for the use of thermophiles lack classical maturity of processes with mesophilic bacteria and yeast (Coolbear, Daniel, and Morgan n.d.). Alkaliphilic *Bacillus* spp often produce enzymes active at alkaline pH, like, alkaline α-amylase, protease and carboxy methyl cellulose (Horikoshi 1092).

PHYSIOLOGY OF AMYLASE PRODUCTION

Production of α-amylase by submerged fermentation (SmF) and solid state fermentation (SSF) has been thoroughly investigated and so on influenced by various physicochemical factors. Most notable among these is the formation of growth zone, medium pH, phosphate concentration, inoculum age, temperature, aeration, carbon source and nitrogen source (Saranraj and Naidu 2013). Most reports within the mold are limited to a few species of mesophilic fungi when attempts made to specify cultural contexts and select the best types of fungus to produce at a commercial level (Gupta et al. 2003; Mishra and Behera 2008).

Physicochemical Parameters:

The effect of various physicochemical parameters, including carbon and nitrogen source, surface acting agents, phosphate, metal ions, temperature, pH and agitation have been learned (Gupta et al. 2003).

Substrate sources

α-amylase is an inducible enzyme and usually made in front of starch or its hydrolytic product, maltose (Markeberg, Carlsen, and Nielsen n.d.). More reportsis found in α-amylase infusion, different species of *Aspergillus oryzae* suggest that the normal inducer molecule is maltose. There is a report, a 20-fold increase in enzyme activity where maltose and starch are used as inducers in *Aspergillus oryzae.* Similarly, strong infusion of αamylase with starch and maltose in the case of *Aspergillus oryzae* has been reported (Jin, Van Leeuwen, and Patel

1999). Apart from maltose, in some species, another carbon sources such as lactose, trehalose, α-methyl-D-glycoside also acted as inducers of amylase. Not only is the source of carbon, but also the mycelial / age condition affects the synthesis of α-amylase by *Aspergillus oryzae* (Goto et al. n.d.; Gupta et al. 2003).

For optimal induction by maltose, there are reports that 5 days starved non-growing mycelia were the most appropriate. α-amylase production is and under the pressure of catabolite with glucose and some sugar, like many other inducible enzymes (Saranraj and Naidu 2013). However, the role of glucose in sugar α-amylase production in some cases is contradictory. α-amylase production by *Aspergillus oryzae* was not suppressed by glucose rather; a small amount of enzyme is formed in its presence. However, xylose or fructose are suppressed and described as very stressful though they support the positive growth of *Aspergillus nidulans* (Gupta et al. 2003; Saranraj and Naidu 2013).

Sources of carbon such as glucose and maltose have been used for α-amylase production. However, the use of starch is always promising as well all over. The value of something unusual substrates such as lactose, casitone, fructose, oilseed cakes (Alberto et al. 2002) and starch to process contaminated water (Gupta et al. 2003).

Cui YQ *et al.* (Cui, Van Der Lans, and Luyben 1997) also used for production of α-amylase during agroprocessing by-product, wheat bran was used for economic production of α-amylase by SSF. The use of wheat in liquid surface fermentation (LSF) in the production of αamylase from *Aspergillus fumigates* and *Clavatia gigantea*, respectively, has also been reported. High α-amylase activity from Aspergillus fumigatus has also been reported using α-methyl-D-glycoside (a synthetic maltose analogue) as a substrate.

The report has been made for the use of low molecular weight dextran in combination with either Tween 80 or Triton X100 for a-amylase production in the thermophilic fungus

Thermomyces lanuginosus. (Amanullah et al. 1999) Which declared, Triton X-100 had no effect, whereas Tween 80 increases the α-amylase activity 27-fold (Gupta et al. 2003).

Nitrogen sources

Organic nitrogen sources have been a preference for the production of α-amylase. For the production of α-amylase from *Streptomyces* sp., *Bacillus* sp. and *Halomonas meridian* yeast extract has been used (Agger et al. 1998; Gupta et al. 2003).

In conjunction with other nitrogen sources such as bactopeptone in the case of *Bacillus* sp., ammonium sulphate in the case of *Bacillus subtilis*, ammonium sulphate and casein for *C. gigantea* and soybean flour and meat extract for *Aspergillus oryzae*, again yeast extract has also been used. When used as an additional nitrogen source, yeast extract increased the productivity of α-amylase by 156% in *Aspergillus oryzae* than when ammonia was used as a sole source (Agger et al. 1998; Gupta et al. 2003).

Various organic nitrogen sources are also reported to support high α-amylase production by various bacteria and fungi. However, living nitrogen sources namely, beef extract, peptone and com steep alcohol support high α-amylase production by bacteria, soybean meal and amino acids *Aspergillus oryzae*. (Saranraj and Naidu 2013)CSL is also used for economical production and efficacy of α-amylase from mutant *Bacillus subtilis*. Outside from this, and various salts of inanimate matter such as ammonium sulphate for *Aspergillus oryzae* as well as *Aspergillus nidulans*, ammonium nitrate *Aspergillus oryzae* and Vogel salts of *Aspergillus fumigatus* has been reported to support better α-amylase production in the fungus (Gupta et al. 2003; Saranraj and Naidu 2013).

Amino acids in combination with four vitamins and has been reported to affect α-amylase production. However, no conclusion can be reached about this for the role of amino acids and vitamins in improving digestion production of α-amylase in various microorganisms as

reports vary widely. Amylase production of *Bacillus amyloliquefaciens*increased by 300 in the presence of glycine (Saranraj and Naidu 2013). The effect of glycine has not been as a source of nitrogen instead affected the amylase production by controlling pH and later amylase production increased. Alanine, DL-nor Valine and D-methionine were effective production of alkaline amylase by *Bacillus* sp. However, the role of amino compounds was considered as a nitrogen or a source of carbon, but as amylase stimulators, synthesis and excretion. Reportedly only asparagine provided good enzyme yields over time. Importance of arginine to α-amylase production from Bacillus subtilis has also been well documented (Gupta et al. 2003; Saranraj and Naidu 2013).

Role of phosphate

Phosphate has an important regulatory role in the synthesis of the primary and secondary metabolites in microorganisms and just as it affects biological growth and production of α-amylase. Significant increase in enzyme production and status in *Aspergillus oryzae.*

Phosphate levels above 0.2 M have been reported. Similar findings were confirmed in *Bacillus amyloliquefaciens* where low phosphate levels resulted in very low cell density and no αamylase production (Chul Chung et al. 1995; Gupta et al. 2003). In contrast, high phosphate concentration inhibits the formation of the enzyme production of *Bacillus amyloliquefaciens* (Gupta et al. 2003; Justen et al. n.d.).

Role of other ions

K^{+}, Na+, Fe^{2+}, Mn^{2+}, Mo^{2+}, Cl^{+}, SO_4^{2+} had no effect while Ca^{2+} inhibited amylase production by *Aspergillus oryzae*. Mg^{2+} played a significant role and production was reduced by 50% when Mg^{2+} was released in the medium. Na^{+} and Mg^{2+} show a combined stimulation of enzyme production by *Bacillus* sp. (Bocking et al. 1999)The addition of zeolite to regulate ammonium ion in *Bacillus amyloliquefaciens* has led to an increase in α-amylase yield. A

negative relationship between α-amylase production and growth rate was observed in *Streptomyces* sp. in the presence and absence of Co^{2+}, the presence of Co^{2+} improves biomass levels by 13 times, although it reduces the enzyme yield (Cui, Van Der Lans, and Luyben 1997; Gupta et al. 2003).

pH

Within the visible parameters, the pH of medium growth plays an important role by morphological variability in the body and enzyme production. PH changes are observed during the growth of organism. It also affects product stability in the medium. Most of these types of *Bacillus* strains are used for commercial production of bacterial α-amylases by SmF have optimum pH between 6.0 and 7.0 for growth and enzyme production. The same is true of species used for enzyme production by SSF. In most of the cases the pH used is not specified externally, pH 4.2 in the case of *Aspergillus oryzae*, 8.0 in *Aspergillus oryzae* and 6.8 for *Bacillus amyloliquefaciens* (Agger et al. 1998; Gupta et al. 2003).

In fungal processes, the preventive dose of some media elements sometimeseliminate the need to control the pH. The pH value acts as an important indicator of the beginning and end of enzyme synthesis. It is reported to be *Aspergillus oryzae* accumulated amylase in mycelia there grown in an area devoid of phosphate or sulphate and was released when mycelia were replaced in an alkaline environment (Gupta et al. 2003; Saranraj and Naidu 2013).

Temperature

Temperature influence on amylase production is related to the growth of organism. Among the fungi, most of the amylase production studies are conducted with mesophilic mold within the temperature range 25 to 35 °C. Excellent α-amylase yields were present achieved at 30 to 37 °C in the *Aspergillus oryzae* (Saranraj and Naidu 2013). α -amylase production has also been reported 55 °C is a Thermophilic fungus *Thermonosporafusca* also at 50°C by

Thermonosporal anuginosus (Saranraj and Naidu 2013) amylase produced at a very wide range of this temperature between microbes. Ongoing amylase productions from *Bacillus Amyloliquefaciens* at 36°C were reported. However, temperatures up to 80 °C have always been used for the production of amylase from Hyperthermophile *Thermococcus profundus* (Gupta et al. 2003; Prieto et al. n.d.).

Agitation

Agitation force contributes to mixing and oxygen transfer rates in most fungal fermentations and thus influences mycelial morphology as well as product design (Saranraj and Naidu 2013). It is reported that a high speed is sometimes dangerous for mycelial growth and thus can deplete enzymes production. However, it is reported that variations in mycelial morphology as the effect of changes in the level of disturbance do not affect the production of the enzyme at a specific time growth rate. Agitation power up to 300 rpm often used for production amylase from various microorganisms (Gupta et al. 2003; Marco et al. 1996).

FERMENTATION STUDIES ON BACTERIAL AMYLASE PRODUCTION

Impact of environmental conditions on regulation of extracellular enzymes in batch cultures are well-written. Too much work on morphology and physiology of α-amylase production by *Aspergillus oryzae* during batch cultivation has been done. Likewise, the morphology of the *Aspergillus oryzae* was critically affected by the growth Ph (Saranraj and Naidu 2013). In a series of research, the authors noted that at pH 3.0 to 3.5, freely dispersed hyphal elements were formed. At a pH range of 4 to 5, both pellets at fragmented hyphal fragments were observed and at higher pH 6, pellets were the only growth forms recorded. Some groups have written similar recognition for other types of *Aspergillus oryzae* (Eriksen, Jensen, and Olsen n.d.). Very good growth was found to be at the temperature of 35°C. It showed that when

glucose was depleted, biomass production stops and α-amylase production increases rapidly (Gupta et al. 2003; Laderman et al. 1993).

One report states that the inoculum quantity did not affect morphological changes in *Aspergillus oryzae* in air-borne bioreactors and pellet size decreased as the air velocity increased (Saranraj and Naidu 2013). In the case of α-amylase production with *Bacillus flavothermus* in bulk cultivation in 20 L fermentor, α-amylase production and Biomass culminated twice as well as higher activity received 24 hours later. It was noted that the kinetics of enzyme synthesis was more than that related growth rather than non related growth kind. Similar findings are cited in another report by *Bacillus amyloliquefaciens* (Gupta et al. 2003; Saranraj and Naidu 2013).

Ongoing and fed-batch cultures have always been seen as the most effective in the production of enzyme and several study groups for the effectiveness of these cultures. Production of α -amylase from *Bacillus subtilis* has been enhanced greatly by expanding the investment in bulk with fed-batch performance (Flickinger and Drew 1999). Great enzyme activity about 54% larger than the two-phase feed group operating at a feed rate of 31.65 ml h-1 medium, rather than found in the culture of a single stage collection. Effects of a controlled maltose feed at a feed level of 4 g h-1 of α-amylase once glucoamylase production from *Aspergillus oryzae* in a rotary tube fermentor (RTF) have been learned (Monfort et al. 1996). At a feed rate of 1 g h^{-1} yields of α -amylase was twice as large as that of the group customs. When fed-batch planting was performed on an RTF test scale with a feed value of 24g h-1, biomass and amylase yields were higher than those found in a laboratory scale jar fermentor (Gupta et al. 2003). Solid model simulation values biomass yield, residual sugar residue as well a certain level of α-amylase production has been proposed to mimic most test data accurately. In addition, it is obtained from the chemostat to test the specific level of α-amylase production decreased to 70% with increase biomass concentration by a given amount of purification. The

change in the dilution rate in the ongoing culture can be used to find different scales enzymes, in much the same way (Pritchard 1992). It was more than that showed that high α-amylase production occurred in continuous culture with a purification rate of 0.15 h-1 and amylase activity in the culture was low with a purification rate above 1.2 h-1. In contrast, in *Bacillus* sp. growth change from collection to continuous cultivation led to the selection of non-α-amylase producing variants (Gupta et al. 2003; Saranraj and Naidu 2013). Decreased enzyme production was also associated with morphological and metabolic fluctuations between continuous cultivation. Industrial exploitation of the enzyme SSF production is limited to processes which includes mold and it is generally believed to be these techniques are not suitable for the cultivation of microbes. The use of the SSF method in the production of α-amylase and its specific benefits on the other hand it has been discussed extensively (Gupta et al. 2003).

CONCLUSION

As evidence of various reports, such amylases are among the most important enzymes used in different industries. Research on amylase has been developed at a tremendous rate over the past fifty years and possible industrial use of the enzyme especially in the solid waste management they have been identified. Major barriers to using commercially available amylase yield, are stability and cost of amylase production. However, bacterial segregation has been read extensively by many researchers. In addition, there is a need for more efficient amylase in various categories, which can be achieved with chemical modification of existing enzymes or by protein engineering. In the light of modern biotechnology, amylases are now benefiting the importance of biopharmaceutical applications. However, their use in food and staple based industries are a huge market and thus the need for amylase will remain high in these sectors.

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Table 1: Commercial applications of Amylase enzyme

Industry	Applications
Beer	Mashing
Distilled beverages	Mashing
Baking & milking	Reduction of dough viscosity acceleration of fermentation process, increase in loaf volume, improvement of crumb score and softness maintenance of freshness and softness
Chocolate, coco	Manufacture of syrups
Corn syrup	Manufacture of high maltose syrup
Pharmaceutical and Clinical	Digestive acids
Confectionery, candy	Sugar recovery from scrap candy
Cereals	Precooked baby foods, breakfast foods
Flavour	Clarification (starch removal)
Feeds, animal	Pig starter rations
Vegetables	Liquefying soups
Textiles	Digesting fabrics

SRJ

OVERLOADING DETECTION SYSTEM AND SEAT BELT DETECTION SYSTEM IN ELECTRIC VEHICLE USING ARDUINO

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ABSTRACT

Vehicle overloading is a well-known problem that has been linked to detrimental impacts on traffic safety, collisions, and greenhouse gas emissions. In addition to being dangerous, exceeding a vehicle's maximum allowable weight is also prohibited, which involves a number of hazards and fines. Country's economic growth is determined by the expansion of its transportation infrastructure. The structure of transport is increasing and sales of electric vehicles are on the rise. Vehicle overloading and drive without seat belt are most common causes of accidents in densely populated areas. Vehicle overloading would increase the effort required for engine performance, which would increase fuel consumption. Overloading of electric vehicle would increase incidences of road accidents, damage to vehicles, lower speeds, delay to vehicular traffic, increase the effort of battery performance. If you drive without a seatbelt, you risk being struck by a frontal air bag that is opening quickly. So, force could injure or even kill you. This device can continue to identify vehicle overloading and also detect if you not were seat belt. The main innovation we are making in this field is that as soon as the vehicle is overloaded, there will be a warning to the driver with a display and buzzer to draw his attention to the overloaded condition. As a result, the user will never be able to start his vehicle unless he reduces the load on the vehicle. Because it keeps the vehicle from carrying an extra burden and signals the dial to wear a seat belt, this profession is regarded as a protected occupation. Additionally, it lengthens the lifespan of the road. Hope it will play a significant role in the government sector if it is put into effect. This system will stop overloading. In addition, the engine and battery life will be extended and improve vehicle safety.

Keywords: Road safety, Overloading, Seat belt detector

I. INTRODUCTION

Nowadays, safety is really important factor in any field. Many car manufacturers now priorities passenger safety by incorporating a variety of features such as anti-lock brakes, air bags, and other safety features. If we don't keep safety, many accidents happen on the road. Overloading of vehicles and driving without seat belts are most common causes of accidents. As we can see, the globe is going toward electric vehicles. In electric vehicles, overloading presents numerous issues. Overloading has been identified as a safety and cost concern, and the National Department of Transportation has included an anti-overloading campaign in its road to safety agenda (H.D.Kattimani, 2017). Vehicle overloading can harm not just one but many lives. Electric vehicle overloading is very harmful not only for man but also for our vehicle. Overloading of electric vehicle can shorten the life of our electric vehicle.

The velocity and kinetic energy of the vehicle will increase with its weight. (Rupal Shah1, 2016). This reduces the vehicles' ability to stop quickly and causes were and tear on the brakes and tyres, which may have long-term consequences (H.D.Kattimani, 2017). Seat belt helps to hold driver and passengers in the seat and this keeps them from being thrown out of the vehicle by force and hitting the dashboard, windshield which can causes serious head injuries.

Overloading an Electric Vehicle will pose the following risks (H.D.Kattimani, 2017):

Different vehicles are designed for different maximum weight capacities. Similarly Electric vehicles also have load capacity. As a result, if the limit weight is exceeded, stopping the car is difficult. Vehicle will be less stable, difficult to steer. In electric vehicles, over overloading increases the load on the motor. As a result, the motor requires more power from the battery to run, weakening the battery. This can cause battery issues. The suspension system also becomes weak due to overloading. Tyres can overheat and wear quickly in overloaded electric vehicle.

Drive without Seat belt will pose the following risks:

Buckling up is one of the safest choices drivers and passengers can make. Seat belt helps keep safe and secure inside vehicle. In a collision, not using a seat belt can cause full ejection from the car in the event of an accident. Which is almost always fatal. Seat belt helps keep you safe and secure inside our vehicle.

COMPONENTS

LOAD CELL: (as shown in fig.1) is a one type of force transducer. Load cell converts a force such as tension, compression, pressure, or torque into an electrical signal (Neeli Sreekeerthan1, 2020)

Figure 1: Load cell

ARDUINO UNO: The Arduino Uno (as shown in fig.2) is one type of open-source microcontroller board. The Arduino Uno is an open-source microcontroller board created by Arduino CC that is based on the Microchip ATmega328P microcontroller. An open-source electronics platform called Arduino is built on simple hardware and software. A motor can be started, an LED can be turned on, and something may be published online by using Arduino board.

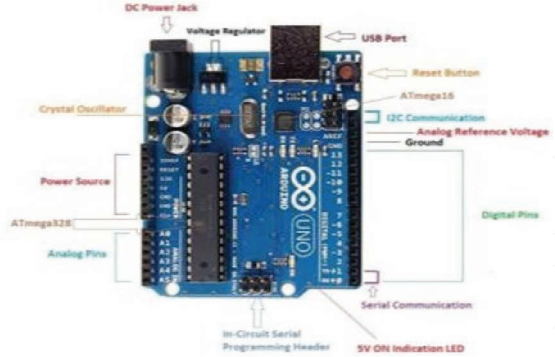

Figure 2: Arduino Uno

DC MOTOR: Any of a group of rotary electrical machines known as DC motors transforms electrical energy from direct

current into mechanical energy (Neeli Sreekeerthan1, 2020).

It is possible to control the speed of a DC motor across a large range by adjusting the field winding current or the supply voltage. A conversion from alternative to direct current, either onboard or outside the vehicle, is therefore required. DC motors can be divided into three groups: stepper, brushed DC motor, and BLDC or brushless DC motor. Electric side windows in electric vehicles can be extended, retracted, and moved around with the use of brushed DC motors.

Due to their inexpensive price, these motors are appropriate for a variety of applications (as shown in fig.3).In this project if the load cell will detect the weight, then with the help of arduino, relay will cut the power of the motor and stop the motor from rotating.

Figure 3: DC Motor

This motor's benefits include simple speed control and the ability to tolerate a rapid rise in load. It is the optimum traction motor because of all these features.

High maintenance required by brushes and commentators is the fundamental disadvantage of DC series motors.

PUSH SWITCH: The push switch (as shown in fig.4) is a mechanical device is usually used to turn on and off the control switch appliance that is widely used (Neeli Sreekeerthan1, 2020). Here we are mount push switch in the belt holder. This switch gives us information whether the seat belt is worn or not. When pushed in, a push-to-make switch permits current to flow between its two contacts. The circuit is disrupted when the button is released. Normally Open (NO) Switch is another name for this kind of switch. (Examples include the doorbell, the power switch on a computer case, the calculator buttons, and individual keyboard keys.)

Figure 4: Push Switch

LIQUID CRYSTAL DISPLAY: A flat-panel display (as shown in fig.5) or other electronically manipulated optical device that makes use of liquid crystals' ability to modulate light is known as a liquid-crystal display (LCD) (Neeli Sreekeerthan1, 2020). Liquid crystal material is placed between two sheets of glass in a liquid crystal display (LCD). Liquid crystal molecules are parallel to the glass surface in the absence of any applied voltage between clear electrodes. There are 16 input and output pins in it. Here LCD is mounted on the display. They generate images by utilizing liquid crystals in their operation. The display screen contains embedded liquid crystals, and some kind of backlight is utilized to illuminate them. It will give us the information of the load of the vehicle and about seat belt.

Figure 5: LCD

BUZZER: It is an electrical signaling tool that creates a buzzing noise and resembles a bell (Neeli Sreekeerthan1, 2020). A buzzer or beeper is a mechanical, electromechanical, or piezoelectric audio signaling device (piezo for short) (as shown in fig.6).

Buzzers and beepers are frequently used as alarm clocks, timers, train horns, and to validate human input such a mouse click or keyboard. Active buzzers have oscillators, which produce noise when powered.

Direct current is changed into pulse signals, typically at a specific frequency, to make it function.

PL= GVW-KW

Oscillators and DC signals aren't used by passive buzzers to generate sound, though. In this system, the buzzer will sound when there is an overload in the vehicle and if the seat belt is not worn.

Figure 6: Buzzer

II. METHODOLOGY

The load cell sensor is mounted on the car chassis and sends the entire load of the vehicle to Arduino Circuit (Arduino is an open source of electronic platform; it is able to read inputs). Arduino will show the total load in the display. Then, based on sensor data Arduino circuit will detect overweight in Electric vehicle. If overweight is detected in Electric vehicle then Arduino circuit will change the relay contacts. These relay contacts are connected to motor (Mr. Shardul Singh Gurjar, 2019). The relay will turn off the power of the motor and stop the motor from rotating. When we start our Electric vehicle, the load cell will detect the load first design of the system is shown in fig.5

GROSS WEIGHT OF VEHICLE**:** The Gross weight of a vehicle (GVW) is the weight of the empty vehicle plus the weight of the maximum payload that vehicle was designed to carry. Gross vehicle weight rating is what the maximum weight that the vehicle may safely carry including the vehicles chassis, body, Battery, driver, passengers, established by the manufacturer. A vehicles payload capacity is the maximum amount of weight it can safely carry. It calculates (Neeli Sreekeerthan1, 2020)

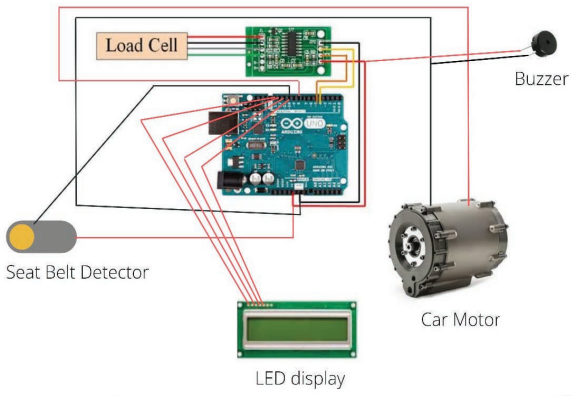

Figure 7: Design of the system

If a load is detected, the buzzer will sound for 5 seconds shown in fig. 5. After that the relay will not allow the motor to start until there is an additional load in vehicle. As a result of this the Electric vehicle will not start. As the same there will be a limit switch in the buckle of the seat belt. If the belt is not wearing, the

sensor will send a signal to Arduino circuit and the buzzer will sound for 5 seconds. The direct signal will go into Arduino if belt is not worn. In this condition the relay will not allow the motor to start until the seat belt is worn. As a result of this the Electric vehicle will not start. Both systems are connected to each other.

