

Vidya Prasarak Mandal's



# B. N. Bandodkar College of Science, Thane



NAAC Re-Accredited 'A' Grade, Best College Award, University of Mumbai,  
Recipient of FIST 'O' level Grant from DST, RBNQA Commendation Certificate  
Recipient of Grant for Star College Scheme, DBT,  
Selected as College with Potential Excellence.



## Proceedings of the International Conference on **Emerging Technologies For Sustainable Agriculture**

**6<sup>th</sup> & 7<sup>th</sup> January, 2017**

*Organized by*

**Department of Biotechnology and Microbiology**

*In collaboration with*

**Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli**

*Edited by*

Ms. Zahera Momin, Ms. Rutuja Gaikwad,  
Dr. Kalpita Mulye, Dr. Jayashree Pawar

Proceedings of the  
2<sup>nd</sup> International Conference on  
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Jnanadweepa, Chendani Bunder Road, Thane (W) 400 601. Maharashtra*

*in collaboration with*

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## *Convener's Address.....*

*India is known to be an Agriculture country. From age old days agriculture is our main source of income and our economy is based on agriculture.*

*India has its own natural resources which include fertile soil, abundant water, very good sunlight and diverse ecological conditions. This has helped us to be the agricultural country. But as we are aware some of the natural resources are not directly controlled by human and many are getting wasted. Similarly some are not utilized to the fullest extent.*

*For this to make agriculture as sustainable business lot of research is ongoing all over the world. It includes improving the conditions of soil, water, to get constant supply of water, exploration of new varieties of plants/ animals for their food value/ nutritive value, bringing such plants under cultivation practices, animals under domestication.*

*But in addition it also includes experiments like increasing the yield from crop, gene transplantation, hybridization, and creating genetically modified crops/ animals.*

*It gives me immense pleasure as the convener of the conference to expose the students to such research happening in the world. This will help them to use their knowledge in the field of research they choose.*

- *Dr. (Mrs.) M. K. Pejaver*



## *Organizing Secretaries' Address.....*

*A safe and sufficient food supply, grown in an environmentally responsible manner is essential to ensure wellbeing of mankind. Biotechnology as an interdisciplinary science holds considerable promise to meet challenges in agricultural production. New approaches in biotechnology have developed high yielding, more nutritious disease resistant crop varieties. They have also reduced the need for fertilizers and other expensive chemical additives responsible for causing environmental pollution. Plant biotechnologists play a key role in the massive in vitro production of improved crop varieties through tissue culture as well as preservation of germplasm.*

*In view of conventional farming getting deviated from sustainability, the youth need to adopt new technologies to increase agricultural productivity, and feed the growing world population along with protecting the environment. Hence, there is an urgent need to engage youth in agriculture. Considering current non professional approach practiced by most stakeholders, dissemination of the knowledge to the youth- the probable future farmers- who would play a key role in bringing in an agricultural reform is mandatory. With the intention to become instrumental in shaping up 'agro-professionals', we decided to bring together leading academic scientists, research scholars and industry professionals to exchange and share their experiences and research results about various aspects of Agriculture and Biotechnology.*

*This international interdisciplinary platform would also provide a premier interdisciplinary forum to present and discuss the most recent innovations, trends, concerns and practical challenges encountered along with the solutions adopted in the form of invited talks, research paper presentations, poster presentations and discussions. Such platform would initiate a dialogue amongst all the stakeholders and be instrumental in generating and motivating young Agro-Entrepreneurs.*

*Organizing this International conference was a great enriching experience for us and has turned out to be a memorable journey!!*

*- Dr. Kalpita Mulye and Dr. Jayashree Pawar*

## ***Editor's Note....***

*This publication contains the proceedings of the two-day International Conference on "Emerging Technologies for Sustainable Agriculture" held at VPM's B. N. Bandodkar College of Science, Thane on January 6-7, 2017.*

*Over the years, B. N. Bandodkar College of Science has been well-known for its efforts in initiating state and national level scientific gatherings for inculcating research aptitude in students. This is the 2nd International conference and we believe it will help in widening the horizons further.*

*The theme of the conference is Emerging Technologies for Sustainable Agriculture. Sustainable Agriculture is economically viable, socially supportive and ecologically sound. It can indefinitely sustain itself without degrading the land, the environment or the people. Hence, one of the aims of the conference is to kindle interest amongst the young minds about Agricultural Biotechnology which may prove as an impetus towards the betterment of society and ecosystem.*

*The conference seeks to provide a platform for an open dialogue amongst the eminent personalities contributing to the field of Agricultural Biotechnology viz. Academicians, Industry professionals, Scientists from Research Institutions, Agro-Entrepreneurs and all the other participants. This interdisciplinary scientific gathering will prove to be a great opportunity for getting acquainted with the most recent innovations through invited talks, research paper presentations and poster presentations.*

*Through this proceeding we have made a humble effort to put together the vast knowledge disseminated through the conference as well as the pre-conference workshops.*

*We are grateful to Vidya Prasarak Mandal, the convener Dr. M. K. Pejaver and the Organizing Secretaries Dr. Kalpita Mulye and Dr. Jayashree Pawar for giving us the opportunity to work on the conference proceedings. Thanks are also due to the entire teaching and non-teaching staff of the Department of Biotechnology & Microbiology.*

*- Ms. Rutuja Gaikwad & Ms. Zahera Momin*

## SCHEDULE

Time	<b>DAY I: 6<sup>th</sup> January 2017</b>
08.45 - 09:30	<b>Registration</b> and breakfast
09:30 - 09.50	Inaugural function
	<b>TECHNICAL SESSION I</b>
	<i>Chairperson: Dr. M. A. Deodhar Rapporteur: Dr. Bela Nabar</i>
09.50 - 10.30	<b>Key note address:</b> Biogeochemical interfaces in soil: Formation, properties and function  <b>Dr. Kai U. Totsche</b> Department of Hydrogeology, Institute of Geoscience, Friedrich-Schiller-University Jena, Burgweg 11, Jena, Germany
10:30 - 11:10	<b>Invited talk 01:</b> Culturable Microbes and Metagenome of Plant Ecosystem for Sustainable Agriculture  <b>Dr. P. U. Krishnaraj</b> Department of Agricultural Microbiology, College of Agriculture, Vijayapura University of Agricultural Sciences, Dharwad, India
11:10 – 11.30	<b>Tea &amp; Coffee Break</b>
11.30 - 12.10	<b>Invited talk 02:</b> Sustainable Farming  <b>Dr. Rajas Warke</b> Director, Agriculture Division, Warkem Biotech Pvt. Ltd., Mumbai, India
12.10 - 12:50	<b>Invited talk 03:</b> Role of IPRs in agricultural biotechnology  <b>Dr. S. R. Torane</b> Deputy Director of Research, Dr. B. S. Konkan Krishi Vidyapeeth, Dapoli, Maharashtra, India
12.50 - 13:45	<b>Lunch</b>
	<b>TECHNICAL SESSION II</b>
	<i>Chairperson: Dr. P. U. Krishnaraj Rapporteur: Dr. Lolly Jain</i>
13.45 - 15.15	<b>Oral Paper presentations</b>
15.15 - 15.55	<b>Invited talk 04:</b> Functional analyses of G-Protein Coupled Receptors (GPCRs) in rice blast fungus  <b>Dr. Subhankar Roy Barman</b> Associate Dean, Department of Biotechnology, National Institute of Technology, Durgapur, West Bengal, India.



15.55 - 16.15	<b>Tea &amp; Coffee Break</b>
16.15 - 16.55	<b>Invited talk 05:</b> Buldhana Urban – Social Banking Model  <b>Dr. Sukesh Zamwar</b> CEO – Buldhana Urban Credit Co-Op Society India, Maharashtra, India
	<b>DAY II: 7<sup>th</sup> January 2017</b>
09:00 - 10.30	Breakfast and <b>Poster presentations</b>
	<b>TECHNICAL SESSION III</b>  <i>Chairperson: Dr. S. R. Barman    Rapporteur: Dr. Minal Dukhande</i>
10.30 - 11:10	<b>Invited talk 06:</b> Unravelling plant metabolism through the integration of heterogeneous data from metabolomics, genetics and informatics  <b>Dr. Asaph Aharoni</b> Department of Plant Sciences, The Weizmann Institute of Science, Rehovot, Israel
11:10 - 11.30	<b>Tea &amp; Coffee break</b>
11.30 - 12:10	<b>Invited talk 07:</b> Molecular Markers and its Utilization for Enhancing Breeding Technologies <b>Dr. N. B. Gokhale</b> Incharge , Plant Biotechnology Centre, College of Agriculture, Dapoli, Dr. B. S. Konkan Krishi Vidyapeeth, Dapoli, Maharashtra, India
12:10 - 12:40	<b>Invited talk 08:</b> Modulation of Plant-Metal Interactions by Rhizosphere Bacteria  <b>Dr. G. Archana</b> Professor and Head (I/c), Department of Microbiology, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India
12:40 - 13.40	<b>Lunch</b>
	<b>TECHNICAL SESSION IV</b>  <i>Chairperson: Dr. Aruna K.    Rapporteur: Dr. Tara Menon</i>
13.40 - 14.20	<b>Invited talk 09:</b> Crop Protection from Lepidopteran insect pests: Scope and challenges  <b>Dr. Ashok Giri</b> Sr. Scientist, Plant Molecular Biology Unit, Division of Biochemical Sciences, CSIR-National Chemical Laboratory, Pune, Maharashtra, India
14.20 - 15.50	<b>Oral Paper presentations</b>
15.50 - 16.10	<b>Tea &amp; Coffee Break</b>

16.10 - 17.15	<b>Valedictory Address:</b> Genomics Accelerating Plant Research in India  <b>Dr. Malali Gowda</b> <ul style="list-style-type: none"><li>• Professor, Genomics Discovery Program, School of Conservation, Life Science and Health Sciences, Trans Disciplinary University, Foundation of Revitalization of Local Health Traditions (FRLHT), Bengaluru, India</li><li>• Founder, DNA life</li><li>• Technology Advisor, Bengaluru Genomics Center, India</li></ul>
17.15 - 17.30	<b>Concluding and vote of thanks</b>

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# SECTION I

KEYNOTE ADDRESS

AND

INVITED TALKS





Keynote Address

## **BIOGEOCHEMICAL INTERFACES IN SOIL: FORMATION, PROPERTIES AND FUNCTION**

***Kai U. Totsche***

*Professor and Chair of Hydrogeology, Friedrich-Schiller-University Jena, Germany*



Soil sustains life. It is vital for the production of food and fibre which helps to feed an ever-growing population and also for the provision of the Earth's primary renewable resources. Soil is the focal and connecting link between the information, matter and energy cycles of the hydrosphere and the atmosphere in the critical zone and plays a central role as a transformer, buffer, accumulator and filter of water, dissolved and suspended particles. The drinking water most people use comes from groundwater reservoirs. Its quality and quantity are largely controlled by the nature of the soils it passes through. And more: About 25% of the atmosphere's carbon dioxide comes from biological oxidation processes in the pedosphere, which contains twice as much carbon as the atmosphere and up to three times the carbon in all vegetation. Soil is built up of a dynamic and frequently hierarchically organized system of aggregates with the microaggregates <math><250\mu\text{m}</math> as essential structural units in nearly all soils (Totsche et al. 2016). The different contacting organic, inorganic and biological components define a complex and hierarchically structured and extremely large and heterogenous

biogeochemical interface (BGI, Totsche et al. 2010) to soil's liquid and gaseous phases, which extends through and within the voids and pores of soils aggregate system. The physical and chemical heterogeneity of these interfaces is the source of a multitude of habitats and supports a vast biological and functional diversity and abundance. The processes at these BGI and within the aggregates ultimately control the fate and transport of nutrients and contaminants. The talk stresses the role of the aggregate associated biogeochemical interfaces for storage and turnover of water, elements, pollutants, and information. It also sheds light on the formation studied with natural and artificial soils (e.g. Pronk et al. 2016) and properties of BGI using sophisticated spectro-microscopic techniques (Rennert et al. 2012) and their role for the fundamental functions of soils that are the basis for all soil-based ecosystem services including plant productivity, water and air quality ultimately.