III. SETUP PHASES

PHASE: 1(Connection of Load Cell & amplifier)

This section provides a step-by-step breakdown of how the entire study project was put up (Neeli Sreekeerthan1, 2020). Once the parts have been corrected and connected, sample screenshots will be shown. Load cell is connected to the amplifier using jumper wire.

PHASE: 2(Arduino)

The software that we use here is Arduino IDE.

The main features are:

CODE AREA: You will enter all of your code here.

VERIFY: This enables you to translate your code into a format that the Arduino can understand. The info section will display any syntactic issues you made in your code.

UPLOAD: This accomplishes the same thing as verify but, if the code is successfully verified, sends your code to your Arduino.

INFO PANEL: This will display any issues that occurred during code compilation or uploading to your Arduino.

Programming of Arduino:

```
#include <HX711_ADC.h>
#include <Wire.h>
const int BUTTON_PIN = 7; // Arduino pin connected to button's pin
const int RELAY_PIN = 2; // Arduino pin connected to relay's pin
const int buzzer = 10;
int Relay = 9;
int val;
void setup () {
Serial.begin(9600);          // initialize serial
pinMode(BUTTON_PIN, INPUT_PULLUP); // set arduino pin to input pull-up mode
pinMode(RELAY_PIN, OUTPUT);      // set arduino pin to output mode
LoadCell.begin(); // start connection to HX711
LoadCell.start(2000); // load cells gets 2000ms of time to stabilize
LoadCell.setCalFactor(968.303); // calibration factor for load cell => strongly dependent on your individual setup
  pinMode(13, OUTPUT);
  pinMode(Relay, OUTPUT);   //
}
```

IV. RESULT AND DISCUSSION

We can monitor and control the overload and seat belt detection using this system as shown in fig 8).

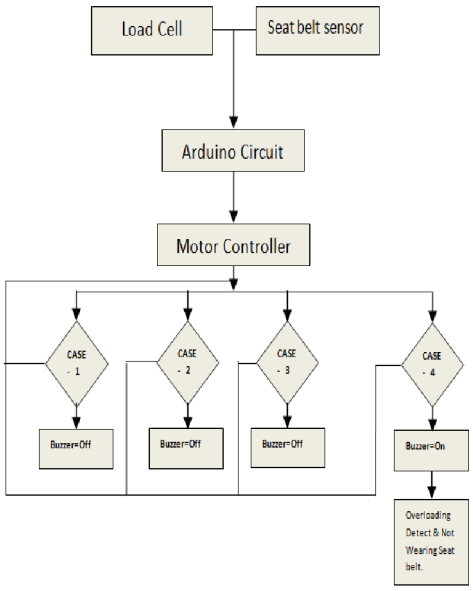

Figure 8: Flow Chart of Result

Case 1: The payload is around 90% to 95%. It can be considered as a Kerb weight of any vehicle. And Seat belts are worn. In this condition relay will allow to start the motor.

Case 2: The load is up to 95% of pay load. It can be considered as a safe load of any vehicle.

Case 3: The load is between 95% to 100% and Seat belts are worn. The load is between 95% to 100% and Seat belts are worn.

Case 4: Overloading Detected and seat belt not worn.

In this condition buzzer will sound for 5 second. And the display also shows that there is overload in vehicle and seat belt is not worn. In this case, the relay prevents the motor from starting.

V. CONCLUSION

Accidents resulting from overloading in electric vehicles will be prevented and this technology detect when a driver is not wearing a seatbelt while driving. With the help of this system also conductive to a reduction in serious damage to people's lives and property. The problem of traffic due to overloading will also be removed. The usage of this system in an arduino based Electric vehicle can improve the efficiency and effectiveness of electric vehicle life management. The primary goal of this essay is to lessen accidents caused by overloaded cars and trucks. We can control the ignition of the vehicle by connecting a load cell and microcontroller to it. Nowadays, the majority of automakers provide a reasonably inexpensive on-board weighing solution based on load sensors. Additionally, the sensor industry's solutions built on strain gauge sensor technology appear to have a bright future. As a result, this system is easy and convenient for determining Electric vehicle load and whether or not seat belts are worn, as well as successfully resolving the problem of overloading and driving without seat belts. These systems are anticipated to be used in new ways to police heavy vehicle and traffic laws. As a result, this technique makes it easy to understand the vehicle load and efficiently address the issue of vehicle overloading.

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A REVIEW: MICROBIAL DEGRADATION OF PLASTIC WASTE

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ABSTRACT

Environmental threats from the accumulation of plastic trash are getting worse. The demand for biodegradable plastics and the biodegradation of plastic waste have grown in significance over the past few years as a result of the excessive use of plastics and mounting strain on the capabilities available for the disposal of plastic waste. Utilizing microorganisms to biodegrade plastic is one method for minimising plastic pollution. The potential of bacteria that may degrade plastic as described by many researchers has been examined in the current review.

Keywords: Plastic wastes, Biological degradation, Methods of degradation study, Analysis of degradation, Biodegradable plastics.

INTRODUCTION:

Plastics are organic polymers. They are formed of carbon and hydrogen, together with numerous organic and inorganic compounds obtained from fossil fuels, as well as nitrogen, sulphur, and other components [1]. Bakelite, the first plastic to be made in bulk for commercial use, was created in the early 1900s [2]. These long chain polymers are well known for their distinctive qualities, including strength, malleability, low weight, simple manufacture, and low cost [3]. All areas of the economy now use plastics and plastic products. Plastics are frequently

employed in packaging, agriculture, architecture, construction, and medicine. Plastic is the mother industry to hundreds of components and products that are manufactured and used in our daily life [4]. Natural plastics, semi-synthetic plastics, synthetic plastics, thermoplastics, and thermosetting plastics are the different types of plastic [1]. Nylon, polycarbonate, polyethylene terephthalate, polyvinylidene chloride, urea formaldehyde, polyamides, polyethylene, polypropylene, polystyrene, polytetraflouro ethylene, polyurethane, and polyvinyl chloride are some of the plastics that are used in daily life [5]. Approximately 308,000 tonnes of plastic are used each year in India alone for food packaging. Around 140 million tonnes of synthetic polymers are manufactured each year, and the use of polyethylene is growing at a pace of 12% globally (MT). Due to the vast amount of polyethylene that is amassing in the environment, its disposal raises serious ecological concerns [6]. In India, waste management has grown to be a significant problem due to the country's rapidly urbanising population. Plastic garbage makes around 5% of the municipal waste produced [6]. By 2050, 33 billion tonnes of plastic garbage are anticipated to have been produced, assuming that the current patterns of rising plastic manufacturing continue [7]. According to estimates, just 10% of plastics are recycled globally and 14% are burned; the rest 76% end up in landfills or the environment [2]. The majority of waste plastics are not biodegradable and require thousands of years to break down or disintegrate. Reuse, reduce, and recycle strategies are increasingly often utilised to address the issue of plastic waste [1]. Land filling, incineration, and recycling are the methods used to dispose of the plastic trash [8]. Due to its practicality and affordability, land filling is the primary technique for disposing of plastic trash in most nations, especially developing ones, but the accumulating plastic debris has taken up a lot of space. The incineration of plastic garbage can lessen the requirement for landfill space and recover thermal energy, but it also has the potential to produce secondary pollutants that have an adverse impact on the environment, such as dioxins, carbon monoxide, nitrogen oxides, and others. Despite

the fact that mechanical recycling has taken over as the main recycling technique and is used to reuse thermoplastic wastes, the qualities of most recovered materials are considerably impaired after many processing cycles, and the resultant commercial values are thus constrained. Chemical recycling is an option that can recover the monomers and other compounds from plastic trash, but its effectiveness depends on how affordable the procedures are and how effective the catalysts are [9]. Utilizing biodegradable plastics might help to reduce waste to some extent; as a result, interest in degradable plastics is expanding [10]. The most efficient, environmentally benign, and socially acceptable approach for managing plastic is biodegradation [18]. Biodegradable plastics made it possible to rethink waste management tactics [8]. Biodegradable plastics are those that can be broken down by biological processes, primarily microbial ones [5]. Bacteria have the capacity to break down the majority of organic and inorganic materials, including lignin, starch, cellulose, and hemicelluloses, thus the biodegradable polymers are made to break down swiftly by the microbes [10]. Some of the biodegradable polymers (BPs) that have been created have already been released on the market. The biodegradable plastics poly (lactic acid), poly(-caprolactone), poly (butylene succinate), and poly (butylene succinate-co-butylene adipate) (PBSA) are used to make a variety of goods (bottles, containers) [13]. By allowing microorganisms in the environment to break down the molecules of plastic films, it is possible to biodegrade plastics in a way that is less hazardous to the environment and more harmless to humans. A change in a plastic's chemical structure that results in a harmful alteration in its qualities is known as degradation [4]. The enzymatic breakdown of the plastic provides nutrients to the bacterium. The energy and carbon needed for their growth and development are sourced from plastic garbage. It is an essential component of how the natural environment recycles resources [11]. Microbes connects to microbial surfaces to form colonies and begin the enzymatic breakdown process during biodegradation. The hydrolytic link in polymers is broken down by microbial enzymes, which then transform

the polymer into its simpler monomer, dimer, or trimer form. Again, it is degraded by microorganisms through aerobic and anaerobic metabolism, producing carbon dioxide, water, and methane as a by-product [12]. Esterases, lipases, and cutinases are a few enzymes that have the impressive capacity to hydrolyze polymers, including poly (ethylene adipate) (PEA) and poly (caprolactone) (PCL). Fungal species, including *Candida cylindracea, Rhizopus delemar, R. arrhizus, and Achromobacter sp.* are the sources of enzymes like esterases and lipases [3]. The rate of biodegradation decreases as the molecular weight of the polymers increases, and this relationship between biodegradation and molecular weight is direct [4]. Over 90 species of bacteria and fungi, including *Bacillus megaterium, Pseudomonas sp, Azotobacter sp, Ralstonia eutropha, Halomonas sp, D. nigrificans, and Penicillium simplicissimum,* are capable of degrading plastics [4]. Utilizing microbial strains created by selection, strain improvement, and genetic manipulation, the wide metabolic capacity of bacteria may be used for bioremediation of plastic wastes [4].

THE IMPACT OF MICROBES ON PLASTIC BIO-DEGRADATION

Different microorganisms break down various plastic groups, and their involvement in the breakdown of plastic is quite important. Studies published by various researchers are displayed in Table 1

TABLE 1: Microbial agents implicated in the breakdown of plastics

Microorganisms	Types of plastics	Source of the microbes	Degradation Efficiency	Reference
Pseudomonas putida	Milk cover	Garden soil	75.3%	[15]
Brevibaccillus borstelensis strain 707	Branched low-density polyethylene	Soil	11%	[16]
Streptomyces sp	LDPE	Garbage soil	46.7%	[17]
Micrococcus luteus	Plastic cup	Forest soil	38%	[18]
Bacillus cereus	Polyethylene	Dumpsite soil.	7.2-2.4%	[19]
Arthrobacter sp. and Pseudomonas sp	High-density polyethylene (HDPE)	dumped sites	12% and 15%	[20]
Aspergillus oryzae leads	High density polyethylene films	High density polyethylene (HDPE) film buried in soil	72%	[21]
Pseudomonas stutzeri	Low density polythene and polythene	soil from the plastic dumping site	73.38% reduction	[22]
Aspergillus versicolor and Aspergillus sp	LPDE in the powdered form	Sea water	4.1594 g/L	[23]
Masoniella sp	Plastic cup	Forest soil	27.4%	[24]
Phanerochaetechrysosporium and Pseudomonas aeruginosa	Polythene carry bags	Plastic dumping sites	50% and 35% respectively	[25]
Aspergillus niger and Streptococcus lactis	Polythene bags and plastic cups	Medicinal Garden soil, Sewage Water Soil, Energy Park soil, Sludge Area soil, Agricultural Soil	12.25% and 12.5% respectively	[26]
Serretiamarscence	Polythene carry bags	Polythene dumping site	22%	[27]
Bacillus amylolyticus	Garbage Soil	PE	32%	[28]

BIODEGRADATION OF PLASTIC

The term "biological degradation of plastics" refers to the process by which some microbes that use them to meet their energy needs create physiologically active enzymes that break down complex polymeric molecules into simpler ones. The three main stages of polymeric biodegradation are bio-deterioration, bio-fragmentation, and assimilation [3].

Bio-deterioration:

The first stage of polymers' biodegradation is bio-deterioration. Microbes attach to plastic polymers in the first phase, forming a thin biofilm on the plastic's surface. Next, they produce EPS, which catalyses the different mechanical degradation events. Under different temperature, drying, PH, and moisture content conditions, it affects the physical and chemical characteristics of polymers [14].

Bio-fragmentation:

It is a lytic process in which enzymes and free radicals are secreted by microorganisms when polymers' molecular bonds are broken, leading to the production of low molecular weight plastics such monomers, dimers, and oligomers. Another name for the procedure is depolymerisation. Lipase, proteinase K, pronase, hydrogenase, and other enzymes produced by microorganisms are used in the biodegradation of plastic [3].

Assimilation:

The assimilation stage refers to the process by which the monomers, dimers, and oligomers generated by the bio-fragmentation process are absorbed into the cells of microorganisms through the cellular membrane. Some of the broken molecules are recognised by cell

membrane receptors of microorganisms, allowing them to pass through the membrane and enter the cells. Cell membrane receptors can't recognise the plastic pieces, thus additional biotransformation events are needed to create the products that can be readily dispersed into the cell. The molecules that entered the cell go through further metabolic processes to produce adenosine triphosphate, fresh biomass, a variety of primary and secondary metabolites, and packing vesicles. Organic acids, aldehydes, terpenes, antibiotics, and other simple and complex metabolites are released into the extracellular environment during this process. Additionally, internal metabolites such as CO, N2, CH2, H2O, and other salts undergo full hydrolysis and reach the mineralization stage after being released into the environment. Microorganisms may mineralize monomers, dimers, and oligomers either aerobically or anaerobically [3].

Aerobic biodegradation

In order to convert monomer, dimer, and oligomer materials into simpler ones, aerobic bacteria use oxygen (O2) as an electron acceptor. The final products of the process are CO2, H2, and biomass, also known as aerobic respiration. [3].

Anaerobic biodegradation:

Anaerobic biodegradation is the term used to describe the breakdown of complex polymers into smaller units without the presence of oxygen by bacteria, with the production of CH2, CO2, H2O, and biomass as by products. In the absence of oxygen, anaerobic microbes use alternative sources as their electron acceptor for biodegradation, such as nitrate, sulphate, iron, manganese, and carbon dioxide [3].

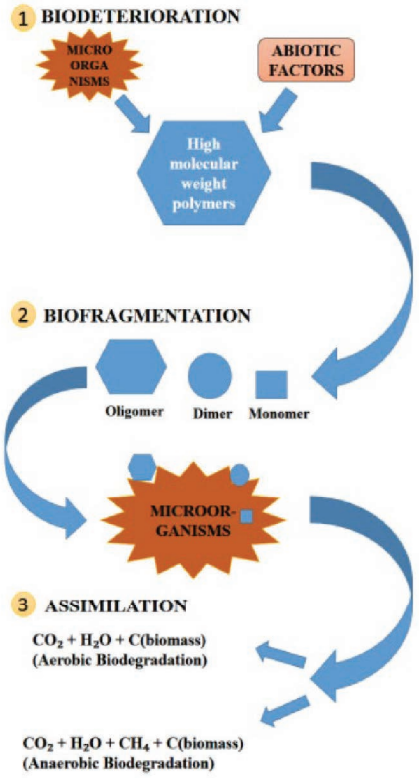

Figure: A- [3] Process of Biodegradation

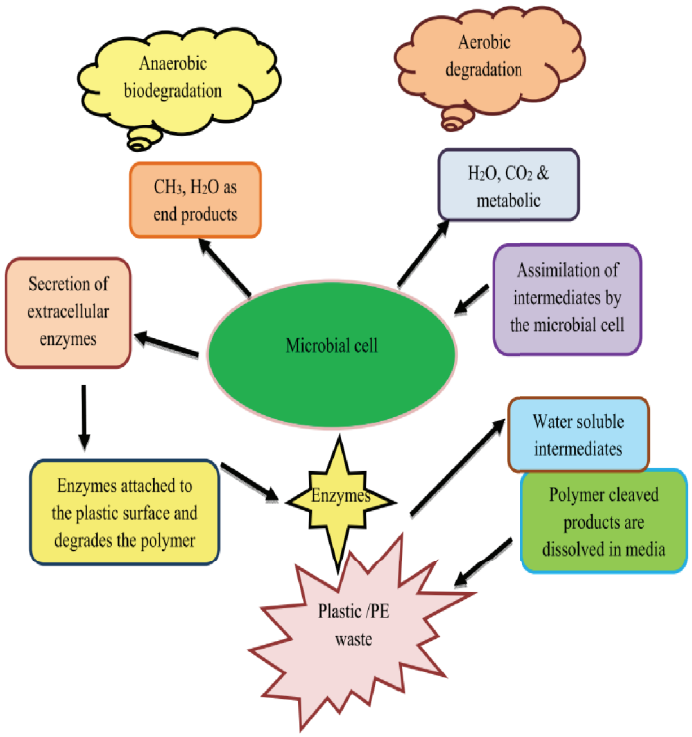

Figure: B- [14] Process of Aerobic & Anaerobic Degradation

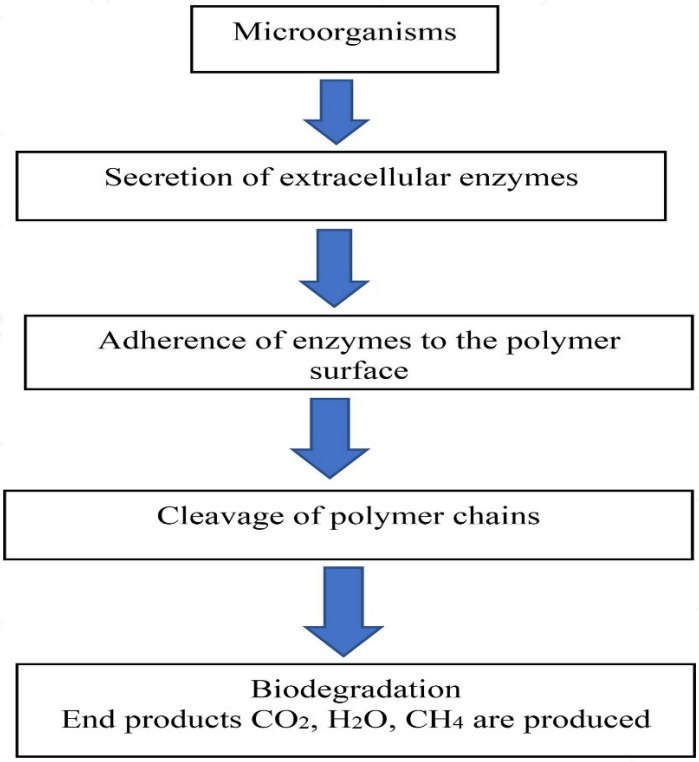

Figure: C- [45] Mechanism of enzymatic biodegradation of polymer.

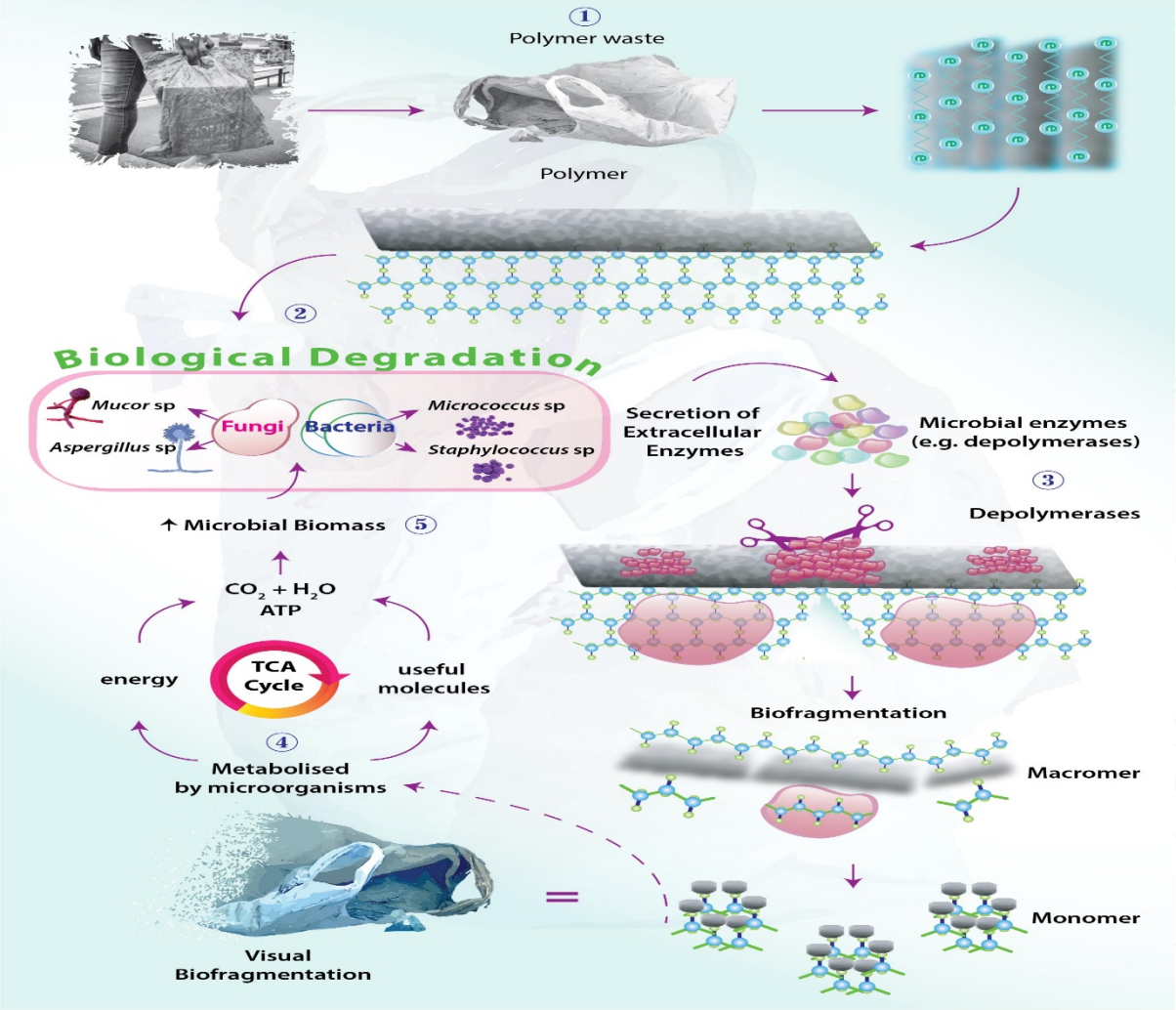

Figure: D- [45] Biodegradation of polyethylene.