Further information can be found here: [www.hydro.uni-jena.de](http://www.hydro.uni-jena.de), [www.spp1315.uni-jena.de](http://www.spp1315.uni-jena.de), and [www.madsoil.uni-jena.de](http://www.madsoil.uni-jena.de).

## CULTURABLE MICROBES AND METAGENOME OF PLANT ECOSYSTEM FOR SUSTAINABLE AGRICULTURE

**Dr. Krishnaraj P U**

*Professor and Head*

*Department of Agricultural Microbiology, College of Agriculture, Vijayapura  
University of Agricultural Sciences, Dharwad*



Microorganisms dominate the earth. The structure of microbial communities from many environmental samples is highly complex and diverse. The studies of these microbes have been based on the organisms that are amenable to *in vitro* cultivation. A large fraction of the diversity in an environment is still unknown due to difficulties in enriching and isolating microorganisms in pure culture. Correspondingly, the diversity of enzymes catalyzing a certain reaction is only partially known. The classical and cumbersome approach for isolating microbes with a particular activity or products from environmental samples is to enrich, isolate, and screen a wide variety of microorganisms for the desired activity or product.

Recent analysis using molecular and biochemical approaches have led to the realization that only 99 % is culturable. This has grossly limited our understanding of the ecology, physiology and genetics of these predominant life forms. The advent of genomics and large-scale gene sequencing has led to the development of a new concept: the metagenomics- the genomics of the uncultivable. The genomic and genomic enabled approaches could provide a way to directly understand the microbial diversity and activity in its geochemical and ecological context.

If the diversity of chemistry produced by the culturable bacteria is an indicator of the chemical capacity of the uncultured bacteria, then many molecules, and perhaps useful chemicals, remain to be discovered from soil microorganisms using advanced molecular

biology approaches. The approach in metagenomics that aims at creating a biodiversity library is to directly isolate the DNA from diverse environmental samples, purify, restriction-digest and clone into suitable cloning vectors to construct complex environmental (metagenomic) libraries and study heterologous gene expression in various model species. A number of reports indicate that the metagenomic approach is technically feasible and can reveal novel biology and chemistry. Such approaches have led to the identification and synthesis of novel compounds including terragine, violacein, polyketide antibiotics. A major reason for the isolation of DNA from enrichment cultures arose from the difficulties associated with the isolation of high-molecular-mass DNA from microbial habitats. However, during the last decade different approaches for the isolation and purification of bacterial DNA from a variety of environments have been reported and have been used for the construction of DNA libraries leading to the isolation of genes with desirable industrial traits.

However, there is still enough importance to use the culturable bacteria. Especially, under an inoculums consortia mode. More recently, attention has been on the water stress management using microbes through what is referred to as induction of systemic tolerance (IST)' in plants under different abiotic stresses such as drought and salinity through physical and chemical changes in the rhizosphere and plants. The microbes are known to colonize the rhizosphere of different plant species and confer additional beneficial effects finally

stimulating plant growth various mechanisms. Certain microbes produce cytokinins and antioxidants such as catalase, which result in ABA accumulation and ROS degradation, respectively and degradation of the ethylene precursor ACC by bacterial ACC deaminase releases plant stress and rescues normal plant growth under drought and salt stresses and emitted by PGPR down regulate hkt1 expression in roots but up regulate it in shoot tissues, orchestrating lower Na and recirculation of Na<sup>+</sup> in the whole plant under high salt conditions. Production by PGPR of IAA or unknown determinants can increase root length, root surface area and the number of root tips. However, to be successful, they must be proficient to colonize the roots, must survive, multiply and compete with other microbiota and be in soil for at least the time needed to express their plant growth promotion/protection activities. It has been recognized that a consortia of microbes

perform better in many occasions and niches.

Many interactions between organisms in the soil are based on the emission and perception of volatiles and they work as communication signals for species-specific interactions. Beneficial microorganisms can stably colonize the rhizosphere of various plant species, and promote plant growth by improving soil structure and moisture retention as well as by enhancing plant mineral-nutrition absorption, These need to be considered in the future as valuable info-chemicals to understand the entire integrity of the ecosystems and their ability to provide conditions for augmenting plant growth in adverse conditions as well. Biochemical interactions, metagenomics and metatranscriptomics should be studied leading to rhizosphere engineering/microbiome modification to optimize plant functions of interest and increase crop yields.

## SUSTAINABLE FARMING

*Dr. Rajas Warke*

*Director: Molecular Biology and Virology, HiMedia Laboratories Pvt. Ltd.,  
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Green revolution of 1960's brought dawn to the industrial agriculture. It showed crops grown in monoculture, with new hybrid varieties, mechanization of agricultural practices, use of chemical fertilizers and pesticides boosted the agricultural productivity exponentially. But this productivity didn't sustain for long. It started showing negative effects on cost of crop production, productivity measurements and farm enterprise budget. Uncontrolled use of agricultural fertilizers and pesticides led to contamination of water bodies such as ground water, wells, streams, rivers etc. This started showing its harmful effects on aquatic ecosystem, hypoxic zones, on agricultural workers and posed threat to food safety. Such agricultural practices with excessive fertilization and pesticide application adversely affected the soil quality, physico-chemical properties through soil erosion, compaction, acidification, salinization, and none the less biological health of the soil. Industrial agriculture completely depends on non renewable energy sources especially petroleum which cannot be sustained indefinitely. This type of agriculture uses and depletes natural resources & pollutes environment which in turn loses its productivity.

Warkem Biotech Pvt. Ltd. has approached modern agriculture with a Scientific and Holistic view of sustainable agriculture. Sustainable is derived from Latin word 'Sustinere' (Sus – from below and tenere – to hold) to keep existence or maintain long term support or permanence. Sustainable agriculture uses the principle of Ecology & studies relationship between organisms and their environment which in turn helps in

conservation. It ensures economic viability of farm operations, enhances productivity with improved nutritional value of yield & upgrades the quality of life for the farmer and the society as a whole. Warkem Biotech understands the exact need of sustainable agriculture and has focused on innovation and manufacturing of highest purity genotypically confirmed and potent Non-Toxic BioPesticides, BioNematicides and BioFertilizers not only reducing the total reliability on toxic chemical pesticides, fertilizers etc. but also increasing productivity and improving overall soil health.

In an endeavor to empower the farmer, Warkem has developed and launched a Free Online Portal ([www.warkembioagri.com](http://www.warkembioagri.com)) where any farmer can enter soil NPK test values obtained using Warkem portable soil test kit and select the crop to display Government standards for that crop. Free advice is immediately seen on the website so that farmer can add the exact amount of nutrients that are deficient in his soil.

### **Warkem BioAgri Solution (Inputs) For Sustainable Farming:**

Warkem Biotech Pvt. Ltd. was started in 1978 with the mission "Make In India" world class products with indigenously developed technology manufactured in State-of-Art facilities to make India self-reliant. With a vision to Empower The Farmer, Warkem Biotech has developed novel solutions like Portable Soil and Petiole Testing Kits, BioStimulant, BioFertilizers, BioPesticides, BioNematicides, Soil conditioners, Adjuvants, Agricultural Diagnostic kits, Insect attractants or traps etc. These products are reliable, easy-

to-use, non-toxic, biodegradable and environment safe to allow sustainable farming for farmers following conventional agriculture,

organic agriculture, biodynamic, permaculture, agro-ecological systems, low input agriculture and more.

## ROLE OF INTELLECTUAL PROPERTY RIGHTS (IPR) IN BIOTECHNOLOGY

***Dr. S. R. Torane***

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Intellectual property is an intangible creation of the human mind, usually expressed or translated into a tangible form that is assigned certain rights of property. Intellectual property rights (IPR) can be defined as the rights given to people over the creation of their minds. They usually give the creator an exclusive right over the use of his/her creations for a certain period of time. The different types of Intellectual Property Rights are such as i) Patents, ii) Copyrights, iii) Trademarks, iv) Industrial designs, v) Integrated Circuits layout design, vi) Geographical indications of goods, vii) Biological diversity, viii) Plant varieties and farmers rights, ix) Undisclosed information or trade secrets.

George Alfred DePenning is supposed to have made the first application for a patent in India in the year 1856 He was granted a patent for his invention such as "An Efficient Punkah Pulling Machine".

The some of the patents in biotechnology are namely 1) Cohen-Boyer recombinant DNA patent, 2) Cell line derived from human leukemia patient (1984), 3) Genetically engineered corn (1986), 4) Harvard Onco - mouse patent (1988), 5) Isolated human bone marrow stem cells patent. (1991)

Potential Areas for Investment in the Indian Biotechnology Sector are 1) Agriculture and Food Biotechnology 2) Industrial Biotechnology 3) Preventive and Therapeutic Medical Biotechnology 4) Regenerative and

Genomic Medicine 5) Pharmaco-genomics 6) Bio-engineering and Nano-biotechnology 7) Bio-informatics and IT Enabled Biotechnology and 8) Clinical Biotechnology and Research Services. Certain products in recombinant research are also known by their trademarks such as Pfizer, Mahico, Monsanto etc. Trade secrets often include private proprietary information or physical material that allows a definite advantage to the owner. Trade secrets in the area of biotechnology may include material like (i) Hybridization conditions, (ii) Cell lines, (iii) corporate merchandising plans or (iv) customer lists. Unlike patents, trade secrets have an unlimited duration and, therefore, may not be required to satisfy the more difficult conditions laid down by law for patent applications.

Choice of proper form of intellectual property protection in biotechnological products is essential. The intellectual property in biotechnology can be protected by patents, trademark, copyrights, and trade secrets. Pace of technological development plays important part if it is rapid then a trade secret approach may be preferable to patenting. Secondly associated costs of invention protection is also important. The cost of secrecy may exceed the cost of obtaining a patent, but it may be more expensive to enforce the patent, so that one will have to decide, whether trade secret is a better protection. However patent provides a better strong legal protection which is not applicable in trade secrets.

## FUNCTIONAL ANALYSES OF G-PROTEIN COUPLED RECEPTORS (GPCRS) IN RICE BLAST FUNGUS

**Dr. Subhankar Roy Barman**

Department of Biotechnology, National Institute of Technology, Durgapur



We have analyzed and characterized a total of twelve Conserved Fungal-specific Extra-cellular Membrane-spanning domain (CFEM) subfamily of GPCRs predicted in *M. oryzae in silico*. Primary structure analysis showed all the proteins are highly hydrophobic in nature due to the high content of non-polar residues. The aliphatic index computed by Ex-Pasy's ProtParam infers that these proteins are thermostable for a wide range of temperature. Secondary structure analysis shows that these proteins have predominant  $\alpha$ -helical structures. The very high coiled structural content of most of the sequences is due to the rich content of more flexible glycine and hydrophobic proline amino acids. The number of cleavages and the site of cleavages of the putative GPCRs were calculated by the Ex-Pasy's peptide cutter tool. Different GPCR prediction tools were used to compare the presence of seven transmembrane domains in these proteins. The presence of disulphide (-S-S-) bonds in these proteins was predicted by CYS\_REC tool, and the 3D structures were generated by threading method obtained from PHYRE2, which were visualized in Swiss-PdbViewer. Overall, this is first time *in silico* analysis of physico-chemical properties as well structural biological studies, which provide insight into tertiary structure of the twelve putative CFEM sub-family GPCRs in *M. oryzae*. This will be useful to understand the mechanism of these GPCRs and also to predict ligand partners based on which different fungicides can be designed to combat this deadly fungal pathogen. Further, we have identified and characterized a unique Conserved Fungal-specific Extra-cellular Membrane-spanning