THE ROLE OF ENZYMES IN THE BIODEGRADATION OF PLASTICS:

Numerous bacteria produce numerous kinds of crucial enzymes for the biodegradation of plastic. Potential catalysts for the decomposition of plastic component polymers include the enzymes laccase, lignin peroxidase, manganese peroxidase, lipase, esterase, and amylase. The polymer biodegradation process involves the reactions of hydrolysis and oxidation. Esters, carbonates, amides, and glycosidic linkages from different degraded polymers are hydrolyzed by hydrolase enzymes to generate monomers. Oxidizing and reducing processes of ethylene, carbonate, amide, urethane, and other substances are also catalysed by oxidoreductase enzymes [1].

FACTORS INFLUENCING THE BIODEGRADATION OF PLASTIC

1. **Water availability:** When there is moisture present, microbe-produced enzymes function well.
2. **PH value:** Hydrogen ion concentration influences how well enzymes function.
3. **Molecular weight and size of polymer:** The molecular weight affects the rate of breakdown. Polymers with a higher molecular weight and greater size are challenging to break.
4. **Temperature:** At high temperatures, degrading enzymes may get denaturated and lose their integrity.
5. **Enzyme characteristics**: Each enzyme is different from the others in terms of structure, and it has specific catalytic activity for microbial breakdown.
6. **Nature of plastic:** Due to their hydrophobic non-polar character, plastics derived from petrochemical sources are challenging to degrade.
7. **Morphological characters:** Degradation rate is influenced by polymer shape. Comparatively speaking, crystalline polymers are less likely to degrade than amorphous polymers
8. **Additives:** Chemical substances (dyes, fillers, etc.) added to polymers to catalyse biodegradation are known as biodegradable additives. Through the attraction of bacteria to polymers, it accelerates the pace of breakdown.
9. **Bio surfactants:** Chemical substances known as bio surfactants lower surface tension and alter the interfacial characteristics of polymers. It accelerates biodegradation when environmental circumstances are not suitable.

DETECTING MICROBIAL DETERIORATION OF PLASTICS USING THE MAIN APPROCHES

Parameter analysed	Method	Signs of biodegradation of plastic	References
Molecular weight	Nuclear magnetic resonance (NMR), gas chromatography/ mass spectrometry (GC–MS), gel permeation chromatography (GPC)	Decrease in the mean molecular weight	[29], [30], [31], [32], [33]
Microbial biomass	Analysis of protein (biomass) yield	Increased amounts of microbial protein in the presence of the polymer	[30]
Chemical composition	FTIR spectroscopy	Increase in the carbonyl index; formation of intermediates with additional functional groups(carbonyl/carboxyl) and alkanes; appearance of novel peaks corresponding to carbonyl and ether bonds	[30], [31], [32], [33], [35]
Hydrophobicity	Measuring the contact angle; BATH test	Decrease in the contact angle (increased hydrophilicity)	[37], [40]
Degree of crystallinity	Fourier-transform infrared (FTIR) spectroscopy	Increase in the degree of crystallinity due to preferential degradation of amorphous PE	[39], [40]
Residual mass of polymer	Gravimetry (weighing)	Decrease in the mass of plastic	[30], [31], [32], [33], [35]
Glass transition temperature	Differential scanning calorimetry (DSC)	Decrease in the glass transition temperature	[33], [41]
Tensile strength (TS) and extension at break (EAB)	Tensiometry	Decrease in TS and EAB (in the course of LDPE degradation	[32]
Features of plastic surfaces	Scanning electron microscopy (SEM), atomic force microscopy (AFM)	Changes in the structure of the surface: formation of micro flaws, holes, uneven relief	[30], [31], [32], [33], [35]

ADVANCES IN IMPROVED PLASTIC BIODEGRADATION EFFICIENCY:

In order to speed up deterioration, polymers are utilised in the form of thin films or powder since the rate of polymer breakdown by microorganisms is dependent on the size of the contact surface. Plastics may be subjected to several physical variables that alter their structure and increase their susceptibility to breakdown. Heat and UV exposure are the two most often used therapy modalities. Plastics' hydrophobicity, which makes it difficult for cells to adhere to plastic surfaces, considerably hinders microbial decomposition [7].

CONCLUSION

Plastics are used in the biodegradation of waste plastic. A beneficial plastic waste treatment that must be employed to maintain the environment's quality and address the issues brought on by plastic trash is bacteria degradation. The negative impacts of plastic pollution on living things must be made more widely known. The identification of more depolymerases produced by the microorganisms that break down plastic should be the focus of future research. Enzymatic degradation may be facilitated by the introduction of physical pre-treatment such mechanical grinding and beta-irradiations. To increase the activity and stability of depolymerases, which will assist the improvement of enzymatic degradation efficiency, methodologies of rational protein engineering and directed evolution are required. Recombinant DNA technology and genetic, molecular analysis for locating genes producing plastic-degrading enzymes can expedite and enhance plastic waste clean-up. It should be highlighted that just a small number of biological niches have been examined for the presence of microorganisms capable of breaking down plastic, and extending the range of habitats examined might likely lead to the isolation of novel target microorganisms.

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Observation and Analysis of Top Free Surface Features on $In_2Te_{3-X}Sb_X$ (x= 0.3) Single Crystal

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ABSTRACT:

The manufacture of ionising radiation detectors, solid-state electrodes, photosensitive heterostructures, solar cells, and ionic batteries depends on the III-VI compound semiconductors. In this work, $In_2Te_{3-X}Sb_X$ (x=0.3) single crystals were produced using the Bridgman technique at a growth rate of 0.35 cm/hr and a temperature gradient of 60 °C/cm. Under an optical microscope, numerous fascinating characteristics seen on the top free surface of a single crystal during growth were investigated. The investigation of potential growth processes implicated in the emergence of the defining growth features is covered.

INTRODUCTION

The compound In_2Te_3 is a member of the class of semiconductors having layered crystal structures. Ionizing radiation detectors, solid-state electrodes, and photosensitive heterostructures may all be made using In_2Te_3 single crystals [1-2]. Z.C. Medvedeva asserts that the hexagonal structure of the α-In_2Te_3 phase has the values a=4.0 Å and c=19.24 Å [2-3]. The layer-structured compound α- In_2Te_3 is regarded as an outstanding material for use in ionic batteries [3, 6] and solar cells [4, 5], and several investigations on its electrical and optical characteristics have been published [4-8]. In_2Te_3 single crystals with a stable phase may also be formed by adding impurities while the crystal is growing, as is known [1-2, 5,

9]. It is generally known that changeable band gaps, refractive indices, and lattice characteristics, which rely on the relative numbers of chalcogen atoms in the compound, may be achieved for pseudo-binary II-VI semiconductors [10]. For photovoltaicapplications, both elements are crucial, particularly when thin film heterostructures are takeninto account. We have been working with the $In_2(Se, Te)_3$ system since we need to tune bandgaps [4, 11].

Five atomic sheets with the Te-In-Te-In-Te structure make up each layer [1-2]. Strong covalent forces hold these sheets together, but the interaction between neighbouring layers is considerably weaker and is of the Vander Walls type [1-2, 4, 12]. One of the significant III-VI layered compounds, indium selenide (In_2Te_3), has a low density of 2 dangling bonds and is a promising material for the production of hetero junctions with a very low density of interface states [1-2, 4, 13]. As an example of a two-dimensional system,the III-VI semiconductors, which crystallise with a layered structure, have been thoroughly studied [1-2]. A compound semiconductor with the general formula A_2B_3 with a hexagonal structure is indium telluride (In_2Te_3) [1-2, 4, 14]. The differential resistance between the different phases of In_2Te_3 can be used to create an ultrahigh-density phase-change memory [4, 15, 16]. Additionally, it may be used in the production of rotating polarizer optoelectronic devices. The use of In_2Te_3 in solar cells [2, 17] and phase-change optical recording media [2,18-19] is another intriguing application.

EXPERIMENT:

A single crystal of $In_2Te_{3-X}Sb_X$ containing Antimony concentration at 10 atomic, was grown by the Bridgeman method. The top free surface features and the perfection of the crystal were judged using optical microscopy several research groups have investigated the use of antimony as a surfacing during the growth of III-VI compound semiconductor [1-2,

20-21]. In the present work, $In_2Te_{3-X}Sb_X$ Single crystal contains 10 % at. of Sb were grown from melt by Bridgeman method [20]. Many factors are involved in the technique for a successful result. The most important controlling factors are the purity of the constituent metals, the effect of premixing the constituent metals, the temperature gradient in the specimen and the furnace and the speed of growth of single crystals. Growth of homogeneous single crystal of $In_2Te_{3-X}Sb_X$ (x=0.3) presented a good deal of difficulties. Indium, Tellurium and Antimony presumably aid two dimensional growths and increase the thickness for the dimension [1-2, 20, 22-23].

MATERIALS AND METHODS:

Nuclear Fuel Complex in Hyderabad, India sold indium, tellurium, and antimony, all of which were 99.999% pure (5N purity). Using a semi-microbalance and a filled quartz ampoule that was about 10 cm long and 1 cm in diameter, the stoichiometric quantities of the substance were precisely weighed up to 10 micrograms [1-2, 24]. The quartz tube was retained within the alloy-mixing furnace and vacuum sealed at a pressure of around 10^{-5} torr [1-2]. To ensure homogenization and full reaction in the molten charge, the material in this mixing unit was kept in the molten state by rotating the tube at 10 rpm at 650 ^{0}C temperature for about 48 hours [1-2, 24]. After that, the tube's rotation was stopped, and the material was kept in the molten state for an additional 24 hours. After then, it was gradually brought to room temperature [1-2, 24]. Typically, a reasonably homogenous chemical was created by this method. The Bridgeman Method was used to grow the ingot once it had been so prepared [24].

RESULT & DISCUSSION:

The $In_2Te_{3-X}Sb_X$ (x=0.3) compound prepared as discussed above was used for growing single crystals by zone melting method [1-2, 24]. The starting ingot was about 3 to

4.5 cm in length and 0.8 to 1 cm in diameter [1-2]. The temperature profile of the zone-furnace is shown in figure-1. First the ingot was zone levelled. The temperature gradient across the two solid-liquid interface was obtained to be about 60 ^{0}C / cm by controlling the furnace temperature within ± 5 ^{0}C, giving a zone length of about 2-3 cm [25-32].

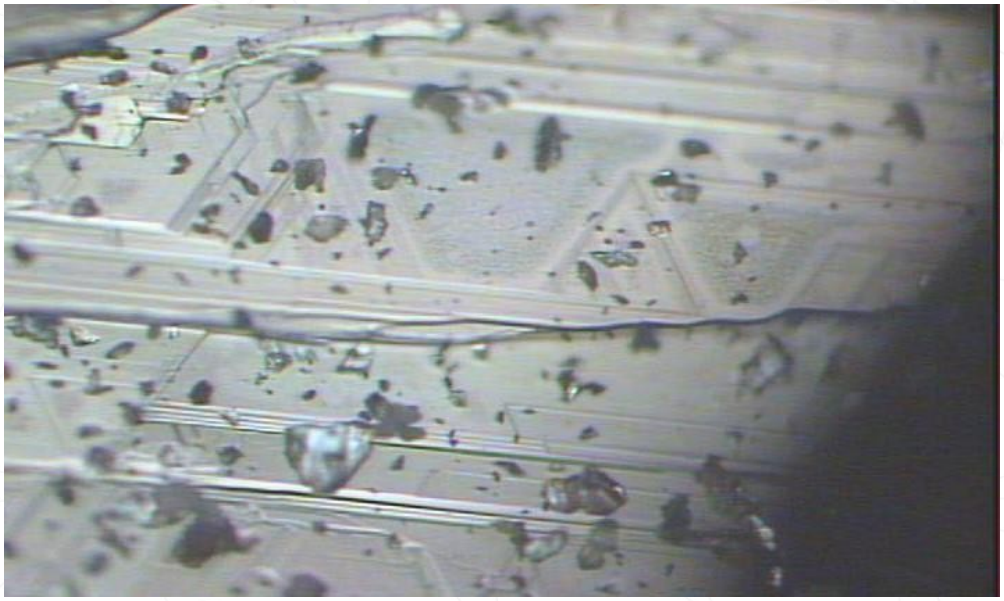

Figure- 1: Triangular growth of the top free surface of $In_2Te_{2.7}Sb_{0.3}$crystal

The typical growth feature in figure- 1 shows triangular growth feature observed on the top free surface of the samples. It is interesting to note that the edges of the triangular growth patterns are strictly parallel to one another [26-27]. It has been pointed out that the triangular features observed on the cleavage plane are the outcome of some phenomena and the pattern has a step like structure [26, 28].

Finding the indices of the planes responsible for the triangle pattern's edges and their orientation is now crucial. To establish the orientation of the triangle pattern's edges and the indices of the planes responsible for them, a stereographic approach based on Reed-Hill's [27-28] three trace analysis was used. It was discovered that the planes belonged to the {111} family and that the edges had the direction operations $[\bar{1}10]$, $[10\bar{1}]$ and $[01\bar{1}]$. The observed triangular features on the cleavage plane are due to traces resulting from the intersection of

three inclined faces of the tetrahedral pyramid, with the cleavage face. Bhide et. al. [28-31] have reported the surface studies of the dendritic growth of Germanium crystal. They have observed triangular growth features aligned along the central lines of dendrites and on the opposite faces of the platelet [28].

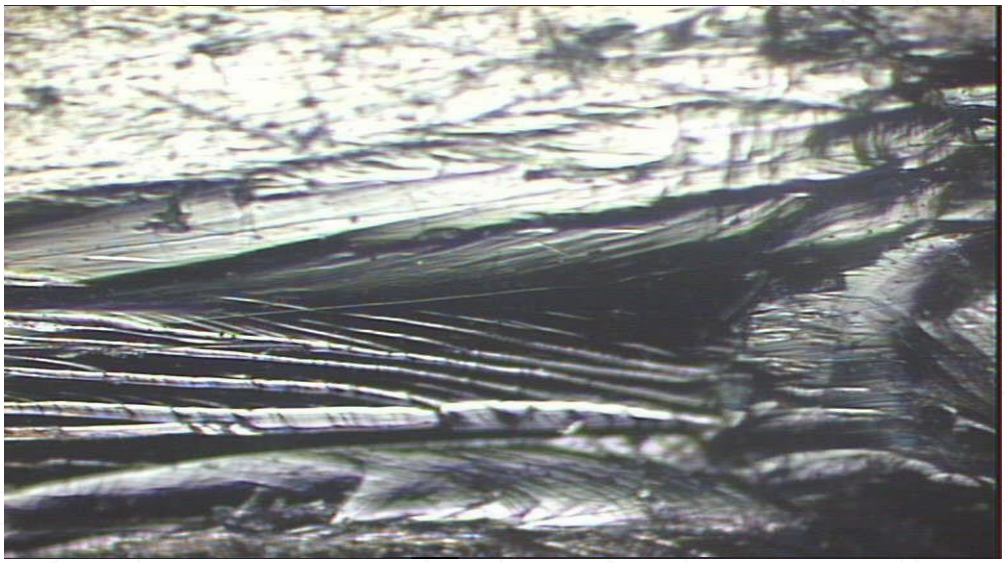

Figure. 2. Shows the top view of the as-grown free surface of $In_2Te_{2.7}Sb_{0.3}$crystal

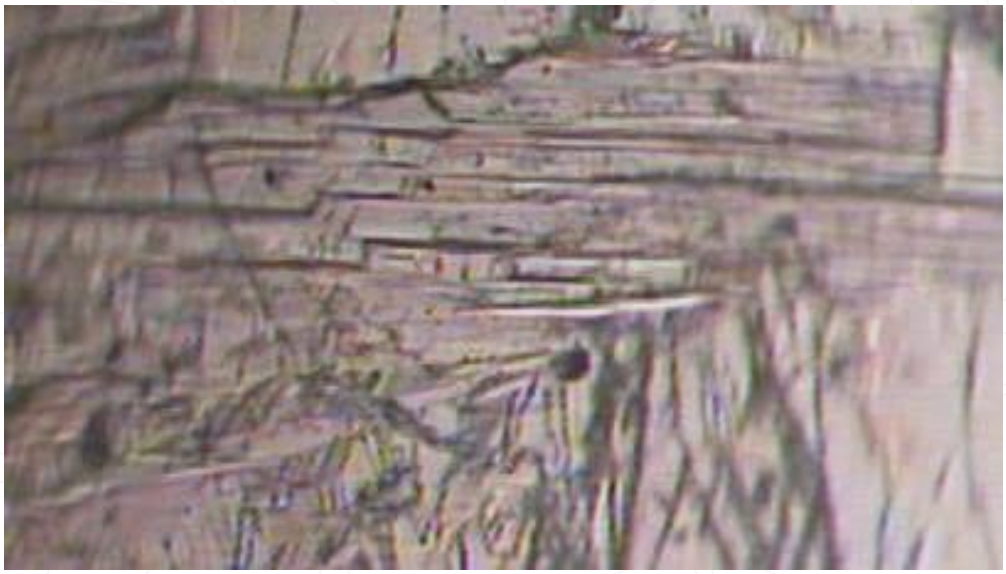

Fig- 3. Parallel Striation was observed on the top free surface of $In_2Te_{3-x}Sb_X$ (x= 0.3) crystals.

Typically, the crystal cleavage was easier in the central region than in the peripheral one. Fig. 2 is a photograph showing the peripheral region at a higher magnification [1-2, 28-32]. They are parallel and nearly equally spaced indicating crystallographic associations. In this regard, some crystallographic planes like {111} may be responsible for these features. This is because the layer growth mechanism has been reported to give rise to such striations (Desai et al) [33].

On the majority of crystals, parallel-line striations may be detected. These striations are growth layers that were created on the surface as a result of the microscopic growth rate fluctuating or the periodic incorporation of contaminants [4, 24, 27, 32]. According to Fig. 3, the parallel growth layers are spreading out and becoming rather thick at the crystal's top surface. Evidently below the development patterns are parallel striations to the primary axis [4]. It is conceivable that the growth pattern occurred during the period of growth where there was a plentiful supply of ions. When the reactant concentration is high, the growth layer's thickness rises [4].

Figure. – 4. Step Strain On {110} Crystal Consisting of Somewhat Higher Step Parallel to $[1\bar{1}0]$.

According to Pandya's 1973 Ph. D. thesis, transitory thermal currents created by temperature instabilities—which are often brought on by gradients, both radial and longitudinal—can be blamed for the striated morphology of the as-grown crystal surface [1-2, 4, 27, 33-37]. The radial gradients should then be precisely linked to the striations on a surface normal to the growth axis. There should be no radial temperature gradient, ideally [1-4]. The gradients produced by the ampoule and material combination, in addition to the properties of the furnace, cannot often be removed because of the huge disparity between their thermal conductivity. These characteristics were seen in all the crystals generated using the Bridgman method [4].

As shown in figure 4, reveals multiple steps on the {110} faces. The step's height isn't incredibly low, thus it's likely that a grouping of lesser stairs produced these steps. Only if the growing of {110} face of crystals occurs via a step flow process can the production of such large steps be comprehended. It was discovered that {110} potash alum is an F- face from a periodic bond chain (PBC) [32, 33, 38] analysis of several alums performed by Encvort [33, 36–39] because its growth layer contains the two interconnected PCBs < $\bar{1}$ 1 0> and < 001>.

The step orientations on the {110} crystal layer is predicted to be parallel to the two directions < $\bar{1}$ 1 0> and < 0 0 1> because, according to Harman in his research article from 1980, the morphological PCB theory on an F- face steps usually run parallel to periodic bond chains [40-41]. The core of the PBC has exclusively strong bonds, whereas the core of the PBC contains both lesser interactions and strong bonds. The PBC < $\bar{1}$ 1 0 > is stronger than the PBC < 001>, it may be inferred [4, 33-41].

CONCLUSION: -

- The intersection of two sets of family {111} planes result in the triangular characteristics seen on the crystal's top free surface. Due to the angle of the < 112> direction with the

cleavage plane, it has also been discovered that no more than three edges of the hexagonal type design may be seen on the cleavage plane.

- The top free surface of the crystal as it is developed has a shallow hillock, which shows that the layer growth mechanism predominates during crystal formation.
- These steps often come from triangular growth hillocks that are sometimes seen above inclusions under the crystal surfaces. It is clearly implied that these hillocks are growth spirals based on the relationship between the hillock centres and dislocation outcrops.

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SRJN

Halophilic Rhizobacteria: A Flexible and Eco-friendly tool for Saline Stress Tolerance in Plants

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Abstract

Increasing salt levels have generally been a problem for the economy and agriculture on a global scale. This abiotic stress has an impact on over 20% of the planet's geographical area. By changing plant processes, salt stress has a negative impact on the growth, development, and production of crops. To deal with such detrimental effects, several adaptation and mitigation techniques are needed. Few of the current techniques are long-term and financially unviable; therefore, a straightforward, inexpensive method that effectively reduces salt stress is urgently needed. Although salt-tolerant and/or resistant types have been bred and developed, they have only been effective as a short-term fix. PGPR is a group of bacteria that colonises the roots of different plants in a variety of ways and exhibits notable behaviours from the perspectives of biology and ecology. Salt tolerance and/or salt dependency traits are present in a large number of isolated microorganisms from the saline and sodic soil. Furthermore, these halophilic PGPR (microorganisms) are renowned for reducing biotic and abiotic stressors, as well as for positively promoting crop plant development and increased biomass synthesis. Elevated levels of K+/Na+ ratio, ACC deaminase production, specific antioxidants, compatible solutes, and reduced ethylene synthesis are a few of the primary mechanisms used by these bacteria, which ultimately confer tolerance to salt stress. Halophilic or salt-tolerant PGPR are thus important tools that can be used to recover the practise of saline irrigation. In order to achieve sustainable growth and higher yields from crop plants grown in salty locations, the current review emphasises the various aspects and mechanisms of halophilic PGPR.

Keywords: Halophilic, Plant growth, PGPR, Salt stress.

1.Introduction

The challenge of increasing crop output to feed the expanding population has existed from the beginning of agricultural operations. The current growth rate of agricultural production is insufficient to satisfy the anticipated food demand of 10 billion people in 2050, as emphasised in the 2018 Global Agricultural Productivity (GAP) Index (GAP Report, 2018). Crop productivity is also hampered by a number of abiotic variables, such as temperature, salinity, drought, pesticide and fertiliser application, soil pH, and heavy metal contamination (Ahmad, 2014). Salinization of arable land is one of these and is regarded as a serious threat to agricultural production. One of the nine significant risks to soil functions mentioned in the Food and Agriculture Organization's (FAO and ITPS, 2015) study "The status of the world's soil resources" is soil salinization. Salts are the single most detrimental element that restricts plant growth globally. One of the many ways that climate change, a current-day hot subject, has impacted Earth is the rapid emergence of saline landscapes, which ultimately contributes to global food insecurity and decreased agricultural production (Bharti et al., 2016).