(CFEM) domain containing *PTH11* like G-protein coupled receptor (GPCR), responsible for Water wettability, Infection, Surface sensing and Hyper-conidiation (*WISH*), a pathogenicity gene in the blast fungus of rice, *Magnaporthe oryzae*, one of the deadliest pathogens of rice. The mutant is nonpathogenic due to a defect in sensing hydrophobic surface cues and appressorium differentiation. This defect is evocative of wild type strains such that conidia germinate and undergo early differentiation stages, but appressorium maturation is diminished on non-inductive hydrophilic surfaces. No functional appressoria were found in the  $\Delta wish$  mutant, suggesting that the protein encoded by *WISH* protein is required for appressorium morphogenesis and is also involved in host surface recognition. We have assayed the facets of pathogenesis and the results indicated involvement of *WISH* in preventing autolysis of vegetative hyphae, determining surface hydrophobicity of vegetative hyphae and maintenance of cell-wall integrity. *WISH* gene from *M. oryzae* strain B157 complemented the  $\Delta wish$  mutant, indicating functional authenticity. Exogenous activation of cellular signaling failed to suppress *WISH* defects. These findings suggest that *WISH* GPCR senses diverse extracellular signals to play multiple roles and may have effects on *PTH11* and *MPG1* gene especially as an upstream effector of appressorium differentiation. It is for the first time that a typical GPCR containing seven transmembrane domains and involved in the early events of pathogenesis has been functionally characterized.



## BULDANA URBAN: SOCIAL BANKING MODEL

*Dr. Sukesh Zamwar*

*CEO, Buldana Urban Credit Co-op Society, Maharashtra, India*



Buldana urban is largest co-operative credit society in Asia Pacific region and whose size is around 10000 crore and member base is around 1000000. Total branches 401 and ware houses around 360 and employee around 7000. Buldana urban has received the highest award of cooperative in the Asia pacific region in the form of CUMI award and the highest national award from NCUI in the credit union sector.

Buldana Urban coop credit society has increased GDP of one million people by twice. This was possible due to the execution of three innovative strategies, which are as follows:

- 1) four pillar system
- 2) closed loop economic system
- 3) social banking

Buldana urban is an example of **EVOLUTION OF SOCIAL BANKING**

Principle behind social banking **“PEOPLE’S MONEY SHOULD BE UTILISED FOR WELL BEING OF PEOPLE”**

Buldana urban also provides loan like gold loan, ware house loan, term loan, cash credit

loan, farmer loan and other loans like personal loan, crop loan, vehicle loan etc.

It has also executed several projects like dam excavation to solve the water problem of Buldana city and nearby villages at Yelgaon. This help in increasing the capacity of dam by 50 million cubic liters.

Buldana Urban offers water ATMs, Vedic schools, hotels, bridges, spinning mills and even guest houses at religious places such as Shirdi and Tripati. Buldana urban plants 50000 trees every year. It has adopted a village of population 50000 and is trying to implement facilities like biogas, biomass, solar and wind energy. It is trying to provide urban amenities in rural area, like roads, potable water, street lights, underground drainage etc.

Buldana urban has started school at various places under Buldana Urban Charitable Trust named as Sahakar Viday Mandir. It has also developed innovative strategies like Low cost spinning mill, water ATM etc.

## UNRAVELLING PLANT METABOLISM THROUGH THE INTEGRATION OF HETEROGENEOUS DATA FROM METABOLOMICS, GENETICS AND INFORMATICS

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The term METABOLISM describes all the chemical reactions and interactions that take place in a biological system. The regulation of metabolic pathways is constantly tuned in order to suit the needs of development and fitness. Our main research objective is to unravel networks of genes and proteins which coordinate the activity of metabolic pathways during plant development and stress response. An integrated investigation of several members of the Solanacea family (particularly tomato, potato and eggplant), rather than studying a single plant, provided us with unprecedented insights to metabolic biology in these species. Most if not all processes characterized, impact to a certain degree key quality, nutritional and post-harvest traits of these crop plants. Integrating cutting-edge

transcriptomics, proteomics and metabolomics tools together with genes co-expression assays were of great value in making several key discoveries. In a recent example, combined co-expression analysis and metabolic profiling in tomato and potato led to the discovery of the multi-step, core pathway leading to the formation of the renowned Solanum Alkaloids. This class of cholesterol-derived molecules represent important anti-nutritional compounds in these crop plants. The talk highlights several advanced technologies and genetic research tools and the invaluable knowledge on core metabolic traits obtained through combining them in a single study. Most if not all could be applied in the coming years to the study of key traits in other, less studied plant species.

## MOLECULAR MARKERS AND ITS UTILIZATION FOR ENHANCING BREEDING TECHNOLOGIES

**Dr. N. B. Gokhale**

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Dr. B. S. Konkan Krishi Vidyapeeth, Dapoli*



### Introduction

Genetic markers are one of the advances which have occurred in the genomics era. Among genetic markers, molecular markers mainly because of their abundance, are the most widely used of them. Development of molecular markers has greatly altered genetics and plant breeding. Genetic markers indicate the genetic differences between not only the organisms but also within the species. Some studies which were conducted during the last decade of the 20<sup>th</sup> century reported numerous DNA markers that have been utilized in plant breeding programs. Apart from the application of molecular markers in the construction of linkage maps, they have numerous applications in plant breeding such as assessing the genetic variations within cultivars and germplasm. The most interesting application of molecular markers is marker-assisted selection (MAS). Suitable DNA markers should be polymorphic in the DNA level and can be expressed in all tissues, organs, and various developmental stages. Compared with traditional breeding programs, molecular markers can increase the efficiency and effectiveness of breeding programs. DNA markers have developed into many systems based on different polymorphism-detecting techniques or methods (southern blotting – nuclear acid hybridization, PCR – polymerase chain reaction, and DNA sequencing) (Collard et al., 2005), such as RFLP, AFLP, RAPD, SSR, SNP, etc.

### Diversity analysis

Assessment of genetic variation in Mango (*Mangifera indica* L.) Cv. Alphonso is fundamental for the conservation of genetic resources and utilization in breeding programme. The objective of this study was to assess the genetic variation in mango (Cv. Alphonso) at various locations in Konkan region. RAPD profile for all Mango plants of various location (Cv. Alphonso) were generated with 38 random decamer primers. Out of 38 primers screened 10 primers gave scorable DNA fragments and each of the 10 random primers revealed polymorphism. These primers generated 201 DNA fragments in the average range of 348.3 bp to 812.2 bp, of which 140 were polymorphic. The average level of polymorphism generated by the primers was high (67.37%). The primers OPF-20, OPM-12, and OPU-08 produced distinct RAPD patterns (100% polymorphism) for all the Mango plants.

Assessment of genetic variability within *Vigna unguiculata* (L.) Walp. is fundamental for the conservation of genetic resources and its utilization in hybridization programme. The objective of this study was to estimate the genetic diversity among 30 genotypes of cowpea through molecular characterization by using RAPD markers. RAPD profiles for all 30 genotypes were generated with 20 random decamer primers. Out of 20 primers screened 17 primers gave scorable DNA fragments. The primers generated 1238 DNA fragments in the average range of 381.94 bp to 1131.71 bp, of which

908 were polymorphic. The level of polymorphism generated by the primers was high (71.20%). The primers OPA-04, OPA-05, OPC-02, OPC-05 and OPC-08 produced distinct RAPD patterns (100% polymorphism) for all the 30 genotypes. This study could identify the diverse genotype like DCP-11 and Pusa Phalguni for their use in hybridization programme of cowpea.

A total of 35 SSR primer pairs distributed throughout the genome were used for molecular analysis of 23 rice varieties. All 35 microsatellite markers were found to be polymorphic. The average size of amplified products ranged from 121.86 bp to 217.14 bp. A total of 184 alleles were obtained using 35 SSR primer pairs with an average of 5.26 alleles per primer. The number of alleles amplified for each primer pair ranged from 2 to 8. The markers RM-343, RM-112 and RM-224 generated a maximum number of alleles (8). While the primers RM-315 and RM-223 produced minimum number of alleles (2). The PIC values of primers ranged from 0.23 in SSR primer RM 315 to 0.78 in SSR primers RM 318, RM 276 and RM 343 with an average PIC value 0.58 of all the primers. Resolution factor (Rf) values of primers ranged from 0.574 in primer RM318 to 0.763 in SSR primer RM85 with an average Resolution factor (Rf) value 0.675 of all the primers. The minimum Resolution factor (Rf) value is 0.574 of the primer RM-318 while, maximum Resolution factor (Rf) value is 0.763 of the primer RM-85.

#### **Sex specification**

Determination of sex in nutmeg is of utmost importance from the commercial agricultural point of view, since the sexuality cannot be distinguished prior to flower initiation. The use of bio-molecular techniques in sex determination of nutmeg presents a potential theoretical significance and economic value. The nutmeg DNA showed a poor amplification with RAPD primers studied. Out of the total 60 decamer primers

used in the investigation, only 14 primers showed amplification. Primers OPA-14, OPA-15, OPQ-16 and OPQ-04 showed polymorphism which can be used to differentiate male and female plants. A large number of RAPD primers failed to amplify. However, the information generated by few polymorphic primers was able to differentiate in between male and female plants. Such information will help the farmers to identify the sex of the saplings at an early stage and avoid their economic loss. Thus widening the probability ratio of male to female plants 1:9 as compared to present ration of 1:1 increasing more females, thereby increasing the yield of nutmeg almost two folds is possible.

Investigation was conducted to identify sex linked molecular marker for gender identification of kokum (*Garcinia indica* choicy) at seedling stage. To study sex linked marker, sixty RAPD markers and eleven ISSR markers were employed for gender identification of kokum. Among the 60 RAPD primers 20 primers showed amplification out of which OPA-01, OPA-06, OPD-04, OPD-12, OPD-15 and OPQ-12 showed 100% amplification either in male and female. The primers OPA-03, OPA-05, OPD-01, OPD-02, OPD-05, OPD-11, OPQ-09 and ISSR primer UBC-807 showed polymorphism between both male and female plants. Among these 8 primers OPA-05 showed male specific banding pattern ranging from 450 bp-700 bp which was 80% reproducible.

#### **Marker Assisted Selection**

The investigation was conducted to screen Biotic and Abiotic stress tolerance germplasm from 100 germplasm of Rice. Blast resistance alleles were observed in the 32 rice genotypes. Salt tolerant alleles were observed in the 60 rice genotypes. Bacterial Blight resistant genotypes were 16. Gall midge resistant 18 genotypes observed. While in 7 germplasm observed tolerant to drought condition.

### Hybrid Purity Testing

The investigation was conducted for purity testing of five hybrids (Sahyadri, Sahyadri-2, Sahyadri-3, Sahyadri-4, Sahyadri-5.) released by Dr. B. S. Konkan Krishi Vidyapeeth, Dapoli and their parents through SSR markers.

Numerous articles that consist of DNA markers are available. But, if all markers are useful? To answer the question above, it should be noted that desirable genetic markers should have the following features:

- a) Show high level of genetic polymorphism
- b) Be co-dominant (heterozygous individuals can be distinguished from homozygous)
- c) Allelic features should be clearly distinguished in them (so, the different alleles can be easily detected)
- d) Have appropriate distribution throughout the genome
- e) Have neutral selection
- f) Have an easy tracking (the entire process can be automated easily)
- g) Low-cost genotyping
- h) Have a high repeatability (the data can be stored and shared between laboratories). Suitable DNA markers should be polymorphic in the DNA level and can be expressed in all tissues, organs, and various developmental stages.

#### Significance of Molecular markers:

**1) Time saving:** genomic DNA can be isolated from any part of the plant tissue at every stage of its development, and target trait information can be obtained with linked DNA markers

before pollination, thus allowing breeders to carry out more informed genetic crosses.

**2) Stability and reliability:** phenotypic evaluation of genetic traits is often complicated by environmental factors. However, DNA markers are mostly neutral to environmental variation. The breeder can evaluate their material independently of the environmental conditions (environmental conditions can be favourable or unfavourable for morphological and biochemical marker expression).

**3) Biosafety:** diagnostic tests for the presence or absence of traits for disease resistance can be conducted by DNA markers tightly linked to the target gene without resorting to pathogen inoculation in the field or greenhouse. Additionally, molecular markers facilitate introgression of genes into elite cultivars in advance of the occurrence of certain races of diseases or biotypes of insects.

**4) Performance:** evaluation of breeding lines in early generations of the breeding process with DNA markers can allow breeders to reject progenies from the programme and improve the genetic quality of breeding materials.