Almost 20% of the irrigated land on the planet suffers serious damage from salt build-up (Selvakumar et al., 2014). By the year 2050, this land degradation could reach up to 50%. (Hossain, 2019). Due to soil salt contamination, cereal crops such as wheat, rice, maize, and barley have experienced yield losses of nearly 70% (Rajendran et al., 2009; Hussain et al., 2019). Global soil salinization is increasing, and over the past few years, the accelerated rate of salinization has led to food insecurity in a number of nations. Due to the salinization of coastal soil, the delta regions of India, Myanmar, and Bangladesh, which make up the majority of the world's rice supply, are seriously threatened in terms of food security (Abedin et al., 2014; Szabo et al., 2016). By using a variety of inorganic (gypsum, limestone, sulfuric acid and its derivatives, synthetic fertilisers), organic (green and farm yard manure, and industrial waste such press mud), and inorganic (fertilisers) techniques, such soils have so far been reclaimed (Qayyum et al., 2016). Similar to this, biotechnologists and plant breeders are always battling to create crop types that can tolerate salt, whether through natural selection, QTL mapping, marker-assisted selection, or genetic modification through the transfer of salt-tolerant genes from other animals. However, at the field level, a variety of issues have prevented such biological methods for agro-economically significant crops from producing satisfying results for stress tolerance increase (Khare et al., 2018).

Any kind of salt in excess is harmful to plant health. High salinity soils impede the physiological functions of plants. Significant soil activities like respiration, residue decomposition, nitrification, denitrification, soil biodiversity, and microbial activity are negatively impacted by the high salt concentration (Schirawski and Perlin, 2018). Where there is an excessive input of fertiliser into the soil, crop production is lost and the soil becomes highly salinized (Rütting et al., 2018). When high salt index fertilisers are used, an osmotic effect results, making it challenging to obtain the water needed for plant growth. Additionally, it has been noted that farming practises have an impact on how well crops grow in salt soils.

Recently, the use of root-adhering, plant growth-promoting rhizobacteria (PGPR) that live in hyper-saline environments has attracted interest as a different, environmentally benign biological strategy to improve crop output from salt-degrading soils (Talaat and Shawky,2015). These bacteria have been well-documented for promoting improved plant growth (Barnawal et al., 2012; Bharti et al., 2014). These salt-tolerant plant growth-promoting rhizobacteria use their primary mechanisms by colonising the rhizosphere of the plant to fend off harsh environmental challenges and the ensuing disastrous production penalties. Additionally, these microbes produce phytohormones, ACC deaminase, biological nitrogen fixation, siderophores, exopolysaccharides, volatile compounds, and antifungal or antibacterial metabolites. They also mobilise mineral ions, improve photosynthesis, and adjust the osmotic pressure by accumulating osmotically active metabolites like amino acids, sugars, polyols, and betaines (Talaat and Shawky, 2015; Shabani and Sabzalian, 2016). Therefore, these small animals help plants resist several biotic and abiotic obstacles by using a variety of direct and indirect techniques (Ilangumaran and Smith,2017). Recent studies have demonstrated that salt-tolerant plant growth-promoting rhizobacteria (ST-PGPR) can be used in saline agriculture to increase production and boost soil fertility (Grover et al., 2011).

The capacity of these organisms to develop osmo protectants, suitable solutes, and specialised transporters is related to their adaptive responses to salt stress. The bioinoculants ST-PGPR are now being utilised to increase crop yields, protect against phytopathogens, and enhance soil health. The goal of the current review is to use ST-PGPR to raise agricultural productivity in soil that has been impacted by salt. Targeted applications include boosting crop output through the use of bioinoculants. In this paper ,ST-PGPR mediated mechanisms are reviewed, providing fresh thoughts on how to increase agricultural productivity in the face of saline stress.

2. Impact of salt stress on plants

According to earlier studies, plants under salt stress exhibit signs of delayed development, limited growth of new branches, decreased plant height, lower germination rate, and wilted leaves. Through two separate processes, salt stress produces these outcomes. First, salt stress, also known as osmotic stress, causes plants to absorb less water, which inhibits plant growth. Then, if too many salty ions enter the plant's transpiration stream, they will harm plant cells by obstructing photosynthesis, deteriorating ion homeostasis, and oxidising membrane lipids, further reducing plant development and causing ion toxicity (Barba et al.,2011). In conclusion, understanding how plants respond physiologically to salt stress is essential for increasing plants' tolerance to salt(Figure:1).

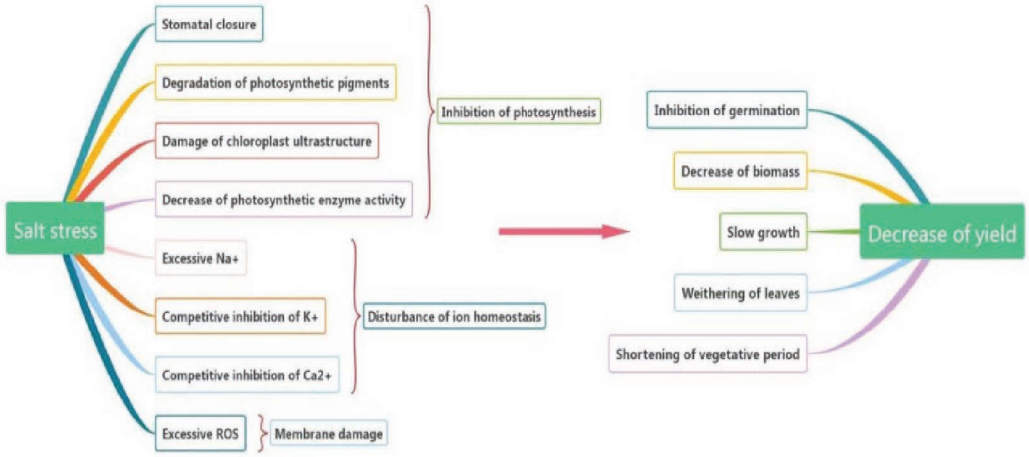

Figure:1 Depiction of plant salt injury (Hao et al.,2021)

2.1 Photosynthesis suppression

The primary source of the nutrients and energy required for plant growth and development is photosynthesis. According to research, salt stress has a considerable impact on plant photosynthetic rate, osmotic potential, water potential, transpiration rate, leaf temperature, and relative water content of plant leaves . Salt stress also has an impact on the elements of photosynthetic structures, including enzymes, photosynthetic pigments, thylakoid membrane proteins, and membrane lipids . Photosynthesis is negatively impacted by salt stress in two ways(Sudhir and Murty,2004). First off, plants stomas close when they are under salt stress, which lowers the level of CO_2 inside their cells. Second, salt stress also affects the photosynthetic membrane system, which results in decreased CO_2 absorption capability, impaired photosynthetic pigments, and other limitations that are unrelated to the original problem.

2.2 Ion homeostasis disruption

The elements of the structural substances of cells are typically mineral ions that are present in plant cells. As parts of enzymes and coenzymes, mineral elements control the activity of enzymes. In the electrochemical processes of osmotic control, colloidal stabilisation, and charge neutralisation, they also play a part. Maintaining the stability of the cell membrane and promoting plant growth and development depend heavily on the balance of ion metabolism. Inadequate levels of other essential ions, such as Ca2+ and K+, as a result of an excessive accumulation of Na+ and Cl, are the primary causes of salt damage. Na+ builds up in plants as a result of the high soil Na+ content(Flower and Colmer,2015).

High concentrations of Na+ lower membrane potential and encourage Cl absorption along a chemical gradient. Some enzymes and cell metabolism are harmed by too much Na^+. High Na^+ concentrations impair cell division and development by causing an osmotic imbalance, membrane malfunction, and increased ROS generation(Yang and Guo,2018) . In addition to impeding plant growth, a high concentration of Cl lowers the amount of chlorophyll present and damages the organelle and cell membrane structure(Munns and Tester,2008) .

2.3 Plant Salt Stress Indicators

Speaking about morphological markers, salt stress is commonly assessed using the weight of the shoot, root, and leaves, the length of the roots and shoots, and the diameter of the shoot(Li et al.,2017). The growth of plants under salt stress can be accurately represented by an indicator called biomass. Under salt stress, plant biomass generally declines, but the rate of decline varies amongst plants.

When it comes to physiological indicators, measuring the concentration of Na^+ or Cl^- in the leaves and roots indicates plants under salinity stress.

Transcriptome investigation of plants under salt stress revealed that some genes' expression altered in response to various salt concentrations. Plant salt stress can be predicted and confirmed using these genes as molecular indicators. Comparatively to morphological and physiological tests, which are less expensive and simpler to carry out, molecular indicators for stress evaluation are infrequently used in practical research. While many morphological and physiological markers, including as RWC, MDA concentration, and chlorophyll content, are activated by both salt stress and other stresses, many morphological markers, such as plant

height and shoot diameter, are only detectable after the plant has already suffered significant harm.

3. Salinity-Responsive Plant Strategies

Salt tolerance varies among plant species depending on the species, climate, type of soil, properties of the soil, and agricultural practises. Halophytes and glycophytes are two categories into which plants are categorised based on their adaptive adaptation against salinity. Glycophytes, which comprise the majority of plant species, cannot survive salinity (Shrivastav and Kumar,2015). The biosynthesis of osmoprotectants, the activation of antioxidant enzymes and the synthesis of antioxidant compounds, ion homeostasis, ion transport and uptake, the synthesis of polyamines and nitric oxide, and hormone modulation are just a few physiological and biochemical strategies that plants have evolved to survive in saline soils(Gupta and Huang,2014).

3.1 Osmoprotective agents

Through constant water inflow, compatible osmolytes safeguard the cell's structure and maintain osmotic balance. These osmolytes, or suitable solutes, include sulfonium compounds, sugars, sugar alcohols, and derivatives of amino acids. The most significant osmoprotectants include glycine betaine, sugars, proline, and polyols(Yokoi et al.,2002). Soluble carbohydrates build up in plant tissues as a reaction to the stressors of salt and drought, resulting in osmoprotection, osmotic adjustment, carbon storage, and radical scavenging. One disaccharide that accumulates in plants under various abiotic stressors and prevents apoptotic cell death is trehalose(Yamada et al.,2003).Intracellular proline, an osmoprotectant, also serves as an organic nitrogen reserve during stress recovery. A subclass of polyols known as sugar alcohols function as complementary osmolytes and ROS-scavenging substances. During times of stress, the cell is protected, and enzymes or membrane structures are stabilised by them.

3.2 Antioxidants

Reactive oxygen species (ROS) are produced and build up in plants as a result of both biotic and abiotic stressors. ROS are powerful compound oxidizers that pose a risk to the integrity of cells (Groß et al.,2013). When dealing with ROS brought on by salt and other stressors, antioxidant enzymes and nonenzymatic substances are crucial. Antioxidant enzyme activity is positively connected with salt tolerance and includes superoxide dismutase (SOD), catalase (CA), ascorbate peroxidase (APX), glutathione peroxidase (GPX), glutathione reductase (GR),

and polyphenol oxidase (PPO). It has been shown that several nonenzymatic antioxidants, including the vitamins C and E, carotenoids, and lipoic acid, can shield plants from oxidative stress(Kojo,2004).

3.3 Polyamines

In the kingdom of plants, polyamines are tiny, polycationic, aliphatic molecules with low molecular weight. The most prevalent polyamines discovered in plants are putrescine, spermidine, and spermine.The advantageous effect maintaining membrane integrity, lowering ROS production, managing the buildup of Na^+ and Cl^- ions in various organs, and regulating gene expression for osmolyte synthesis are all functions of polyamine (Roychoudhury et al.,2011)

3.4. Regulation of Hormones

Abscisic acid (ABA) is a phytohormone that is thought to reduce the negative effects of stress on plants. Under osmotic stress, ABA expression is increased in plants. Salinity results in osmotic stress and a water deficit, which induces the production of ABA to rise in shoots and roots(Cabot et al.,2009). A crucial physiological signal called ABA controls the expression of numerous genes that respond to osmotic and salt stress (Fukuda and Tanaka,2006). Abiotic stressors in plants also cause them to respond with salicylic acid (SA) and brassinosteroids (BR). Under salt stress, rice seedlings produced more endogenous SA and showed an increase in the activity of SA biosynthetic enzymes (Sawada et al.,2006). Plants with exogenous SA and BR treatment have improved salt tolerance(Ashraf et al.,2010)

4. PGPR Enhance Salt Tolerance in Plants

Kloepper and Schroth (Kloepper and Schroth, 1981) coined the term "plant growth promoting rhizobacteria (PGPR)" for these advantageous microorganisms, opening the door for more research on PGPR. Plant growth-promoting rhizobacteria (PGPR) are well known to improve plant growth and disease reduction by several mechanisms such as the production of plant growth regulators, secondary metabolites, and nitrogen fixation, enhancement of the availability of different mineral nutrients, decomposition of organic matter, and induction of resistance in plants against various pathogens (Valencia-Cantero et al.,2007). Salinity reduces the microbial diversity of soils and affects microbial activities such as soil respiration and soil enzyme activity. However, extremophilic microorganisms have specific adaption strategies to

live in saline soils. Several studies revealed that PGPR could enhance salt tolerance in plants via ion homeostasis, the production of antioxidants, ACC deaminase, phytohormones, extracellular polymeric substance (EPS), volatile organic compounds, the accumulation of osmolytes, the activation of plant antioxidative enzymes, and the improvement of nutrient uptake (Egamberdieva et al.,2019)(Figure:2).

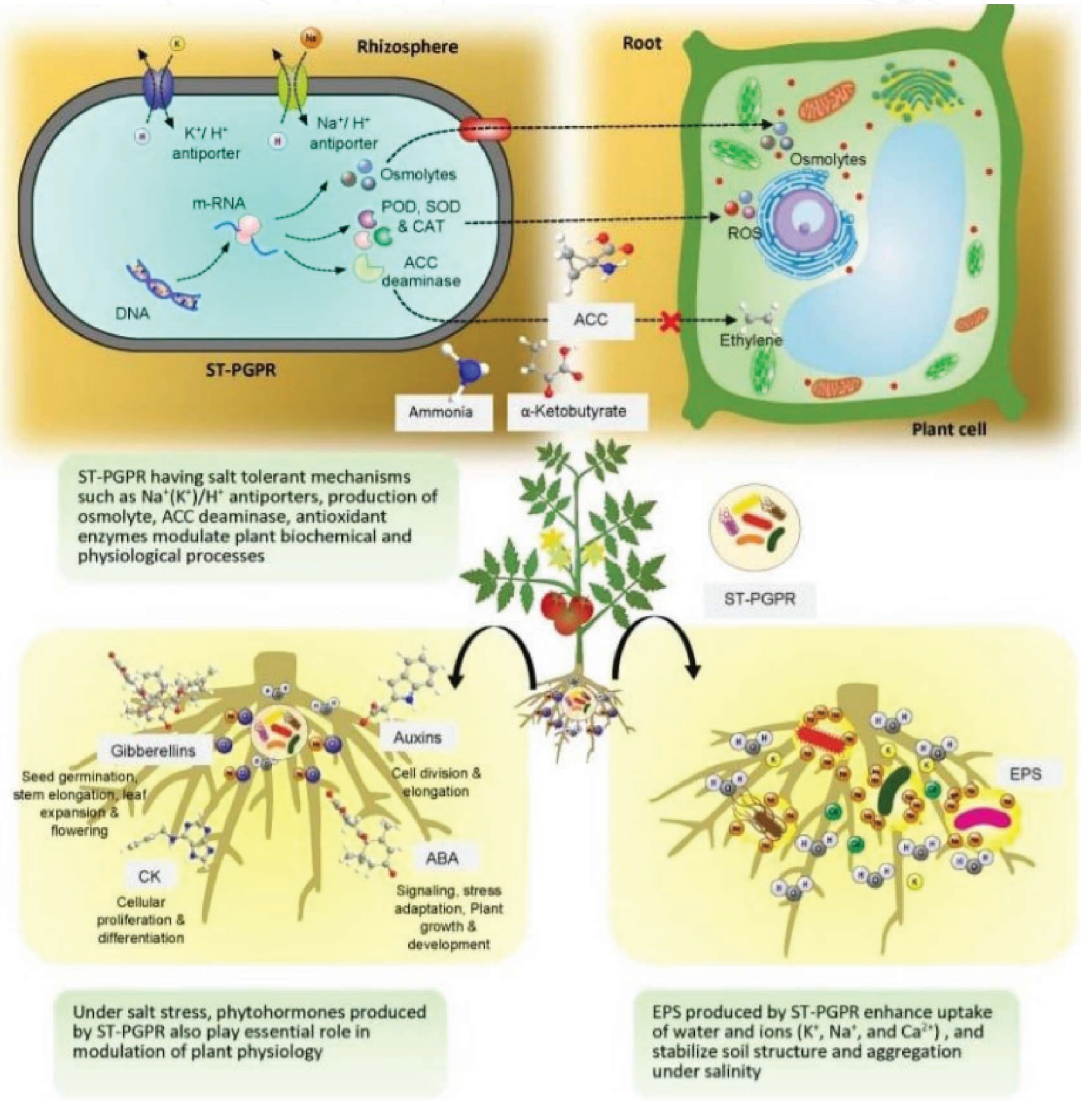

Figure:2 Mitigation of Salt stress by ST-PGPR (Egamberdieva et al.,2019)

4.1. Enhancing Nutrient Uptake and Solubilization

Under salinity stress, a plant's ability to absorb nutrients such as potassium, phosphorus, and nitrogen is reduced. The improvement of nutrient absorption and solubilization is a well-known property of PGPRs (Jaiswal et al.,2016). According to studies, both symbiotic and nonsymbiotic methods used by PGPR boost plants' uptake of nitrogen(Santi et al.,2013). The

well-known example of the symbiotic relationship for the biological fixation of N_2 in plants is *Rhizobium* sp. The establishment of nodules on the roots of legumes in saline soils is successfully accomplished by rhizobium, and the growth of legumes under stress circumstances is enhanced(Sobti et al.,2015). There are other nitrogen-fixing bacteria that are not plant-specific, including *Azospirillum, Azotobacter, Burkholderia, Bacillus*, and *Paenibacillus* (Goswami et al.,2015).

Another crucial macronutrient is phosphorus (P), which is absorbed by plant roots in the forms of H_2PO_4 or HPO_4. P is generally unavailable to plants under natural circumstances due to its precipitation in soil. Under salt circumstances, halotolerant PGPR can turn unavailable phosphorous into available phosphorus by the mechanisms of acidification, ion exchange, and chelation(Choudhary and D. K. ,2012; Etesami and Beattie,2018). In addition to helping grains fill up and enhancing plant tolerance to biotic and abiotic stressors, potassium (K) is crucial for plant metabolisms(Sindhu et al.,2010). It stands to reason that most of the potassium in soil is not immediately accessible to plants. Additionally, this element is less readily available under salt stress.

Even in salt-stressed soils, K-solubilizing bacteria can increase potassium solubilization(Saghafi, et al.,2019). Other nutritional elements including Fe, Cu, Zn, and Mn can be made more available to plants by PGPR(Etesami et al.,2014).

In order to compete with pathogens for iron in the environment, PGPR uses the siderophore synthesis process. Small compounds known as siderophores have a strong affinity for Fe^{3+}. Inducing disease resistance in plants and promoting plant development are the roles of siderophores. Siderophores are produced by several PGPRs, including *Pseudomonas* species, and *Bacillus* species (Aznar and Dellagi,2015). Due to the suppression of proton pumps, salinity decreases the availability of Fe. In numerous plant models, siderophore-producing bacteria have been demonstrated to improve salt tolerance(Kavamura et al.,2013; Ramadoss et al.,2013).

4.2 ACC Deaminase Production

Plants naturally produce the growth hormone ethylene. Plants are more likely to produce ethylene when they are under stress, including salt, which is bad for plant growth. Some PGPR are capable of producing the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which controls the formation of ethylene. Therefore, in salinity circumstances, PGPR can reduce the negative consequences of elevated ethylene levels.Gupta and its colleagues reported

the role of halotolerant bacteria in stress tolerance in *Pisum sativum* L.(Gupta et al.,2021). This is reported that the stress mitigation in *Oryza sativa* L. by application of ACC deaminase producing bacteria (Choudhory et al.,2022).

4.3 IAA production

The primary auxin that stimulates plant growth is indole-3-acetic acid (IAA), which also plays a role in the development of roots, seed germination, and plant stress tolerance. Halotolerant bacteria mitigate the effects of salinity stress on soybean by application of IAA producing ST-PGPR was well documented by Khan and team (Khan et al.,2021). Under salt stress, PGPR regulates other phytohormones such cytokinins, gibberellins, and abscisic acid (ABA). By accumulating suitable solutes and cations, such as Ca^{2+} and K^{+}, in the root vacuoles, ABA reduces the consequences of excessive salinity

4.4 EPS production

In their natural environment, bacteria make extracellular polymeric substances (EPS).Salinity stress has been said to be reduced by EPS-producing PGPB(**Ashraf et al.,2014)**. In fact, the majority of bacteria produce EPS to survive in stressful environments. EPS consists of biopolymers such polyester, polyamides, and polysaccharides. Bacteria commonly develop biofilms when under salt stress. EPS keeps the biofilm's structural integrity intact(Zhang et al.,2011). Additionally, EPS contributes to the development of plant-microbe interactions. Additionally, EPS contributes to the development of plant-microbe interactions. By lowering the Na+ level that is accessible for plant uptake, EPS mitigates the negative consequences of salt stress (**Upadhyay et al.,2011)**. Plant growth and soil aggregation under salt stress improved as a result of the salt-tolerant *Halomonas* variable and *Planococcus rifietoensis* forming a biofilm.

4.5 Additional Mechanisms

The PGPR produces volatile organic compounds (VOCs), which promote plant development and control plant responses to stress. Low-molecular-weight substances like ketones, aldehydes, alcohols, and hydrocarbons make up VOCs. Plants are better able to withstand abiotic stressors thanks to the multiple chemical and physical changes that VOCs released by PGPR can promote . *Paraburkholderia phytofirmans* produced 2-undecanone, 7-hexanol, and 3-methylbutanol, which improved plant development and resistance to salinity stress(**Mantelin**

and Touraine,2004). Plants can be encouraged to tolerate salt by other substances produced by PGPR, such as enzymatic and nonenzymatic antioxidants, bacteriocins, osmolytes, including polyamines, sugars, sugar alcohols, betaines, and amino acids(**Kumar et al.,2020)**.

5. Potential outcomes

Although great work has been made in understanding how saline affects plants, there has been limited success in managing productivity losses in a sustainable way. A saline soil's crop output can be increased by utilising the potential of ST-PGPR. Pan and colleagues claim that the physiological functions performed by ST-PGPR could enhance plant performance in saline environments(Pan et al.,2019). It has been recognised that understanding the mechanisms of osmo-adaptation in ST-PGPR may help to achieve the long-term objective of increasing crop productivity in saline agro-ecosystems (Paul and Lade ,2013). The understanding of ST-PGPR's tolerance mechanisms is still lacking, especially in regards to the role of bacterial genes in osmotic control and plant-microbe interactions (under saline conditions). However, some halophilic bacteria have had their molecular processes for salt tolerance examined in recent years. This showed that, in addition to other processes, bacterial salt-related antiporters like Na^+/H^+ play specialised roles in plants' ability to tolerate salinity. According to previous study , knowing the regulatory networks that ST-PGPR uses to induce salt tolerance in plants could be a useful strategy for reducing salt stress and enhancing global food production(Ma et al.,2019). To effectively utilise microbial reactions to soil salinity, more research on this topic is necessary.