**5) Precise selection of the complex traits:** polygenic traits are often difficult to select for using conventional breeding approaches. DNA markers linked to QTL allow them to be treated as single Mendelian factors. Beside analyzing and selecting the interesting characters, molecular markers allow the researchers also to analyze the wild species with potential interest for the breeding program.

## MODULATION OF PLANT-METAL INTERACTIONS BY RHIZOSPHERE BACTERIA

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Heavy metal contamination of agricultural soils leads to diverse environmental and ecological problems including microbial community shift, plant growth and yield reduction, deterioration of soil and entry of metal in the food chain. A common practice of phytoremediation is to develop plant systems that hyperaccumulate the metal in plant parts. This process may be enhanced by inoculating the plants with specific metal resistant bacteria that promote plant growth and assist metal accumulation in plant tissues. However, high accumulation of metals in limits plant growth. The selection of the microorganisms capable of promoting plant growth in metal polluted environments with minimum or no accumulation of the heavy metal in edible parts is essential. Specially for heavy metal like Cadmium which enters the food chain and accumulates in animal and human tissues causing several health hazards. The traits and mechanisms possessed by certain metal resistant rhizobacteria that ameliorate metal stress to plants will be discussed along with the possible mechanisms how metal accumulation may be reduced by microbial inoculants. A multimetal resistant *Enterobacter* sp C1D was found to enhance growth of crop plants in presence of cadmium, lead and Chromium. The bacterium has multiple plant growth promoting traits which improved the plant growth in presence of these metals.

Another aspect of heavy metal pollution is to understand the affect of phytotoxic metals affects on the soil microbial

community and its interaction with plant roots. Our laboratory used copper as it is an essential metal as well as toxic above threshold levels. The response of rhizobacterial communities upon Cu perturbation was investigated in mung bean (*Vigna radiata*) plants using culture-independent and -dependent Denaturing Gradient Gel Electrophoresis (CI-DGGE and CD-DGGE) fingerprinting techniques. Rhizobacterial community shifts were noticed upon Cu amendment. In group specific PCR-DGGE, a negative impact was seen on  $\alpha$ -Proteobacteria followed by  $\beta$ -Proteobacteria while no significant changes were observed in Firmicute and Actinomycete populations. In presence of Cu, plants showed toxic effects by reduction in growth and elevated Cu accumulation, with root system being affected prominently. It can be concluded that Cu level above 250 mg /kg adversely affected the rhizobacterial communities. Additionally,  $\alpha$ -Proteobacteria was found to be a sensitive bio-indicator for Cu toxicity and is of particular significance since this group includes majority of plant growth promoting rhizobacteria.

Studies on *Ensifer* (*Sinorhizobium*) *meliloti* 1021, a symbiotic diazotrophic bacterium belonging to  $\alpha$ -Proteobacteria, and its plant host *Medicago truncatula* have revealed that high Cu concentration induced morphological changes in Em1021 affecting key molecules in the early symbiotic processes such as exopolysaccharides and lipopolysaccharides. Negative effects were seen in the motility, root attachment and

biofilm formation ability of Em1021. Cu inhibited root hair formation and modified root surface which in turn may have reduced root attachment of Em1021. Proteomic studies revealed that Cu stress resulted in over-

expression of proteins such as GroEL (60 kDa chaperonin) and WrbA (NAD(P)H dehydrogenase) indicating elevated stress status.

## CROP PROTECTION FROM LEPIDOPTERAN INSECT PESTS: SCOPE AND CHALLENGES

***Dr. Ashok P. Giri***

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We have identified several wound-inducible Pin-II proteinase inhibitors (PIs) that have role in plant protection from insect attack. These defensive molecules have been studied for their structural and functional diversity, and further relevance in how they could be useful for designing crop protection strategies. *In vitro* and *in vivo* studies revealed strong activity of PIs against cocktail of insect pest's proteases. Modern tools provide greater impact in improving delivery and application of novel molecules for crop protection. Judicious uses of such molecules via advancement in formulation technologies successfully demonstrated using nano- or micro-based formulation, which contributes to enhance activity, greater stability, and

biodegradability. Microemulsions and nano-materials provided flexibility to modulate according to the PIs to be incorporated or conjugated. We have optimized formulation with a careful selection and design engineering of materials for the maximum loading, greater firmness, enhanced efficacy and controlled release of candidate PIs. In addition, properties like ionic strength change in pH, surface charge, temperature, and particle aggregation were determined for PI-based bicontinuous microemulsions and silica-nanospheres. Our findings suggest that plant PIs can be implicated as potent insecticidal molecules and could be explored using novel delivery systems.



*Valedictory Address*

## **GENOMICS ACCELERATING PLANT RESEARCH IN INDIA**

***Dr. Malali Gowda***

*Professor, Transdisciplinary University (TDU), Foundation of Revitalization of Local Health Traditions (FRLHT), Bangalore*



Agriculture is the backbone for the Indian population. Indian subcontinent harbors a rich diversity of life including crops, livestock and wild populations. Traditionally plant research has confined to usage of plants such as food, medicine or cloth in India. Over 98% of the plants in India have not been studied at the molecular level. Many plant species have extinct from the wild and many are red listed due to large-scale deforestation. Next generation sequencing (NGS) is the recent breakthrough in the life science technology, which pushed the plant research from laboratory to land. NGS

technology has reduced the cost per genome and the speed of DNA sequencing over a million folds. Today we can sequence any plant species and associated organisms including bacteria, fungi, insects and microbiome. We can identify markers for traits, genes and pathways related to any traits. Genomics and Bioinformatics will help to understand the mechanisms underlining the phenotypic characters including disease resistance, drought, yield etc. The predictive or designer plants can be produced using genome-assisted molecular breeding.

**MARKER ASSISTED SELECTION IN PLANTS****Dr. Shivani Patel***Dept of Biotechnology, Shree M & N Virani Science College, Rajkot*

The development of DNA (or molecular) markers has irreversibly changed the disciplines of plant genetics and plant breeding. While there are several applications of DNA markers in breeding, the most promising for cultivar development is called Marker Assisted Selection (MAS).