6.Conclusion

Globally, agricultural crop productivity suffers significantly from salinity stress. Although breeding methods have been employed to increase salt tolerance and production in plants, these methods are not cost-effective and have occasionally failed to pass on salt tolerance to the target species. Complex metabolic and physiological networks are involved in the adaptation of plants to salinity stress. PGPR utilises a variety of methods to assist plants in overcoming the negative effects of salt stress. In order to increase crop yield and salt tolerance, it is crucial to use effective microbial agent formulations as biofertilizers. This is a crucial step toward sustainable agriculture. It is possible to raise the production of agricultural goods and the quantity and quality of organic food by commercialising bacterial agents in microcapsules and providing them to farmers. Further research is required to understand how the PGPR bacteriainteract with soil microflora, complicated environmental conditions, and their ability to survive and tolerate salt in soils.

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Basic aspects of textile dye effluent decolorization by microorganism- a review

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ABSTRACT

Dyes are coloured compounds that, when applied to textiles, provide a permanent colour that is resistant to fading from perspiration, light, water, and a variety of chemicals. Only natural sources were used to create dyes in the past, those produced from natural sources such as plant leaves, roots, and bark. However, as requirements and expectations grew, businesses increasingly reliant on colours made from petrochemicals, or synthetic dyes. They are classed in the colour index based on the chemical structure of their chromophoric group, colour, and application technique. The chromogenic chromophore is a collection of atoms found in dye compounds that are responsible for the colour. Textile dyes are categorised by their chemical structure (azo dyes, nitro dyes, indigo dyes, anthraquinone dyes, and so on) or their industrial applicability. Environmental issues associated with the textile industry are with the water contamination produced by the discharge of untreated wastewater into the environment and it impacts visibility inside the receiving waters, reducing the amount of light available to aquatic plants and animals. Existing dye removal procedures may be divided into three categories: biological, chemical, and physical therapies.

KEY WORDS: Decolourization, Dye, Textile effluent, Waste water, bacteria

INTRODUCTION

Synthetic colors are utilized broadly in the textile industries, a large portion of which are quickly dissolvable in water. In most textile coloring activities, as much as 15% of the colour utilized doesn't interface with the textiles, so they are lost as wastewater [1]. The subsequent effluent presents water contamination issues because of the harmful content. Before 1856, dyes were prepared from natural sources such as flowers, vegetables, woods, root, insects, etc. However, with the increasing needs and demands, industries became dependent on dyes manufactured from petrochemicals, i.e., synthetic dyes. These dyes are soluble in water, easily absorbed, and very fast in coloration as compared to the natural dyes and provide a large versatility in colors. In the current picture, the worldwide production of dyes is nearly 800,000 tons per year. A large amount of dyes produced is used in textile industries. Unfortunately, incomplete exhaustion of dyes onto textile fiber from an aqueous dyeing process leads to a major fraction of dyestuff being released with the wastewater. The released wastewater contaminates water and soil, resulting in a considerable amount of environmental pollution [1, 2]. Issues related with effluent is incorporation of heavy metals into water bodies, an increase in biochemical oxygen interest (BOD), compound oxygen interest (COD), pH and suspended solids [1]. Moreover, the presence of synthetic colors in wastewater makes the water smell obnoxiously, and less adequate as well as the natural light absorption to the water also decreases which influencing photosynthesis and whole oceanic biological systems. Likewise, a large portion of the colors that are delivered into wastewater, including their breakdown items, are poisonous, cancer-causing, mutagenic and teratogenic to people and other life structures [1, 2]. A textile organization in Ghana manufactures and trades African textiles both locally and globally. The manufacture of these textiles includes the utilization of many synthetic

colors and high amount of water, creating a great deal of color wastewater [3]. Textile industries has a treatment plant that treats the wastewater in three distinct stages; natural treatment (utilization of microorganisms benefiting from just the cellulosic materials in the proceeding), ultrafiltration and Reverse Osmosis (RO). Nonetheless, 70% clear water is acquired and reused into the handling plant after conclusive treatment by RO. The leftover30% effluent is treated by physicochemical strategies like adsorption, electrocoagulation, flocculation, particle separation, filtration and ozonation for the decolorization of color containing wastewater. Notwithstanding, these strategies are costly and produce a lot of emission after treatment which requires safe removal [4,5]. Then again, organic treatment techniques are viewed as more appropriate and largely utilized because of their cost feasibility, less sludge producing, and its eco-accommodating nature.

Even in the absence of toxic component in effluent, people in general assume it as pollutant die to the presence of color in the water. When the -C=C- bond, – N=N-bonds, heterocyclic and aromatic rings present in organic color polluted effluents cleaves, the color of the effluent decreases. The absorption of light by the associated atoms shifts from the noticeable to the visible to infrared range and then to electromagnetic range. There are around 12 classes of chromogenic groups of molecule; the most common is azo type and then anthraquinone dye. Azo type dye makes up to 60-70% of textile and tannery dyestuff produced. A dye house effluent ordinarily contains 0.6-0.8 g/L of dyes in which azo type and anthraquinone are mostly used. Consequently, dye and textile effluent treatment has been the interest of area for the research purpose. Wastewater treatment by physical, chemical and biological or the combination of these strategies is already used for the dye color treatment [5].

Many Physical and chemical purification shown in Fig. 1 are used for the treatment of dye containing waste water but due to the high costs their use is limited in high scale process and pollution profile of the effluent. On the other hand, biological methods listed in Fig. 1 are currently observed as specific, energy economic, effective and environmentally friendly as it converts partial or complete bioconversion of organic pollutant to stable and non-hazardous end products. Many bacterial, fungal and algal species have the ability to convert azo dyes using adsorption or degradation process by either aerobic or anaerobic pathway. Isolation of azo dye decolorizing bacteria has been done from soil, water, human and animal excreta and even from contaminated food materials.

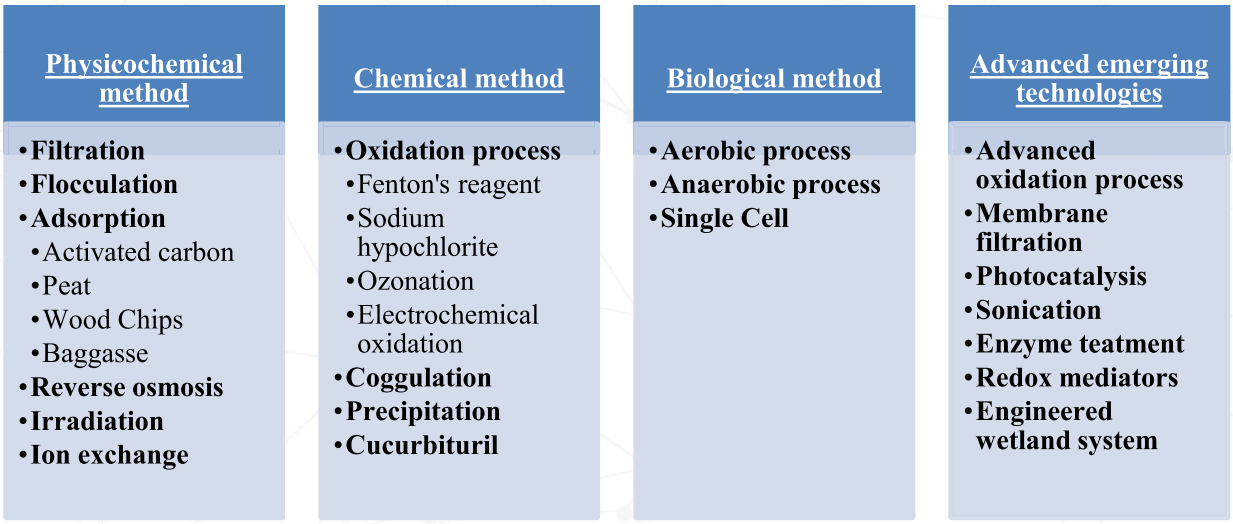


Fig. 1 Different treatment methods for dye containing effluent

However, coloured effluents arising from dye manufacturing and textile industries could be the potential ecological niches for isolating such bacteria. In this review various methods for the treatment of textile effluent containing various classes of dyes, and describe the importance of biological (mainly bacterial) methods are explored.

DYES AND THEIR STRUCTURE

Dyed effluent from industries have the range of 10 and 200 mg/L of dye and a combination of

other natural and inorganic synthetic compounds and added substances. The deterioration of colour compound depends upon the complexity of the dye structures. Based on their molecule compound, they can be acid dyes, basic dyes, azo dyes, sulphur dyes, pigment dyes, disperse dyes and so forth around (Table 1).

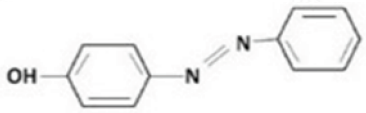
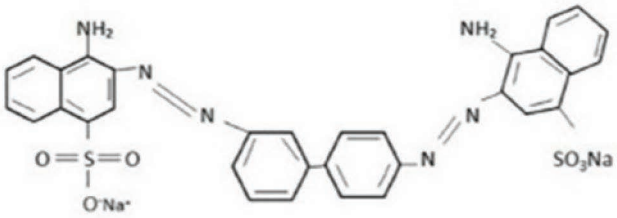
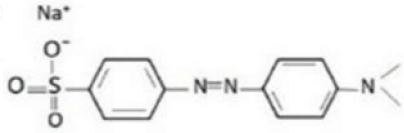
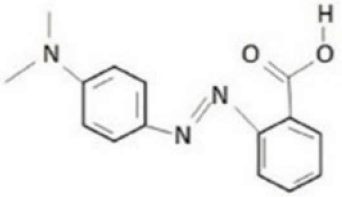
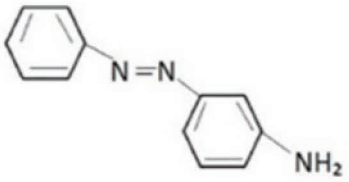
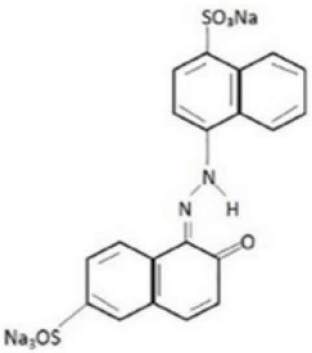
Table 1: Types of dyes

Dye Class	Category	Applications	Properties
Direct dyes	Contains sulphonic acid group, however, these are not the point of attachment.	Cotton, rayon, Paper, leather,and some nylon	Water soluble and anionic dyes
Vat dyes	Like sulphur dyes, however, used inreduced form after treatment with reducing agents	Cotton cellulosic fibers and for rayon and wool	Water insoluble
Basicdyes	Basic amino group protonated under acidic condition, formation of salt linkages	Paper, polyacrylonitrile, modified nylons, modified polyesters, cation dyeable polyethylene terephthalate,and medicine	Water soluble
Reactive dyes	Forms covalent bond with fiberspossessing hydroxyl or amino groups, e.g., dyes with chlorine atom	Cotton and other cellulosic fibersand some extent wool and nylonfibers	Water soluble
Solvent dyes	No water solubilizing group, soluble in organic solvent	Plastics, gasoline, lubricants, oils, and waxes	Solvent soluble, while water insoluble and nonpolar or little polar
Acid dyes	Sodium salts of color acids that contain sulphonic acid or phenolic group	Nylon, wool,silk, modifiedacrylics, paper,leather, ink-jetprinting, and food	Water soluble

Mordant dyes	Contains group that can hold metal in chelate groups or coordination complexes	Cotton and rayon	Water soluble
Disperse dyes	Water insoluble dyes, small, and contain hydroxyl or amino group to give finitewater solubility at definite temperature	Polyester and some amount nylon, cellulose,cellulose acetate,and acrylic fibers	Water insoluble and nonionicdyes

In textile industries 70% of the azo dyes have been used. Azo dyes contain diazotized amine combined with an amine or phenol and at least one azo groups (- N=N-). They are economically savvy and simple to utilize, which make them the most famous manufactured dye. Azo dyes are subdivided into two classes: toxic and nontoxic dyes. Toxic dyes prompt the free and N-acetylated amino groups in dyes that structure the RNA/DNA nitrenium ties, accordingly making the dye genotoxic. These dyes are for the mostly hydrophobic (taken up through bacterial cell and decreased in the cell and causes cell and natural effects), cancer-causing, mutagenic, and lipophilic. Non-toxic azo dyes are non-cancer-causing due to the presence of alkyl, arylamines, and the expansion of carboxyl and sulpho groups. Table 2 showing the basic structure of azo dye and types of azo dyes.

Table 2: Azo dyes and their Structure

Azo dyes	Structure
Basic Structure of Azo Dye	
Congo Red	
Methyl orange	
Methyl Red	
Acid Red	 

BIOLOGICAL TREATMENT OF TEXTILE EFFLUENT

Bioremediation is one of the most important areas of environmental science. In this method microbes are acclimatized with toxic material and develop new resistant strain which would be capable to biodegrade toxic chemicals to less toxic form. The development of new strain occurs by the actions of biotransformation enzymes. There are two processes for the biological treatment 1) biosoption and 2) biodegradation. In biosoption, the dye structure remains intact while in biodegradation the structure has been degraded, destroyed and pollutant split into fragments and forms CO_2, biomass and inorganic compounds. While in biosoption the pollutant entrapped into the matrix of the microbial biomass, then the disposal of these biomass makes problem for complete biocleaning of the effluent. This this method is not practical approach for cleaning of industrial effluents. The effectiveness of microbial decolorization depends on the adaptability of the selected microorganisms. In adsorption method, growing/living microbial cells or dead microbial cells could be used. Adsorption method is more useful when the toxicity of the effluent is very high. And biomass adsorption is more favorable when it is difficult to grow the culture and maintain the microbial population. Variety of microorganism have been investigated for biosorption studies these organism termed as biosorbents include bacteria, microalgae and fungi because the cellulosic composition of the microbial cell wall provides binding sites such as hydroxyl and carboxyl groups. The use of bacterial for biodegradation of dyes began in the 1970s with reports of *Bacillus subterraneus* followed by *Aeromonas*, *Bacillus subtilis* and *hydrophilia Cereus, Klebsiella pneumoniae*. The ability of *Acinetobacterniae* RS-13 and *Acetobacter liquefaciens* S-1 to decolorize methyl red has been demonstrated [6]. Table 3 showing the microorganism used in the bio decolorization of different dyes.

Table 3: Microorganism involved in decolorization of dyes

Type of organism	Organism name	Dye name	Conditions	% Decolorization	Reference
Bacteria	*Pseudomonas sp.*	Reactive Blue 13; (200 mg/L)	pH: 7.0, Temperature: 35 ^{0}C, Condition : Aerobic static Incubation time: 70	83.2	[7]
	Enterobacter EC3	Reactive Black 5; (1000 mg/ L)	pH: 7.0, Temperature: 37 ^{0}C, Condition: Anaerobic, Incubation time:36	92.56	[8]
	Mutant Bacillus sp. ACT2	Congo Red; (3,000 mg/ L)	pH: 7.0, Temperature: 37 ^{0}C, Condition : Aerobic static Incubation time: 37–48	12–30	[9]
	Citrobacter sp. CK3	Recative Red 180; (200 mg/L)	pH: 7.0, Temperature: 32 ^{0}C, Condition : Aenarobic Incubation time: 36	96	[10]
	Desulfovibrio desulfuricans	Rective Orange 96 and Reactive Red (120 mg/L)	pH : acidic Temperature: 28 ^{0}C, Condition : Aenarobic Incubation time: 2 h	95	[11]
Fungi	*Aspergillus flavus*	Malachite green; (18.25 mg/L)	Incubation time: 6 days	97.43	[12]
	Alternaria solani	Malachite green: (18.25 mg/L)	Incubation time: 6 days	96.91	[12]
	Pycnoporus sanguineus	Trypan blue; (20 mg/L)	Incubation time: 24 h	70	[13]
Algae	*Scenedesmus bijugatus*	Tartrazine (5 mg/L)	Incubation time: 6 day	68	[14]
	Cosmarium sp.	Malachite green (10 mg/L)	Incubation time: 24 h	87.1	[15]
	Phormidium ceylanicum FF	Sky Blue; (100 mg/L)	Incubation time: 26 days	80	[16]
Yeast	*Candida krusei*	Reactive brilliant red K-2BP; (50 mg/L)	Incubation time: 24 h	98	[17]
	Pseudozyma rugulosa	Reactive brilliant red; (mg/L)	Incubation time: 24 h	99	[17]

Mixed cultures are especially beneficial, as certain cultures are more adaptable than others; so, Microbial consortia can work together to solve problems. Perform biodegradation activities that nosingle pure strain can satisfactorily complete. Efforts to find pure in the 1970s, bacterial cultures capable of degrading azo dyes were developed. *Bacillus subtilis, Aeromonas hydrophilia*, and other bacteria were discovered in the 1970s. The mechanisms of biodegradation in details are studied by the analytical methods of biochemistry and molecular biology, and this knowledge might be used to control the enzyme system and make changed products. A variety of analytical techniques, including Fourier transform infrared spectroscopy, UV–Vis spectroscopy, infrared spectroscopy (FTIR), gas chromatography mass spectrometry (GC/MS) have been used to determine the oxidoreductive analysis of culture to decolorize the dye. According to biochemistry and molecular biology investigators, this information could be used to manage the enzyme system in order to create modified strains with increased enzyme activity.

FACTORS AFFECTING BACTERIAL DECOLORIZATION

Temperature:

Environmental temperature directly determines the metabolic and enzymatic activity of the microorganism. As a result, temperature is a critical element for the study. All mechanisms related to microbial viability rely on it. Furthermore, Temperature has also been reported to play a role in microbial physiology. Temperature influences on growth rate, biomass yield, and reaction mechanism have also been documented. The decolorization of the dye increases as temperature of rises until it reaches the ideal temperature, and then it drops. This reduction of dye decolorization could be due to the cell viability decrease with high temperatures and also the denaturation of azo-reductase enzyme. The enzyme azoreductase is relatively thermostable

and can function at temperatures as high as 40°C [18]. The maximum rate of color removal is mostly related to the optimum cell culture growth temperature, with an increase of decolorization proportional to the increase of temperature within the optimum temperature range.

pH :

When it comes to decolourization, the medium pH is also crucial. The pH level has a significant impact on dye efficiency. The optimal pH for color removal in bacteria is mostly around 6.0-10.0. While fungi and yeast reported better decolorization and biodegradation activities at acidic or neutral pH. At the appropriate pH, the rate of colour removal is faster, and when the pH is too acidicor too alkaline, it tends to drop quickly [19]. It is considered that pH has something to do with dye transfer through the cell membrane, which is referred to as the rate-limiting step. Generally, changing the pH between 7.0 and 9.5 has little impact on the dye degradation process. The pH tolerance of decolorizing bacteria is critical, since it allows them to thrive in a variety of environments. Table 3 showing the dye decolorization activities at different pH by microorganisms.

Dye Structure and concentration:

Dye Color removal is faster for dyes with simpler structures and low molecular weights. The nature of aromatic ring substituents has been demonstrated to have an effect on the oxidation process. Numerous studies have shown that methyl and methoxy substituents that donate electrons improve the decolorization. The dyes containing the chloro, fluoro, and nitro substitutes inhibit the growth of bacteria. According to a study, by increasing the concentration of dye the decolorization of the dye decreases. This could be because dyes have a harmful effect on the microorganisms. Inadequate cell-to-dye ratio, as well as active site blockage of the

azo reductase enxyme by dye also affects the effectively of decolorization [20].

Carbon and nitrogen sources supplements:

Azo dyes lack carbon sources, and thus without adding any supplement it is difficult to grow the culture on it. Yeast extract, peptone, or a mixture of these complex organic substances used for the growth of microorganism which could decolorize the azo dye. Glucose is most readily available carbon source which increases the rate of decolorization easily. Addition of carbon source does not always increases the rate of decolorization but incorporation of complex nitrogen source seems to increase the rate of decolorization. Because it regenerate NADH, which acts as an electron donor for the reduction of azo dyes by microorganisms, and thus effective decolorization could be achieved. The dye absorbs carbon from a variety of sources. It had also been reported the conversion of soluble substrates, such as carbohydrates, to volatile organic acids or alcohols, such as acetic acid and methanol, which serve as competitive substratesfor methanogenic, sulfate-reducing, and other bacteria [21].

Oxygen and agitation:

Environmental conditions can affect the azo dyes degradation and decolorization process directly. Depending on the reductive or oxidative status of the environment and indirectly, influencing the microbial metabolism and pathways of the dye degradation. Anaerobic conditions facilitate the activity of the reductive enzyme but still a small amount of oxygen is also required for the oxidative enzymes which are involved in the degradation of azo dyes [22].

Electron donor:

The azo dyes and the other organic content of textile wastewater have not any substrate for the

growth of anaerobic bacteria and thus for complete decolorization is not possible. Therefore it is necessary to have an external substrate (electron-donor) supply which could enhance the aerobic decolorization performance. It has been perceived that the electron donors, such as glucose or acetate ions incorporation to the treatment process apparently induce the reductive cleavage of azo bonds. The type and accessibility of electron donors are significant in achieving decolorization in bioreactors worked under anaerobic conditions.

FUTURE PERSPECTIVES

Biodegradation of synthetic dyes using microorganism (fungi ,bacteria, yeasts, and algae) is becoming a favorable approach for the dealing with dye wastewaters. The biodegradation abilities of microorganisms can be enhanced by increasing the adaptability of the microorganism by exposing them to higher concentrations of synthetic organic compounds. [23]. Microorganisms exposed to higher levels of contaminants develop and evolve new pathways for degradation of the same. This occurs through expression of genes encoding for enzymes responsible for degradation using r-DNA technology Alternatively, identification, isolation, and transfer of genes encoding for enzymes responsible for the degradation of the recalcitrant compounds can greatly help in designing microbes with improved degradation abilities. Thus, acclimatization and genetic engineering both can be helpful in designing super-degraders microorganisms. Acclimatization is natural process while in genetic engineering the microorganismis changed by incorporating new genes. Therefore, environmentalists are skeptic about using genetically modified organisms as it will create new environmental problems.

CONCLUSION:

Dyes effluent creates not only ecological and environmental pollution, but also medical problems. Regulations are becoming even stricter so, there is an crucial requirement for

technically possible and cost effective management methods. Different physical and chemical methods have been employed for the treatment of synthetic dyes wastewaters. These methods have their own limitations, like high cost, low effectiveness, limited flexibility, and production of sludge, etc. In contrast, bioremediation is a cost-effective, efficient, bio friendly, and environmentally gentle method for removal of dyes from industrial effluent. The literature reviewed in this paper indicates that a physicochemical parameters influence the decolorization performance, optimization of these is very much essential.

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21st Century and Cutting Edge Technology for Front-End Web-Development (React & Angular): A Programmer Approach

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ABSTRACT

We are living in a planet where several processes of our work have been automated and still it is going on. One of the channels to automate the process is web application and the role of front end developer are very important. The standard of the web designing and requirement of new features are increasing and it is expected from the developer to implement the same in very short time. So, developer should use the framework endorsed by the developers across the world instead of reinvent the wheel. Angular and React is one of the best open source framework for front end web development. It's crucial to pick the right frontend development framework or library like angular and react while creating scalable online applications. The research work explain the depth about React and Angular, its comparison, limitations and its real time usage that is useful in business. The researcher has found that presently for building dynamic apps for mobile, web, and other platforms, React & andgular framework is a fantastic tool, which is utilized by around 10.2 million websites globally. Component is a complete function which include template, style, logic function [1].This study will outline the benefits and drawbacks of both framework and library according to different criteria by assessing the research data on many areas.

Keywords: Virtual DOM, JavaScript, React, Library, Angular, Web Application Development, Frontend Development

INTRODUCTION

One of the most distinguishing aspects of an organization is the user experience. It has a significant impact on visitors as well as on SEO. So, it's necessary to make responsive and user-friendly web application by using various front-end technology and framework. The framework helps to develop such applications in effective manner in terms of easy & fast coding, understanding on in-built features & functionalities. Angular and React is open source framework and very popular among front end developers. Its only help to develop application in fast track but also it is very useful to integrate with API of different applications.

Objectives

The objectives of the paper are as follows:

1. Understanding on React & Angular
2. Identification of parameters to choose the React and Angular
3. Assessment on the difference and limitations between React and Angular

Web Technologies: React JS & Angular

The field of web development is always changing. The technologies that became the most well-liked in the development sector are react, angular, vue, and many more. AngularJS, was originally made public in 2010 and is built on JavaScript, one of the most widely used programming languages. The ability to transform HTML texts into dynamic content was Agular's standout feature at the time of its debut, which laid the road for its further growth and success. In 2016, six years later, Angular had a complete overhaul. Typescript, a relatively new programming language, was used by Google to fully rewrite the Angular framework.

We have seen the React technology evolve steadily over the last couple of years with frequent upgrades. Among JavaScript frameworks, React is the most well-liked and frequently hailed as the finest. Jordan Walke, a Facebook programmer, finally developed React.js in 2013, which completely altered how people see web development moving forward.

A JavaScript component library called ReactJS is used to build user interfaces for websites and software. A library is a collection of already written code that may be used to create a product. A JavaScript package called React JS is used in website building to create interactive components. React gives developers access to a vast array of pre-existing solutions. With the aid of React, programmers may build substantial online apps that can modify data without refreshing the page. Reacts primary goals are to be quick, scalable, and easy to use. Angular is a Typescript-based open-source JavaScript framework Because In order to create a usable package that a browser can comprehend, Angular created a framework based on typescript that can generate HTML, CSS, and JavaScript code with the aid of transpires**.** As developers spend more time developing the UI, the developers will not spend a lot of time devoting themselves to implementing the function and logic [5].

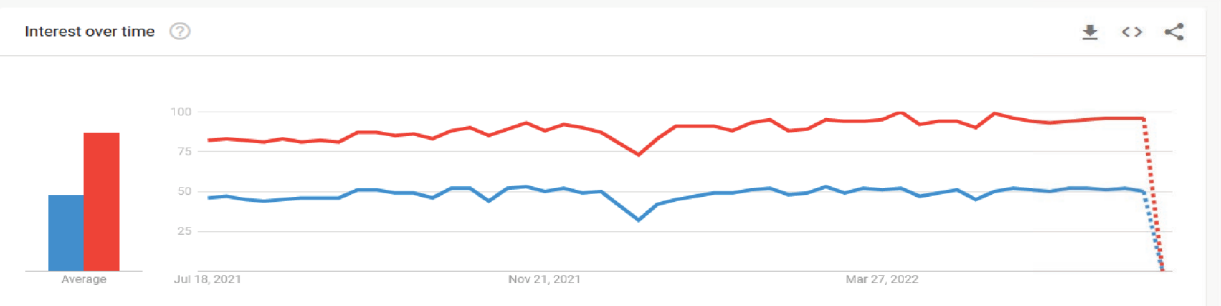
Review Literature

In 2021 Yongkang Xing , JiaPeng Huang and YongYao Lai had listed the pros and cons of framework and library .They analyze the react , angular and vue in different aspects [6]. For massive commercial applications, especially in e-Business, Angular has the most complete capabilities and functionalities. Live streaming, communication, blogging, and small- to medium-sized apps are all ideal for React and Vue.In 2017 Naimul Islam Naim wrote a thesis on ReactJS: An Open Source JavaScript Library for Front-end Development and conclude that ReactJS is proven as the fastest rendering library. With large amount of data it has been successful for

delivering a good user experience. Also he stated that ReactJs is less complicated and fast rendering library. The main goal of a single page application is to reconstruct the Web application around the Web2.0 page interaction principle, so that all the operations are done on a page, all of which is controlled by JS [2]. In 2020 Vinuta Hutagikar and Vinay Hegde shed light on advantage and disadvantage on different frameworks. Moreover , they comprises the frameworks and According to the findings, the three frameworks like angular , react and vue are appropriate for SPA development based on the aims and viability of the application.

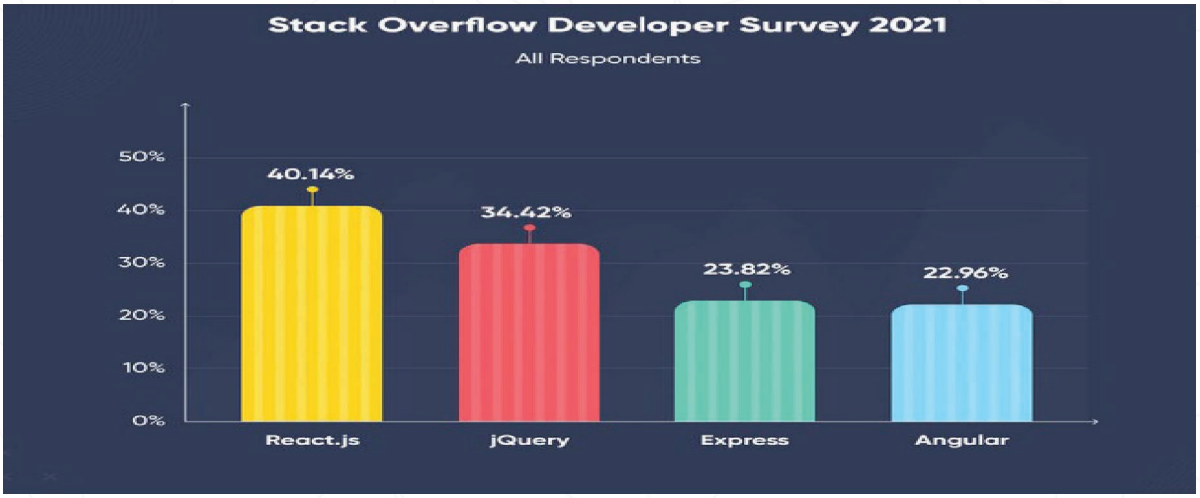
DIVERGENCE BETWEEN ANGULAR AND REACT

A single comparison structure for web programming languages cannot be found or defined. As a result, the researcher presents many approaches and points of view to illustrate various comparison techniques.



Interest over time (World Wide) Red: React, Blue: Angular

Compare Breakdown by region



Stack Overflow Developer Survey 2021

Languages	**React**	**Angular**
Structure	JavaScript Library	Full Fledged JS Framework
Size of Library (Package Size)	Small	Big
Execution Time	Fast	Slow
DOM Implementation	Virtual	Real
Rendering	Fast	Slow
Version Migration	Simple	Complex
Compatibility	Full Backward Compatibility	Updates Required
Data Flow	Unidirectional	Two-way Data Binding
Basic Prerequisite	JavaScript	Typescript
Development	Not Constant	Constant
Client	Facebook, Instagram, Twitter, Airbnb, Netflix, PayPal, Uber.	Gmail ,Google Cloud Platforms, AdWords, Forbes, YouTube, Wix, telegram

Bundle size	Small	Big
Page loading speed	Fast	Slow
UI Components Flexibility	More	Less (Restricted to the in built options)
Git-hub Popularity	42.62%	20.39%
Version	16.8.4	7.0
Packaging	Strong	Medium
Technical support	Persistent Technical Service	Persistent Technical Service but API is not durable.
Dependencies	Managed Automatically	Need to install Third party tools
Popularity	More	Less compared to React

React is used to create UI components for any project with rapidly changing data, but Angular is mostly used to create complicated enterprise-grade apps like single-page apps and progressive web apps.Every six months, Angular releases one significant upgrade with a six-month depreciation period. The distinct packaged contributions make up the entire framework. Due to the constant development process, the update method is simple yet cumbersome. React-based scripts may be simply updated to leverage newer APIs.The Facebook UI serves as a good example of how reliable the APIs are.

Contrary to Angular, migration is simple and updates are seamless. The primary releases offer quick libraries yet are independent and reliable. For its applications, Angular employs a genuine DOM tree that is produced by the web browser. Real DOM is perfect for a single page that occasionally gets modified. The size of the library is another factor that makes Angular-based apps slow. For instance, Up Work is one of the most popular apps that uses Angular since there is less need for frequent updates to the user feed. React makes use of a virtual DOM and is ideal for web pages that frequently need content modifications. The library is more dynamic and light weight due to its trivial scope. For instance, Instagram uses react as it necessitates frequent updates to the user feed. By executing the code it has been observed that more powerful, more testable, and more

scalable are reactive forms that are built around observable streams. They are more predictable because they are synchronous. Gulp is an automatic task running tool based on Nodejs [4].

PARAMETERS FOR ANGULAR AND REACT FOR FRONTEND DEVELOPMENT

Parameter (Application Point)	React (Application)	Angular (Application)
Choose between Angular and React	• Suitable for unique application with a high level of customization , a lot of dynamic, data-intensive, and constantly changing content • Good at choosing among the Best packages • Application that require versatility , require best SEO and need high speed	• Suitable of enterprise-grade application with functionalities like progressive, single-page dynamic, and native web apps • More Suitable for Mobile App Development

It also depends on details of the project, the developers' present skill set, and budget.

Learner Criteria	React	Angular
Student Learning	• Easy learning curve • Resource and tutorials available	• Difficult learning curve • No clear manual documentation and Community support

Devloper Criteria	React	Angular
Prerequisites for React an angular development	• Good at choosing among the Best packages	• Require knowledge of Typescript

	• The programming team is at ease with JavaScript and HTML.	• If staff is conversant with Java and C# as both of these languages share many similarities with Typescript

Migrating between Reacts versions is quite easy. Because of its virtual DOM implementation and rendering optimizations, React is superior to Angular. Jquery—a fast and simple JavaScript framework, which has many features including the chain grammar and highly efficient CSS selector, flexible animation system and event system, rich plugins and the solutions on browser compatibility issues [3]. Building single-page web apps, animated mobile apps, progressive web apps that operate offline, business apps, e-commerce websites, and more is made easy with Angular. Building Angular-based front-ends for small-scale apps increases complexity. React, on the other hand, works well for complex front-end apps that emphasize an interactive user interface (e.g., Netflix or Uber).Strong developer collaboration is necessary to master a large-scale application created with React [7].

React is best suited when as the app scheme grows, components are anticipated to be shared across several applications. And The process of developing a product involves a countless number of elements with various and regularly changing states, including dynamic inputs, active or inactive navigation items, buttons that may be enabled or deactivated, and user login and access rights.

LIMITATION

React web applications that allows attackers to trick users into taking accidental actions without their knowledge is CSRF. Although it operates against the will of the genuine user, it does not take their identity. Limitations of Angular are limited SEO options and their low performance.

DISCUSSION AND CONCLSION

For startups, small enterprises, and midmarket corporations, React and Angular provide utterly unique methods to web application development. Both technologies are strong and adaptable, yet neither is superior to the other in any way. Also in this fast upgrading technical world There are several open source platforms available for creating web apps.ReactJS is superior to Angular when comparing the two frameworks since it is difficult to find the time to learn a new one given how frequently the front-end industry changes [8]. On the contrary, While Angular is alone capable of handling multiple things on its own without depending on any additional help, so, it may seem tricky at first. However, the benefits foresee the more extended concept and overweigh the time invested. Which tool best satisfies the requirements of the user project must be determined before making any final selections. In this study, the benefits and drawbacks of each framework were discussed. This research presents details on both different front-end Web technologies used in the web development process. Reacts component-based architecture helps the user to save time and money on development. An interface may be disassembled into reusable parts that allow to creation of dynamic user interfaces. Users of React may dramatically reduce their development time with the help of features like Virtual DOMs, JSX, excellent state management, and reusable components that are independent of one another. React has a shorter ramp-up time since it has a less difficult learning curve. With two-way data binding, Angular makes sure that data is constantly in sync at all levels. The research area will be expanded in the future to cover more front-end development strategies and examine their guiding principles for creating web apps.

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SRJN

Portraying Mechanics of Seed Biopriming with Plant Growth Promoting Rhizobacteria

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Plant beneficial microorganisms such as rhizobacteria, rhizobia, arbuscular mycorrhizal fungi and *Trichoderma* etc. can reduce the use of agrochemicals and enhance plant yield, nutrition and tolerance to both biotic and abiotic stresses. Yet, large scale applications of these microbes have been hindered by the high amounts of inoculums per plant or per cultivation area needed for successful colonization and consequently the economic feasibility. Beneficial microbes are applied to the soil and plant tissues directly or through seed inoculation, whereas soil application is preferred when there is risk of inhibitors or antagonistic microbes on the plant tissues. Seed priming, where seeds are hydrated to activate metabolism without actual germination followed by drying, increases the germination, stand establishment and stress tolerance in different crops. Seed priming with living dense bacterial inoculum is as "biopriming" that necessitates the application of plant growth promoting rhizobacteria. It increases speed and uniformity of germination; also ensures rapid, uniform and high establishment of crops; and hence improves harvest quality and yield. Seed biopriming authorize the bacteria to enter/adhere the seeds and also habituation of bacteria in the prevalent conditions. This review is focused to discuss cognet mechanics employed by PGPR that assists seed to sustain healthy growth of plants.

Keywords: Germination, Health, PGPR, Stress, Seed priming

Introduction

In the year of 2013, Reddy spotlighted "bio-priming" technique as innovative skill for treatment of seed that incorporates biological and physiological facets of plant disease control. It is recently used as an alternative technique to control many seed borne pathogens as well as soil borne pathogens. Biopriming of seeds denotes standard tactic for introduction of disease resistance via bio control agents. Priming of seeds with helpful microorganisms and bio control means has been testified more proficiently for the management of diseases and pests as equated to other available methodologies (Prabha et al., 2019).

The seed biopriming is a recently adapted method of seed priming. Seed priming is a pre-sowing treatment which leads to a physiological state that allows seed to germinate more proficiently. The preponderance of seed a treatment are based on seed imbibition allowing the seeds to go through the first reversible stage of germination but does not allow radical protrusion through the seed coat. Seeds keeping their desiccation tolerance are then

dehydrated and can be stored until final sowing (Stanley et al., 2016). This review summarizes biorpiming techniques by using various PGPR strains for crop improvement.

Various types of seed priming techniques

There are many numbers of priming techniques are being convenient for the horticultural and forest tree crops. They are mentioned as below.

1) Among them, first one which is known as hydro priming which relies on to soak seed in purified water and re-drying to original moisture content prior to sowing. As per method, there is no use of additional chemicals requires so it is consider as a cheap and ecofriendly techniques (Taylor et al., 1998).
2) The second method is known as osmo-priming which involves soaking seeds in salty solution with low water potential instead of purified water. Salty solution contains low water so water enters in seed slowly which allows steady seed imbibitions and start to activate early phases of seed germination however it restricts radical protrusion (Di Girolamo and Barbanti, 2012).
3) Third method require solid matrix priming which involves mixing and incubation of seeds with wet solid water carrier for certain time period. So, seeds are deteched from matrix, washed and back-dried. McDonald (2000) mentioned that use of solid medium allows seeds to hydrate gradually and enhance natural imbibitions process arising in the soil.
4) In this fourth method, seeds are processed for hormone-priming which have direct impact on seed metabolic processes. Some plant growth regulators like abscisic acid, auxins, gibberellins, kinetin, ethylene, polyamines and salicylic acid can be used in this process. Galhaut et al., (2014) reported used of Gibberellic acid and PEG priming improved photosynthetic properties, antioxidant system, and seedling emergence in the case of clover grown on heavy metal polluted soil.
5) In this fifth method, chemo-priming which uses different chemical solutions used as priming agents. It includes priming with extensive range of both natural and synthetic compounds such as antioxidants like ascorbic acid, glutathione, tocopherol, melatonin etc, H_2O_2, Sodium nitroprusside, chitosan etc. Patade et al., (2012) studied positive effects of chemo-priming with various priming agents under environmental conditions (Patade et al., 2012).
6) In this six method, seeds are soaked with particular solution like limiting nutrient content instead of pure water so it is known as “Nutri-priming”. In this method, seeds received benefits of priming in order to increase seed quality, germination parameters and seedling establishments (Farooq et al., 2012).
7) In this seventh method, Callan et al., (1990) reported biopriming which represents seed imbibitions together with biocontrol agent or PGPR treated seed.

Application of PGPR as inoculants for seed bio-priming

Biopriming treatment with commercial biofertilizers like *B. lentus, B. subtilis, Pseudomonas fluorescens, P. putida and Azospirillum spp* has been reported by Saber et al., (2012) for enhancement of wheat plants. Golami et al., (2009) studied total seedling fresh weight of maize seedling enhanced when they treated with PGPR strains. Mirshekari et al., (2012) observed consortium of *Azotobacter chroococcum* and *Azospirillum lipoferum* in combination with 80 kg ha^{-1} urea and 60 kg ha^{-1} P_2O_5 significantly enhanced the yield attributes such as grain weight, dry matter accumulation, biological yield, grain yield and harvest index of barley seeds. Harman et al., (2004) reported seed priming treatment with *Trichoderma* in maize plants. *Arabidopsis thaliana* modulated root system architecture and enhanced the plant growth because *Trichoderma* sp. synthesized IAA that increased later root formation and root hair growth (Contreras-Cornejo et al., 2009). Seed priming by using PGPR strains increased the lateral root growth for the reason that PGPR strains starts postembryonic root system architecture modification by enhancing root hair growth and lateral root development and by inhibiting the primary root length (Zamioudis et al. 2013). Spaepen and Vanderleyden (2011) observed seed biopriming by using PGPR isolates also increased phytohormones like indole acetic acid which results in increased numbers of roots, root hair and lateral and adventitious roots and expanding root surface area.

In general, those living organisms shows different multifunctional activities like production of plant growth regulators, such as auxins, cytokinins, abscisic acid, and gibberellins, as well as secretion of effect or molecules and secondary metabolites through modulation of various pathways/cascades, are the most suitable for the biopriming method and provides resistance to plant against biotic stress (Singh et al., 2020; Audenaert et al., 2002). Raj et al., (2004) reported *Pennisetum glaucum* seeds treated with *Pseudomonas* spp. strains not only increased vegetative growth but also showed resistance to the plant disease. Many researchers (Ait Barka et al., 2006; Cakmakci et al., 2007; Chitra and Jijeesh, 2021) reported biorpimed seeds showed better plant establishment and enhanced plant yield by increased in numbers of germination rate, increasing root length and volume, increasing the number of lateral roots. Deshmukh et al., (2020) reported biopriming with PGPR could be beneficial to plant health. Sarkar and Bhattacharya (2008) studied mung bean seed soaked with suspension of *P. fluorescens* or *T. harzianum* resulted with reduced root rot disease incidence in pot experiments but it was observed that under field trials, increased root length, shoot length, yield and dry weight as compared to control treatment. Mohamedy and Baky (2008) reported pea seed treatment with *P. fluorescens* or *T. harzianum* resulted with the highest survival as well as lowest root rot disease incidence. Authors also observed that highest plant height, more numbers of leaves and branches/ plant, dry weight of shoots/plant, pod length and diameter, numbers of seed/pod, highest percentage of green pod, seed to pod weight, TSS, highest carbohydrate and protein. Biopriming of maize seeds with *T.harzianum* observed

more effective against infection by Fusarium verticillioides. Data also revealed that this treatment also effective as increased of seed germination, vigour index, field emergence, yield, and test weight in comparison with the control (Nayaka et al., 2008). Begum et al. (2009) studied biopriming with *P. aeruginosa* showed effective treatment in the soybean plant when they were infected with damping off caused by *C. truncatum.*

Sharma et al., (2009) observed that cumin seeds when treated with *T. harzianum* increased the germination of seeds while biopriming with *T. viride* showed good shoot-root ratio in pot trials. Someshwar and Sitansu (2010) prepared *P.fluorescens* inoculum and checked their effect on seeds of chilli, tomato and brinjal over some fungal biopriming agents viz, *T. viride* AN-10 and T. *harzianum* AN-13 while it was equivalent to *T. harzianum* WB-1 in inducing germination of the crop seeds. The highest germination of seed was obtained when crop seeds were primed with mycelial form of inoculum of *T. harzianum* AN-5 and WB-1.

Conclusion

Now-a-days, seed biopriming technique by using efficient microbial agents and their commercial circulation among farmers are essentially needed. The proper identification of PGPR strain, formulation development, delivery mode, trials over field application at different geographical regions among farmers and commercialization of products are extremely necessary because of its ecofriendly application for conservation of soil and plant diversity. Apart from this, studies over the viability of the introduced microorganisms and its mode of work represent another area for instant attention.

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DETERMINATION OF CROP PHENOLOGICAL STAGES AND CROP TYPE USING L-BAND POLARIMETRIC SAR DATA

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1. INTRODUCTION

The main driver of Indian economy is agriculture sector as an approximate 60% of Indian population is directly or indirectly engage in agriculture and contributing 13% of country GDP (Gross Domestic Product). To enhance the sustainable growth of agriculture in synchronization with natural recourses precise crop information at spatial scale is prime focus for various national agricultural programs. Whole globe had witnessed the substantial advancement in the field of ground, aerial and space based remote sensing technology and computing techniques in last three decades. In India as per the need of scientific agricultural community space programs and policy had taken a shape and various space missions were executed. With the success of the previous Indian missions the demand from agricultural scientific community increases from national scale monitoring to field scale monitoring during monsoon (rainy) and post monsoon seasons. Indian space community demonstrated the usage of optical, thermal and microwave sensors at various spatial and temporal resolutions for agricultural applications. To further enhance the scope of remote sensing in agriculture and to harness the potential of Synthetic Aperture Radar (SAR) data an investigation was planned with ALOS L band data. As SAR have a capability to provide data in all sky conditions and can overcome the limitation of optical and thermal during cloudy sky. In past studies were done to map crop using RISAT-1 and RadarSat in homogeneous agricultural patches of India. All past studies were more focused to address mapping of flooded paddy crop. This will generated need to address a greater number of crops using SAR data and to explore to address in season crop phenology in homogeneous and heterogeneous patches. In this study crop mapping and phenology were prime objectives. As listed in literature many researches have been carried out for monitoring agricultural areas using SAR data because of their sensitivity to the moist conditions and surface roughness. Nelson et al. (2004) and Q. Zhang (2011) demonstrated the classification by paddy fields from other crops and land uses. Mandowara et al. (2019) used C- band data to classify different agricultural crops over heterogenous area. Yusoff et al. (2016) identified rubber and oil palm from agricultural areas, Tian et al. (2010) identified crops and Mishra et al. (2011) showed promising results in classification of tall vegetation from short vegetation using SAR data. This study aims to use different microwave frequencies data such as ALOS-2/PALSAR-2 data and C-band Sentinel-1 data for identification of crop types and phenology over heterogeneous croplands and classifying other land uses near agricultural crop land.

2. STUDY AREA

The present study was carried out over two regions of India. (a) Gurdaspur, Punjab (Homogeneous) (b) Anand, Gujarat (Heterogeneous). Gurdaspur is on the main highway to Amritsar (southwest), Punjab's largest city. It is located at 32.0414° N, 75.4031° E. The annual temperature in this region varies in the range of 2°C to 45^{0}C. In winter temperature ranges from 2°C to 20^{0}C. The 80% (650 mm) rainfall in this region received during monsoon season (June to September) and rest due to western disturbance (December to March). Its elevation ranges from about 305 to 381 meters above sea level and having sandy loam soil. The whole region is well irrigated and lies adjacent to Himalayan hill state of Himanchal Pradesh. The study area is primarily a trade Centre for the region's agricultural products; wheat (post monsoon), corn (maize), rice (monsoon), and other crops are grown in the surrounding area. In winter 90% of agricultural area is dominated by wheat crop. Total 45 wheat field were geotagged with crop phenology (Table 1a).

The study area Anand popular known as "Milk Capital of India", for Amul dairy and its vast contribution in milk industry. The Head Office of Gujarat Cooperative Milk Marketing Federation Ltd (GCMMF), which is parent organization for AMUL & other co-operative operations for collection of milk), Vidya Dairy, Institute of Rural Management

Anand (IRMA), NDDB of India and one of the largest University, the Anand Agriculture University are all situated in Anand. It is located at 22.57° North latitude and 72.93° East longitude (Fig. 1) and has an average elevation of 39 meters (127 feet). The region has alluvial sandy and loam type with average depth of 200 cm. Temperature in this region varies from 20.30 ºC (Average) to 33.63 ºC (Average). The average seasonal rainfall here is about 750 mm. Anand is primarily an agricultural district with tobacco and paddy as the predominant crops. The other major crops cultivated are wheat, banana and vegetables such as papaya, mango, onion, cabbage etc. About 30.12 % of land holdings are with small and marginal farmers and the average size of the holdings is 0.96 Ha. Cultivated lands (68.58 %), forest lands (0.20%), open scrub and waste land (9%) and miscellaneous lands (12%). It falls under Agro Climate Region of XIII-Gujarat Plain & hills region.

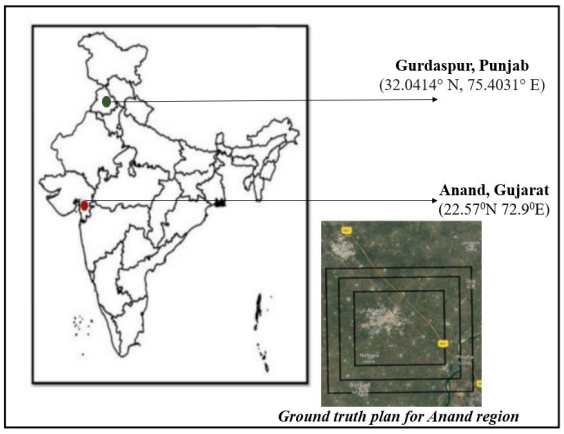

Figure 1. Location map of study area

The texture of the soil is loamy sand. The soil is low in organic carbon and nitrogen, medium in available phosphorus and available sulphur. Status of potassium was found high, while micronutrient status is found sufficient. The GPS survey was carried out across the district using the Trimble® R1 rugged, compact, lightweight GNSS receiver that provides professional-grade positioning information to any connected mobile device using Bluetooth® connectivity. The total ground truth points collected for different crop types is shown in Table 1b for Anand region.

Table 1a. Number of GPS points collected for wheat crop over Gurdaspur district

Sr. No.	Phenological stage	Total ground points
1.	CRI stage	10
2.	Tillering stage	15
3.	Soft dough stage	10
4.	Hard dough stage	10

Table 1b. Number of GPS points covered during field visit at Anand

Sr. No.	Feature class	Total ground points
1	Tobacco	31
2	Banana	14
3	Potato	6
4	Wheat	8
5	Water body	11
6	Bare soil	21
	Total	91

3. Methodology:

A. In situ data

To full the objectives of the study rigorous field data was collected. the field visit was planned in such a way that field data can be collected for two consecutive days nearby satellite imaging date. In the field data collection major land types namely, water bodies, barren land, major crop types and urban/rural settlements. In Gurudaspur district total 45 wheat field were surveyed to record the different phenological stages of crop (Figure 2a). Moreover, in Anand region at 33 crop fields parameters such as crop height, soil moisture, biomass, leaf area index (LAI), plant to plant distance and row to row distance were measured. Additionally, 91 location points have been recorded during the field visit (Figure 2b) as shown in Table 1.

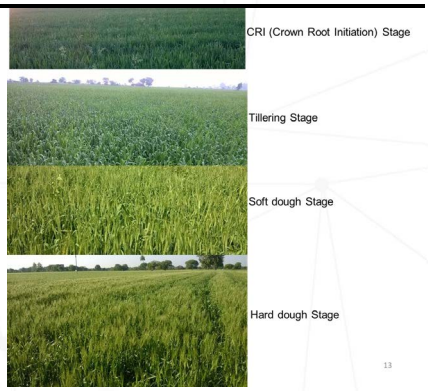

Figure 2a. Ground pictures for different phenological stages of wheat crop

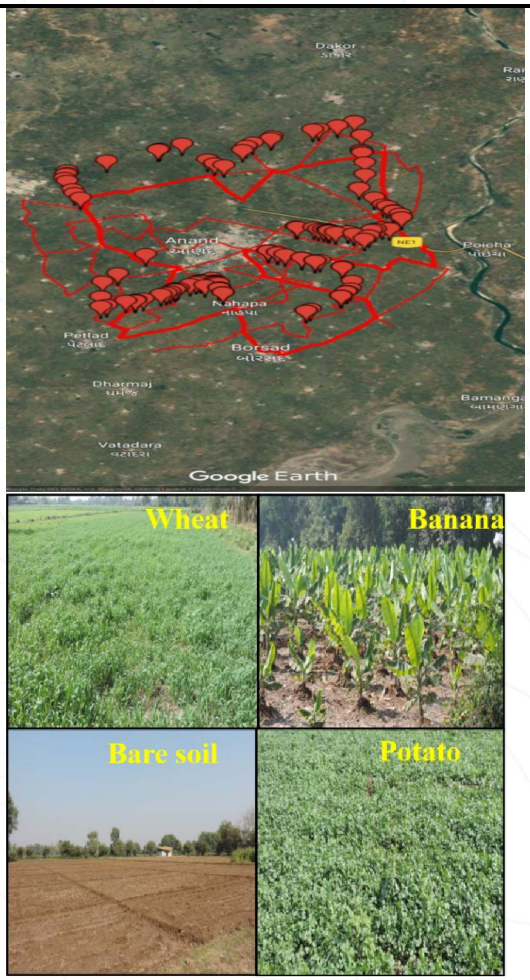

Figure 2b. geo-location and field pictures conditions of ground truth points collected over study area

B. Preprocessing of SAR data

ALOS-2/ PALSAR-2 data:

Sentinels Application Platform (SNAP) software from the European Space Agency (ESA) provides the tools for processing Sentinel-1 satellite data, as well as other satellite data, such as from PALSAR-2. The preprocessing steps are (i) calibration, (ii) multi-look with a 1:2 ratio for azimuth and range (JAXA, 2014), (iii) co-registration, (iv) speckle filtering for reducing the noise in SAR data, (v) geocoded processing and (vi) backscatter coefficient calculation. All PALSAR-2 data were stacked and co-registered together by using the Shuttle Radar Topographic Mission digital elevation model (SRTM DEM). The single product speckle filter was applied based on the Lee speckle filtering method with a window size of 5 × 5. The PALSAR-2 data were geocoded to the Universal Transverse Mercator (UTM) projection 49S with World Geodetic System 1984 (WGS84) datum. The topographic effect was corrected using the SRTM DEM. The results of the geocoded images formed a subset based on study area coverage and were confirmed to have a perfect fit with the Pleiades image. The full-Pol ALOS-2/PALSAR-2 data at 3m spatial resolution was used to carry out study at Gurdaspur, while dual Pol ALOS-2/PALSAR-2 data at spatial resolution of 10m was used for study over Anand region, in order to be coherent with Sentinel 1 A data set.

SENTINEL- data:

The Sentinel-1A IW Level 1 (L1) GRDH (ground-range detected, high resolution) product was used in this study. The Sentinel-1 C band (~ 5.40 GHz) SAR data has dual-polarization (VV and VH) with revisit time of 12 days with spatial resolution of 10 m. L1 data was pre-processed using ESA's open source 'Sentinel-1 Toolbox'. Pre-processing steps include orbit correction, geocoding, radiometric calibration and resampling (seehttps://sentinel.esa.int/web/sentinel/toolboxes/sentinel-1for a detailed description of the processing steps). Since this study is based on the investigation of (low-frequency) seasonal backscattering behavior, speckle filtering was performed for temporal data sets.

C. Polarimetric H-α classification

Cloude and Pottier (1997) proposed an algorithm to identify in an unsupervised way polarimetric scattering mechanisms in the H-α plane. The key idea is that entropy arises as a natural measure of the inherent reversibility of the scattering data and that α can be used to identify the underlying average scattering mechanism. The H-α classification plane is sub-divided into 8 basic zones characteristic of different scattering behaviors. The basic scattering mechanism of each pixel of a polarimetric SAR image can then be identified by comparing its entropy and α parameters to fixed thresholds. The different class boundaries, in the H-α plane, have been determined so as to discriminate surface reflection (SR), volume diffusion (VD) and double bounce reflection (DB) along the α axis and low, medium and high degree of randomness along the entropy axis. Detailed explanations, examples and comments concerning the different classes can be found in the publication from Cloude and Pottier. This methodology shown in Figure 3a is tested only for at parts of Gurdaspur region of Punjab due to data constraints. As per our plan we do not get the temporal ALOS full polarization data for study region

to address full crop growing phase to investigate the whole phenology of the wheat crop.

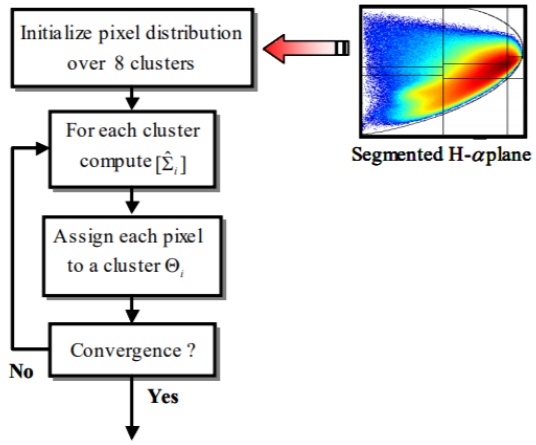

Figure 3a. Unsupervised H-α segmentation scheme

D. Classification based on Backscatter coefficients

Thresholding of backscatter values for various crops, water and urban was calculated with the help of the GT points collected during the visits to get an idea of range of backscatter value for each crop type. For performing supervised classification, region of interests (ROIs) file was generated using ground truth locations (Table 1b).

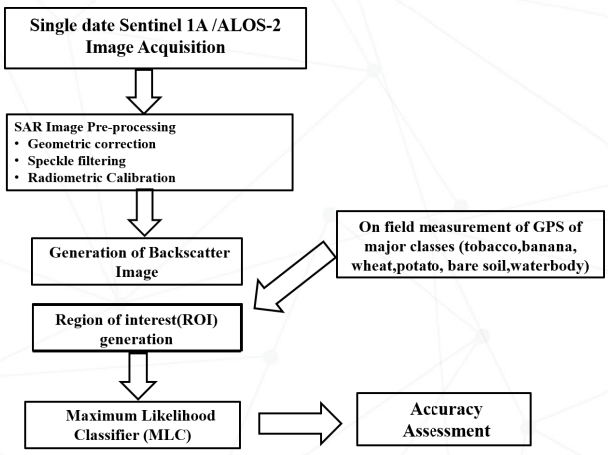

Figure 3b. Overall methodology used in this study

The backscatter coefficient in decibel units (dB) was then calculated using this formula (JAXA, 2017) where the Cf and A are calibration factors with a value of -83 and 32 dB, respectively. Back scatter coefficient (σ) is calculated as:

$$\sigma = 10 \times \text{Log10}(\text{polarization}) + Cf - A \qquad (1)$$

Here, Maximum Likelihood Classifier (MLC) was applied on the backscatter image which is a popular method and is frequently used in the field of remote sensing. The procedure involved in MLC can be described as a pixel with the maximum likelihood is classified into the corresponding class where, the likelihood is defined as the posterior probability of a pixel belonging to class k".

$$Lj = P(j/A) = P(j)*P(A/j)/\varepsilon P(i) * P(A/i) \qquad (2)$$

where, P(j): prior probability of class j; P(A/j): probability density function I.e. PDF or conditional probability to observe A from class j, Mostly, P(j) are assumed to be equal to each other and εP(i) * P(A/i) is also common to all classes. Therefore, Lj depends on P(A/j) or the probability density function. MLC classifier demands for sufficient ground truth data to be sampled to allow estimation of the mean vector and the variance covariance matrix of population. Here, the collected GPS points were used to build the ROI file for the scene and at the time of classification, this ROI file was overlayed on the backscatter image.

4. Results and Discussions:

The polarimetric signature was computed from ALOS full pol L band dataset over parts of the Gurdaspur region. In that region wheat crop was sown at different time scale. This lead made a difference in different phenological stages of crop. The different phenological stages represent different canopy architecture and geometry generate different scattering. To study the effect of different phenological stages of wheat crop H-α decomposition was done. In the Figure 4 the soft and hard dough stage represented by scattering by canopy roughness as in that stage the surface roughness was maximum. The tillering stage was marked as scattering from vegetative surface from random anisotropic scatter and CRI stage was depicted scattering from vegetative surface. In the Figure 4 only wheat crop was present at different phenological stages and conclusion was made as per the ground truth and scattering mechanism. The comparison of decomposition of scattering image showed 70% accuracy with ground data. In few wheat fields the tillering and soft stage gave similar scattering. The accuracy of vegetative field with other classes such as water and rural settlement was high (85%) as compare to within wheat crop phenology discrimination.

To generate and compare the crop classification map using ALOS data over part of Anand region Setinel-1 data is also used. The backscatter coefficients σ_{VH} for C-band varied in range of -17.8 dB to -22.1 dB and for L-band varied in range of -13.4 dB to -23.2 dB for various LULC (tobacco, banana, wheat, potato) as

mentioned in Table 2 and Table 3. Using Maximum Likelihood Classifier (MLC), we concluded that the study area Anand was dominated by tobacco and banana which occupied almost 40% of the area as seen from both C-band and L-band data. The other 20% is dominated by other crops (wheat, potato, cabbage). The rest area is urban and water (Figure 5 and Figure 6). This is very analogous to the ground survey carried out in this area. The kappa coefficient was found to be 0.6 and 0.8 for L-band and C-band respectively. Many workers have reported that the microwave energy in shorter wavelength regions (C band) are more suitable for short crop studies, as they interact more with the crops surface due to lesser penetration compared as compare to microwave energy at higher wavelength (S and L) band. Inoue et al. (2002); Brisco and McNairn (2004) research demonstrated that the backscatter coefficients of higher frequency bands are highly correlated with the weight of heads. Lower frequency bands such as L-band, are better correlated with fresh biomass while C-band is better correlated with leaf area index. L-band data allow identification of well developed 'broad leaf' crops (sunflower and corn), whilst C-band are useful for discriminating different kinds of short crops even in the case of moderate growth (Ferrazzoli et al., 1997). For C band more multi temporal data set is available while for L band we have only two-day data set. This lead to higher classification accuracy observed from C band as compared to L band in heterogeneous agricultural patches.

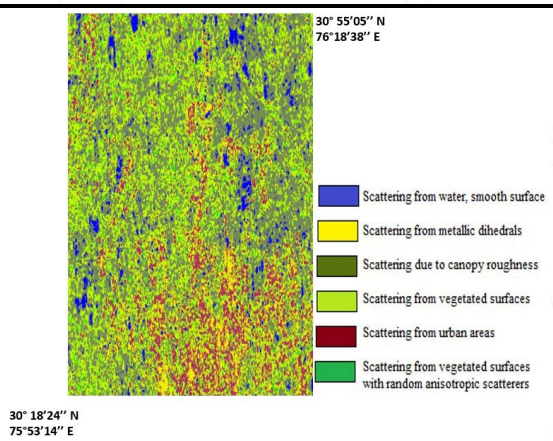

Figure 4: Outputs of H-α segmentation scheme over Gurdaspur, Punjab.

Table 2: Backscatter coefficients (σ_{VH}) range for different crops for C-band

Sr No.	Feature Class	Avg. Backscatter range VH polarization (dB)	Standard Deviation (dB)	Classified (%)
1	Tobacco	-17.8 to -19.2	0.01	24
2	Banana	-16.2 to -17.6	0.008	11
3	Potato	-18.4 to -19.5	0.003	7
4	Wheat	-20.7 to -22.1	0.09	6

Table 3: Backscatter coefficients (σ_{VH}) range for different crops for L-band

Sr No.	Feature Class	Avg. Backscatter range VH polarization (dB)	Standard Deviation(dB)	Classified (%)
1	Tobacco	-15.6 to -17.5	0.11	26
2	Banana	-13.4 to -14.5	0.1	9
3	Potato	-16.7 to -18.5	0.006	7
4	Wheat	-20.6 to -23.2	0.12	4

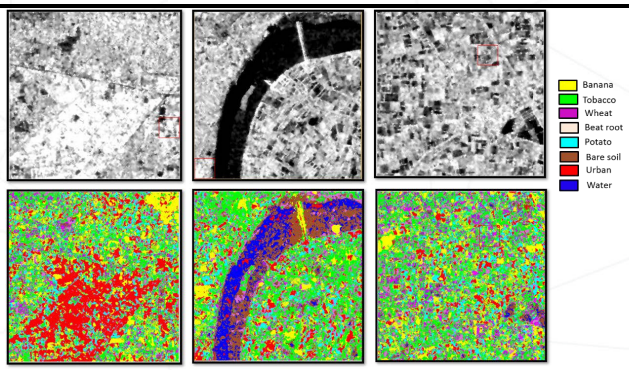

Figure 5: Outputs of MLC classification for ALOS-2/PALSAR-2 L-band data

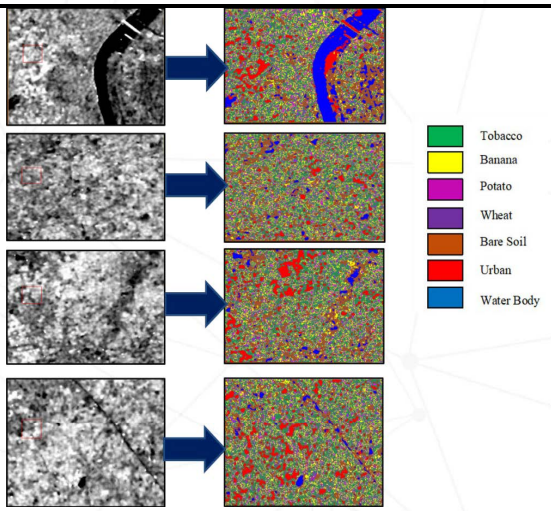

Figure 6: Outputs of MLC classification for SENTINEL-1A C-band data

Conclusion

In this study prime aim was to discriminate crop phenology and crop type using ALOS PALSAR data. We tried to discriminate the wheat phenology using one-time full pol data set with accuracy of 70%. But due to limitation of temporal resolution we do not get any other data from the same study region. This study also demonstrated the application of multi frequency L-band SAR data for crop classification over heterogenous agricultural areas of Gujarat, India. The comparison of L-band SAR data with C band Sentinel SAR data showed for short height canopy C band performed better. To further explore the potential of L band crop classification is planned over region which is dominated by tall crops such as sugarcane and maize. To further enhance the accuracy of crop classification fusion of C and L band is planned by applying different classification accuracies.

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Impact of landfill leachate on ground water quality: A study of Pirana landfill site

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1. INTRODUCTION

One of Ahmedabad's greatest trash generating scales started at the Pirana dump site, which is overseen by the Municipal Corporation of Ahmedabad. leach-ate, a highly polluted liquid that is dangerous to nearby water bodies, including groundwater, as well as nearby agricultural fields, has been found at the Pirana dump site, according to several researchers and local residents. The position of the leach-ate pond under the dumping site, how it percolates and contaminates groundwater, and how this affects the environment and human hygiene at the Pirana land field site are all important considerations for the impact of leach-ate on groundwater.

A landfill site, which routinely burns the trash with daily intermediate and final covers, is sometimes referred to as a garbage dump for the disposal of waste material.(1)

The population of Ahmedabad, one of India's seven major megacities, is above 8 million.(2) Due to the increasing pace of urbanization in Pirana, which is one of the largest cities in India, the population and amount of garbage production have dramatically expanded, directly contributing to the city's pollution.(1) The Pirana landfill site, which has a total area of around 84 hectares, was designated as the Ahmedabad dumping site in 1980.(2) It gets over 4000 terms of trash per day, the most of which is placed in the landfill untreated even though it has to be treated. The Pirana dump site is still a rubbish pile that degrades the appearance of the city and disperses disease-causing organisms through groundwater, farmland, pungent odours from air pollution, etc.(1)

The official stated that a recent estimate in 2021 has rebuilt that the Pirana Mount has 1.25 crore metric tonnes of garbage as opposed to an estimated 80 lakh metric tonne in 2012. At present, 39 machines are processing about 15000 metric tonnes of garbage daily. The pirana landfill site is spread over 80 acres with 3 numbers of 55-meter mounts sitting on 40 acres of land. (1)(2)

The AMC implemented a bio-mining project in 2019 and set up 25 trommel machines in Pirana to separate waste; in a little over 20 months, the AMC was able to process and remove 33 lakh metric tonnes of garbage in 2018. The project was delayed as a result of political unrest because the standing committee had missed the deadline for awarding the trommel machine tenders. (1)(2)

A mountain of cancer is another well-known feature of the Pirana landfill site. Uttar Pradesh is lit to clear the field where air pollution occurs following an incident of paddy-wheat harvesting in Delhi, Haryana, and Punjab.(1)

Gujarat, which has 1.32 lakh hectares of harvested paddy and wheat in the area around Ahmedabad, is the state with the greatest paddy-wheat harvesting area and is likely to experience the same scenario as our neighbouring States in the future due to leach-ate.(1)(2)

By separating it from the rubbish in 2021, the solid waste service was able to designate 24% of the Pirana landfill. According to a Times of India story, the department has been able to handle 33 lakh metric tonnes of trash, and it is anticipated that the Pirana Mount would be completely eliminated in the following three years. According to department representatives, of the 80 acres of land covered by waste, AMC has been able to remove roughly 19 acres.(2)

It is reasonable to predict that cleaning up the Piranha dump site might take between three and six years given its current state.

The government also planned a project to recycle trash across from the Pirana dump, while the Municipal Corporation of Ahmedabad established a scheme to generate power on 13 acres of land using 1 metric tonne of waste from Ahmedabad. (1)(2)

2. SAMPLE ANALYSIS

According to IS 3025, tests to ascertain the properties of water samples were conducted. According to the procedure outlined in APHA 3500, the concentrations of lead (Pb), zinc (Zn), copper (Cu), and arsenic (As) in bore water samples were estimated. The list of examinations carried out on the samples is as follows:(3)(4)

1. PH: This scale is intended to measure how acidic a water-based plan is.(3) (4)

Sr. No.	Sample Description	The temperature of the Sample (°C)	pH
1.	Sabarmati sample	25 °	8.08
2.	Leach-ate (Point 1)	25 °	7.70
3.	Factory (Point 3)	25 °	7.78
4.	Roadside shop (Point 5)	25 °	7.70
5.	Farm (Point 7)	25°	7.99

2. Conductivity: the prohibition on the transmission of heat, electricity, or sound through wastewater.(3)(4)

Sr. No.	Sample Details	Conductance (mS)
1.	Tap Water	1.94
2.	Distilled water	0.08
3.	Sabarmati sample	0.97
4.	Leach-ate	>20
5.	Factory (Point 3)	2.91
6.	Roadside shop (Point 5)	2.62
7.	Farm (Point 7)	2.23

3. Total Dissolved Solids is a measurement of the total amount of inorganic and organic compounds that have been dissolved and are suspended as molecular, ionised, or micro granular particles in a liquid.(3)(4)

Sr. No.	Sample Details	TDDS (ppt)
1.	Sabarmati sample	0.51
2.	Leachate	10
3.	Factory (Point 3)	1.49
4.	Roadside Shop (Point 5)	1.61
5.	Farm (Point 7)	1.18

4. BOD: Readings were recorded in the table in accordance with the approach provided in 4.4.2. BOD5 was further calculated using the provided recipe:(3)(4)

BOD5 (mg/l) = {[D1-D2]-[B1-B2]}/ P
Where: D1 = DO of the sample immediately after preparation, mg/l
D2 = DO of the sample after 5 days incubation at 20°C
B1 = DO of blank immediately after preparation, mg/l
B2 = DO of blank after 5 days incubation at 20°C, mg/l
P= dilution factor

Sr. No.	Sample Details	Burette Reading			BOD5 (mg/l)
		0^{th} Day Reading (IR) (ml)	5^{th} Day Reading (IR) (ml)	FR-IR (ml)	
1.	Sabarmati	33	15.5	16.7	1670
2.	Leach-ate (Point 1)	29	24	29.2	2920
3.	Factory (Point 3)	38.2	27.6	23.6	2360
4.	Roadside Shop (Point 5)	35	23.5	22.7	2270
5.	Farm (Point 7)	30	18	22.2	2220

5. Chemical Oxygen Demand: Every Sample's FAS as well as the blank solution's FAS have been recorded. The following formula was used to determine the COD of each solution:(3)(4)

COD (mg/l) = [(A-B) ×M ×8000]/ Volume of Sample

Where: A = FAS used for sample, mL (table)

B = FAS used for blank, mL (1ml)

M = Molarity of FAS (0.25)

Sr. No.	Sample Details	Burette Reading		The volume of FAS (Ferrous Ammonium Sulphate) 'A'	COD (mg/l)
		Initial Reading	Final Reading		
1.	Blank solution	0	1	1	-
2.	Sabarmati	0	1.4	1.4	320
3.	Leach-ate (Point 1)	0	2.4	2.4	1120
4.	Factory (Point 5)	0	1.3	1.3	240
5.	Roadside shop (Point 5)	0	1.8	1.8	640
6.	Farm (Point 7)	0	1.7	1.7	560

6. Heavy Metal Concentration: Based on the findings of the research facility's investigation, a heavy metal convergence of lead (Pb), zinc (Zn), copper (Cu), and arsenic (As) was discovered.(3)(4)

Sr. No.	Type of substance	Conc .of Lead (mg/l)	Conc .of Zinc (mg/l)	Conc. of Copper (mg/l)	Conc. of Arsenic (mg/l)
1.	Sabarmati sample	-	0.010	-	-
2.	Leach-ate (Point 1)	0.026	0.564	0.105	0.02
3.	Factory (Point 3)	-	0.021	-	0.001
4.	Roadside shop (Point 5)	-	0.004	-	0.002
5.	Farm (Point 7)	-	-	-	0.002

3. METHODOLOGY

There are no sources or infrastructure at the Ahmedabad Pirana dump site to stop leach-ate from getting into the groundwater. There isn't even a collection tank or a system in place for leach-ate treatment.(5)

The following are suggested configurations to stop leach-ate from getting into groundwater and how to handle it.(5)

The first step in preventing leach-ate entering groundwater after clearing the land is to cover the dumping location with a geo membrane layer. This layer provides a strong, lasting, impermeable barrier that keeps rain and waste water from penetrating the earth. The Geo membrane under the dumping side should be left on for the collecting of leach-ate water. While a suggested solution is to collect all wastewater during monsoon precipitation and clean it to reuse the water, preventing leach-ate from entering into the ground. a slope that encourages fluid flow under the effect of gravity and is gathered in artificial leach-ate ponds. Leach-ate ponds should additionally include a Geo membrane on the pond bed to capture polluted fluids on a larger scale and for a longer period of time.(5)

Establish a piped intake from the leach-ate ponds to the storage tank. After collecting contaminated liquid from the inlet pipes, the liquid is sent to the settling tanks, where suspended solid will settle. Then, the water is sent to the RO plant for the first stage of water treatment, where high-pressure pumps are available to speed up the flow of flowing water and send it to the filtration tank, where minor suspended solids are filtered and sent back to pressure pumps with a pressure membrane of 0.0. (5)The approved outlet enables treated water with a tolerable pH of roughly 7 and NO TDS in the water, while the rejected outlet is transported to a secondary RO plant for a second phase of treatment to ensure the requisite water characteristics in both the permitted and rejected outlets.(5) In order to achieve NIL TDS, it is then transported to a resin tank containing anode charges, which absorb charges and thereby reduce the available TDS following treatment. Rejected water is dumped into the rejected pond when water treatment is finished, while approved water is immediately released into the permitted water pond.(5)

Construction, irrigation, and other consumer uses are all permitted for the water. The rejected water cannot be utilized for any purpose.(5) Sludge that is accessible after the treatment procedure is finished is often dumped in landfills or can be dumped on an artificial island that is secluded from the rest of the world. The sludge can be utilized to make composites or to build paver blocks, roadways, and other structures after processing.(5)

4. CONCLUSION

According to test findings and suggested methods to avoid leach-ate, the PH value and total dissolved solids of all surrounding areas are below legal limits, however the COD and BOD values of each sample are much higher than the permitted limits as per the proposed methods (GPCB).

A water sample from the Sabarmati River also suggests the presence of zinc, and the sample reveals the presence of copper, zinc, lead, and arsenic but below acceptable limits. Thus, it can be inferred that the concentration of zinc, copper, lead, and arsenic is

below acceptable limits for an environment that is conducive to low concentrations and that the summer season limits leach-ate percolation.

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Impact of Transits Stations and Land Use with In Its Zoning

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1. INTRODUCTION

Transit stations all over the world have helped to develop the area around them, but the degree of their impact is a hotly contested issue because the influence's zone of effect differs from one location to another and even between stations in the same network. The type and pace of development around a station is influenced by a variety of factors, including the area's ability to develop vacant land, its economic health, population density, and themix of land uses in the area. Quality of the services provided by transfer stations is only one of these influences.

For more than a century, industrialized nations in Europe and America have examined the effects of transit stations on the land use, land value, population density, and other factors in the area that they influence. A review of the research on the impact of transit stations on densities and land uses inside their influence zones found that the extent to which the influence zone of the transit station exists itself varies depending on a number of other supporting variables. [1]

Transit stations have a different effect in developed nations than they do in developing nations in Asia because developed nations in the west also spend a lot of money developing infrastructure facilities that encourage the use of private transportation,which, in turn, hinders the development of public transportation systems to their full potential. Although it is not the sole one, the transit station is undoubtedly a significant main element that, when combined with a number of other secondary variables, leads to the area's rapid development. [1]

The Indian government places a strong emphasis on the need to improve the calibre and reputation of the public transportation system in order to increase patronage and move the nation toward sustainable development.

2. INFLUENCE ZONE OF TRANSIT STATION

The region where customers will walk to the station and use the space is known as the influence zone of a transit station. According to Calvo, Ona, Aran, and Nash (2013), the higher the quality of the transportation service offered, the broader the catchment area will be as the passenger will beready to go farther to get to the station. The capacity, frequency, comfort, and other supports— such as fee integration, the simplicity of switching between modes, last-mile connection, and other amenities provided during travel—all affect the service's quality. Urban places have shorter walking distances than rural ones, and even within urban regions, the central business Centre (CBD) ismuch closer to the station than the suburbs. Because of the advanced infrastructure and well- developed neighboring districts in the core businessCentre, this is the case. [1]

According to the Transit Capacity and Quality of Service Manual, the actual influence zone of the transit station is comprised of the individuals who really use the facility and for whom this service is a practical mode of transportation. Similar to an air-circle with a transit station at its center, the influence zone is frequently described globally in terms of the radius surrounding the station.

The National Transit Oriented Development (TOD) Policy (2017) by the Government of India defines the influence zone of a transit station as being between 500 and 800 meters, which is within 10 to 12 minutes walking distance; however, if the distance between the stations is less than 1 kilometer and there is overlap between the influence zones, the influence zone can only be 500meters from the station.

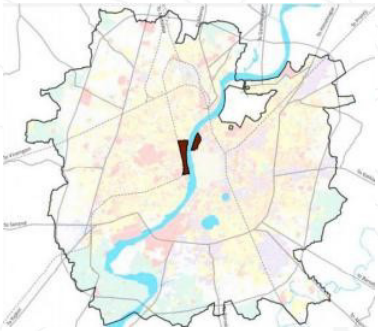

Figure1: Proposed Local Area Plan for CBD Zone

Figure -1 shows how the zone will provide Ahmedabad's core area a unique personality and shape while also enhancing the city's skyline in combination with the Sabarmati Riverfront Development. A lively, mixed-use, transit-oriented, walk able CBD that is appealing for business, entertainment, and tourism will be created thanks to the promotion and incentives provided by this zone. The CBD Zone's maximum permissible FSI is 5.4. *[2]*

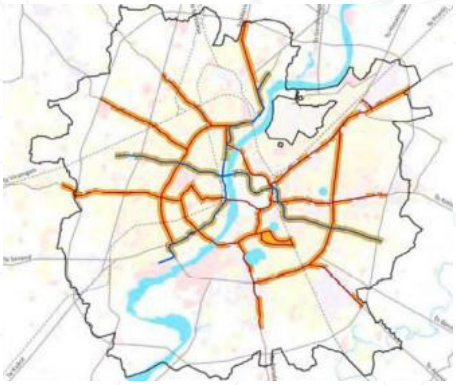

Figure2: Proposed Transit Oriented Zone (TOZ) A

42 sq.km region known as the Transit Oriented Zone is placed on the base zones along the BRTS and proposed MRTS track. The sections shown on the map as the proposed Transit Oriented Zone are: I Metro Transit Corridor (200 m on either side of MRTS); ii) BRTS Transit Corridor (200 m along BRTS route). The maximum allowed FSI in TOZ is 4.0. *[2]*

3. Transit Oriented Development (TOD)

An excellent instrument to promote compact,transit-oriented development that is within walking distance of public transit routes like the BRTS and Metro is the Transit Oriented Z3 One, which was created to encourage such development and to coordinate land use and transportation. Transit Oriented Zone implementation calls for thorough evaluation, meticulous planning, and a stagedapproach. As a result, it is advised that local area plans be created for the numerous corridors and regions in this zone and that compact, mixed-use transportation be implemented. Development that isgoal-oriented by appropriate legislation, incentives,and TP Scheme mechanisms. [3]

Ahmedabad's BRTS network offers excellent connectivity across the city. As a result, the TOZ was established along the BRTS network, and 200 meters on either side of the network were built as high-density zones for the construction of compact cities. The MRTS network was also suggested for development along with the same idea. The areas designated along the transit routes were assigned an FSI of 4.0 and were intended to be developed with a better urban environment, NMT connection, and accessibility across the city.

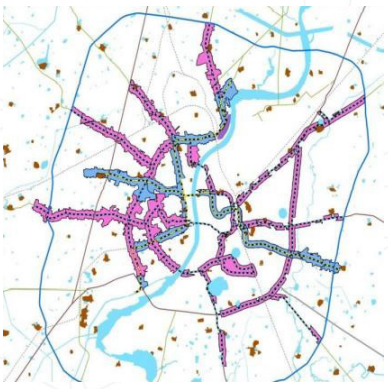

Figure3: Implementation of TOZ

As a component of the Transit Oriented Zone, a total of 82 local area plans have been created.

For the western part of Ahmedabad, 36 LAP areas have been identified, of which 21 have been finished; for the eastern portion, 46 LAP sites have been identified.

Route Length of BRTS: 120.44 km.

Length of MRTS Route: 40.6 km.

40 Sq.km is the total area covered by LAP.

4. IMPACT OF TRANSIT STATION ON DENSITY

In a spatial sense, density is defined as the number of people who live in a certain region. Transit stations effect density in a variety of ways,including an increase or reduction in population density, housing density, employment density, and so on.

The National Transit Oriented Development (TOD) Policy of the Government of India states that in order to increase the population and employment density in the area under the effect of transit stations, a greater Floor Area Ratio / Floor Space Index should be permitted. Depending on the city and its regional features, the minimum FSI should range from 300 to 500% or even more. A higher Floor Space Index (FSI) is proposed in Naya Raipur along transit corridors, in Delhi along Delhi Metro, in Navi Mumbai along transit corridors, andin Kochi along Kochi Metro to tap thedevelopmental impact of the transit stations. The TOD zone in the Government of India's Smart City mission was identified as 500 meters.

The National Transit Oriented Development (TOD) Policy of the Government of India states that the transit station's influence zone should have places for people to engage in activities, such as shopping malls, entertainment venues, such as theatres, and public amenities, such as schools, hospitals, and parks and playgrounds. land uses such as

warehouses, gas stations, cremation sites, prisons, and surface and Impact of Transit Station on Density & Land Use Within its Influence Zone - within walking distance of the inhabitants Multilevel parking needs to be controlled, according to a review of the literature. In order to reduce the use of personal vehicles like two- wheelers, autos, etc., it also insists on making these influence zones more pedestrian-friendly.

5. CONCLUSION

The land use and density inside a transit station's effect zone are affected. The process of changing land use and density takes time, and the amount of time depends on the features of the locale. The modification is advantageous and boosts the area's economy. Due to the development of information technology and the use of personal modes of transportation, the influence of these transit stations cannot be as great as it was in the 1900s on land use and density. The relationship between transit stations and the neighborhoods around them is dynamic and will remain so throughout time. The impact of a transit station is greater in areas where it is complemented by other elements such as vacant land available for development, favorable economic and social conditions in the area, intermodal connectivity provided by the station, already built-out areas with a diverse mix of land uses, population density, etc. Therefore, depending on the features of each place, this influence will vary. [4]

6. SCOPE FOR FURTHER RESEARCH

Since Asian nations like India are exhibiting a high rate of urbanization and the number of people living in these urban centers is growing everyday, there is a need to examine the impact of these transit stations in a larger and comprehensive manner. Since the socio-cultural and economic makeup of the people varies greatly throughout the nations of the Asian Continent, further research is needed to determine the effects of these transit stations on the area under their influence. It's time to comprehend the effects on transit stations within its influence zone in order to formulate a thorough, implementable transit-oriented development policy with development regulations to take advantage of the favorable economic change that will result fromthe operation of these transit stations. [4]

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Case Study on Metro Link from Gandhinagar to Ahmedabad

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1. INTRODUCTION

Government metro project started fromGandhinagar to Ahmedabad metro–link express way to provide a sustainable, convenient, affordable, large scale project throughout the Ahmedabad from Gandhinagar. It has been divided into corridors with directional belts such as north- south corridor and east-west corridor. It has also design no. of population going to use or travel in metro in hour and it has also design platforms for per passenger/ sq.ft. By which they can presume no. of passenger weight, platform can resist and they have also consider safety factor over each and every quantity for safe design with all permissible or allowable criteria.

This project is undertaken by (GMRC) which provides design execution and funding. The total length of rail corridor and every design, planning, and authorized access to land use for metro-link development project. Total budget and per fair charges for public access are provided on their official website of (GMRC) which states that our government is keeping transparence with pubic without any secrecy or any secure access. Theyhave even provided a total cost of project and reports of every minor material detail on their online website. They have also provided details about total length of rails and how many rails are in subways and its underground length.

1.1 PROJECT OVERVIEW

Gandhinagar-Ahmedabad metro rail project is governed by GMRC which resides it's goal to create metro rail for end to end connectivity within shortest span with economic and sustainable with minimum land acquisition, for shortest travel time and list construction cost which directly proportional to public transportation cost GMRC aims to provide effective and affordable transport network for public without any discrimination, withmajor accessibility & mobility.

1.2 PROJECT DETAILS

The total length of Ahmedabad metro rail project phase 1 is 40.03 km in that about 6.5 km is underground and aside that rest of the Railway is elevated section. This project will connect the corners of Ahmedabad city which is North-South corridor and east-west corridor and within that corridor it contains 32 stations. (1)

North-South corridor has length of 18.87 km which will connect Motera stadium to APMC, Vasna and will be passing through Sabarmati, AEC, Sabarmati railway station, Ranip, vadaj, vijaynagar, usmanpura, old High court, gandhigram, paldi, Shreyas, Rajivnagar and Jivraj station. There will be no such underground stations or tunnels are located in North South corridor, it will be completely elevated within these following stations. (1)

The length of east-west corridor is 21.6 km. This corridor originate from Vastral Gaam to Thaltej Gaam and will be passing through Nirant Cross Road, Vastral, Rabari Colony, Amraiwadi, Apparel Park, Kankaria East, Kalupur Railway Station, Ghee Kanta, Shahpur, Old High Court, Stadium, Commerce Six Road, Gujarat University, Gurukul Road, Doordarshan Kendra, Thaltej stations. In east-west corridor there are total 17 stations in which four stations are underground stations; the length of underground railway line is 6.5 km, beside those 13 stations are elevated. In both the

corridors old High court Station will be an interchange station. (1)

North-south Corridor,

- APMC
- Rajivnagar
- Paldi
- Old high court [interchange]
- Vijaynagar
- Ranip
- AEC
- Motera Stadium
- Jivraj Park
- Shreyas
- Gandhigram
- Usmanpura
- Vadaj
- Sabarmati RLY.STN.
- Sabarmati

East-West Corridor,

- Thaltej gam
- Doordarshan
- Gurukul road
- Commerce six road
- Old high court[interchange]
- Ghee khanta
- Kankaria east
- Amraiwadi
- Vastral
- Vastral gam
- Thaltej
- Kendra Gujarat university
- Stadium
- Shahpura
- Kalupur.RLY.ST
- Apparel park
- Rabari colony
- Nirant cross- RD

According to United Nations population estimated Ahmedabad population in 2022 is established to be 845 0228.

Permission required for metro link Gandhinagar Ahmedabad was for 4 million consumers. Since we have already crossed 8 million consumers Ahmedabad is in the state of all population which cannot meet by roadways system for Ahmedabad traffic so with the projected increase in population of the city strengthening of transport infrastructure has assumed urgency for this purpose rail based metro system in the city has considered.

1.3 PROJECT UPDATES:

(a) FUNDINGS:

Name of Corridor	Distance (in km)	Estimated completion cost with central taxes & land cost (INR Crore)
East-West Corridor (Thaltej Gam to Vastral Gam)	20.536	6681
North-South Corridor (APMC to Motera Stadium)	17.23	3994
Interest during construction (IDC)		98
Total	**37.766**	**10773**

Equity of Govt. of India	1990 CR.
Equity of Govt. of Gujarat	1990 CR.
ODA/Loan (JICA)	6066 CR.
Subordinate Debt (SD) from GoG	727 CR.
Total Project Cost:	**10773 CR.**

Fig. project funding's (2)

(b) MILESTONE:

On November 2014, GMRC has sanction order issued by MOUD, GOLL (3)

ON MARCH 2015, Central Government and Gujarat state government are equal partners over converting a special purpose vehicle (SPV) (3)

On October 2015, GMRC has signed a general engineering consultancy contract with SYSTRA S.A. led consortium for Ahmedabad metro rail project phase-1. (3)

Ahmedabad metro rail project phase-1 which provides metro rail services between vastral gam station to apparel park station was inaugurated on, March 2019. (3)

Ahmedabad metro rail train ride between vastral gam station to nirant cross road station. School of AMC for children from Umang deaf and Dump they let the foundation stone for the Ahmedabad metro rail project phase-2 having two metro rail corridors from motera to Mahatma Mandir via Akshardham and from GNLU to gift City with combined length of 28.2 km which is Rs. 5384.17 CR estimated project cost. Ahmedabad metro rail project phase-2 is an extension of North-South corridor of phase-1 from APMC to motera. (3)

Ahmedabad metro rail project phase-2 and links of foundation stone and the ceremony to commence work of Surat metro rail project have been performed by auspicious hands of the honorable prime minister of India Shri Narendra Modi on 18 January 2021.For commence project of Ahmedabad

metro rail phase-2 was performed at Mahatma Mandir sector 13 Gandhinagar. (3)

Tunnel from kalupur launching shaft to Gheekanta station has been completed in the first week of July 2019. On July 2019, 1970 m out of the total scope of 3300 m of underground phase-2 package Tunnel is completed by TBM (tunnel boring machine) from kalupur launching shaft to Gheekanta station break through for both the TBMs are also completed. (3)

(c) PROJECT STATUS:

PRE-CONSTRUCTION PHASE,

Figure: 1

Figure: 2

POST-CONSTRUCTION PHASE,

Vastral Gam Station,

Figure: 3

Figure: 4

Figure: 5

Figure: 6

(D)ENVIRONMENT IMPACT ASSESSMENT:

It is the one of the most important phase of construction which includes EIA(environment impact assessment) report is for the requirement of regulatory agencies and funding agencies (JICA) to provide further funds for project they required a proper confirmation from which includes the impact resulting from the pre-construction of the project by which agencies get notified about environmental clearance into the project by which it gets recognition of environment clearance for notgetting any further obstruction during the project duration. (4) (5)

The MOEF (ministry of environment and forest), government of India, notification of 14th September 2006 and its amendment and list projects in schedule 8(b) "townships and area

development projects" that requires environment clearance. As per notification of metro project does not require environmental from MOEF. (6)

National green tribute said yes for environmental clearance according to 2006 notification schedule 8 (b) but, the Supreme Court gave judgment in 2016 for metro EIA is not mandatory. Where in their statement they says, "Apex codes says eco-clearance not needed for railway, metro project". (7)

On September 2016, THE HINDU published an article about the statement of the Supreme Court which says that, "the Supreme Court stays NGT order asking railway, metro to seek environmental clearance for project". (8) (8)

CONCLUSION:

In the study prime aim was to determine the construction of express metro link between Ahmedabad-Gandhinagar and provide a brief about over-all project which includes from first stone placed to its project worth and how far it progressed. It also enlighten and elaborate about environmental aspect of project which provides brief knowledge of environmental clearance, it also provide and software exposure like SCADA for operation of telecommunication in metro for over watching controlled movements and spread awareness about metro project which is originated in Gandhinagar-Ahmedabad.

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