Marker assisted selection is the breeding strategy in which selection for a gene is based on molecular markers (DNA markers) closely linked to the gene of interest rather than the gene itself, and the markers are used to monitor the incorporation of the desirable allele from the donor source. Selection of a genotype carrying desirable gene via linked marker (s) is called Marker Assisted Selection. MAS greatly increase the efficiency and effectiveness for breeding compared to conventional breeding. As it refers to the use of DNA markers that are tightly-linked to target loci as a substitute for or to assist phenotypic screening. Thus, the fundamental advantages of MAS compared to conventional phenotypic selection are:

- Simpler compared to phenotypic screening
- Selection may be carried out at seedling stage
- Single plants may be selected with high reliability.

These advantages may translate into

- (1) greater efficiency or
- (2) accelerated line development in breeding programs.

For example, time and labour savings may arise from the substitution of difficult or time-

consuming field trials (that need to be conducted at particular times of year or at specific locations, or are technically complicated) with DNA marker tests. Furthermore, selection based on DNA markers may be more reliable due to the influence of environmental factors on field trials. In some cases, using DNA markers may be more cost effective than the screening for the target trait.

Another benefit from using MAS is that the total number of lines that need to be tested may be reduced. Since many lines can be discarded after MAS at an early generation, this permits a more effective breeding design.

**Steps in MAS procedure:**

- ✓ Identification of divergent parents.
- ✓ Generation of a suitable mapping population.
- ✓ Identification of polymorphic probes.
- ✓ Analysis of marker segregation on the mapping population.
- ✓ Establishment of linkage
- ✓ Marker validation

**MAS Breeding Schemes**

1. Marker-assisted backcrossing
2. Pyramiding
3. Early generation selection
4. 'Combined' approaches

**Future Challenges**

It is also critical that future endeavours in MAS are based upon lessons that have been learnt from past successes and (especially) failures in using MAS. Further optimization

of marker genotyping methods in terms of cost-effectiveness and a greater level of integration between molecular and conventional breeding (especially in designing efficient and cost-effective strategies)

represent the main challenges for the greater adoption and impact of MAS on breeding in the near future.

*Invited Talk at 2<sup>nd</sup> pre-conference workshop*

## STATUS OF GM CROPS IN INDIA

***Dr. S. V. Sawardekar***

*Associate Professor, Plant Biotechnology Centre, Dapoli*

*Dr. B. S. Konkan Krishi Vidyapeeth, Dapoli ( MS)*



India has the fourth largest area planted under genetically modified (GM) crops, according to the International Service for the Acquisition of Agri-Biotech Applications (ISAAA). Farmers in India planted a total 11.6 million hectares (mh) under transgenics in 2014, behind the corresponding areas for Argentina (24.3 mh), Brazil (42.2 mh) and the US (73.1 mh). The GM crop acreage in India far surpassed China's 3.9 mh, while equalling that of Canada's 11.6 mh. ISAAA, a New York-based crop biotech advocacy group, has estimated the total global area under GM crops to have touched 181.5 mh last year, up from 175.2 mh in 2013. Since 1996, when farmers first commercially planted transgenics, the area under these crops has risen more than hundredfold from 1.7 mh to 181.5 mh. It represents the fastest ever adoption of any technology in agriculture, said Bhagirath Choudhary, Director at ISAAA's South Asia Office. Significantly, the entire 11.57 mh GM crop area in India last year consisted of Bt cotton. Nearly 96 per cent of the country's cotton area is now covered by Bt hybrids. Bt technology has helped India to treble its cotton output from 13 million bales in 2002 (when it was introduced) to 40 million bales in 2014. China had only 3.9 mh of GM planted area last year, almost fully under Bt cotton. But its

government has allowed commercial cultivation of seven other crops : papaya, maize, rice, poplar, tomato, sweet pepper and petunia.

Moreover, China has sought to actively promote public-private-partnerships (PPP) in GM crop technology. For example, CAAS has licenced its internally developed Bt gene and transgenic phytase maize (which boosts phosphorous absorption by pigs, leading to faster animal growth and higher meat yields) technologies to Origin Agritech, a Beijing-based and US NASDAQ-listed seed company.

We need to extend GM technology to more crops, and also encourage PPPs, so that our farmers benefit from competition and faster commercialization. Indian scientist pointed to the granting of the rights for commercialisation of a Bt chickpea developed by the Assam Agriculture University to Sungro Seeds and that of the Indian Agricultural Research Institute's Bt brinjal to Bejo Sheetal and Ankur Seeds as models for the future. Now that the Maharashtra government has taken the lead in allowing open field trials of five new GM crops, we hope to see more farmers adopting this technology in the coming years.

Invited Talk at 2<sup>nd</sup> pre-conference workshop

## PLANT BREEDER'S RIGHTS

**Mrs. Anushree Lokur**

Associate Professor, Dept. of Microbiology, Ramnarain Ruia College,  
Matunga, Mumbai



India as a member of WTO is required to comply with the TRIPS agreement. To bring India's laws in conformity with TRIPS agreement India has come out with legislation - The Protection Of Plant Varieties And Farmers' Rights Act, 2001 ("PPVFRA") that not only protects the interests of breeders, but also farmers.

Plant variety to be registered under this act should be Novel, Distinct, Uniform and stable. The certificate of registration shall be valid for **nine years** in case of **trees and vines** and **six years** in the case of **other crops** and total period of validity shall not exceed-

(i) in case of **trees and vines**, **eighteen years** from the date of registration of variety,  
(ii) in the case of **extant varieties**, **fifteen years** from the date of **notification** of the variety by the Central Government and  
(iii) in the other cases, **fifteen years** from the date of registration of the variety

Varieties that can be registered are -

- (i) Novel Variety
- (ii) Extant Variety - A variety in public domain
- (iii) Farmers Variety - has been traditionally

cultivated and evolved by the farmers in their fields, or A variety about which there is common knowledge

(iv) Essentially derived variety - Is a variety which is essentially derived from an initial variety, Retains expression of the essential characteristics, is clearly distinguishable from the initial variety & conforms to the initial variety in the expression of essential characteristics except variation which result in the process of derivation

People who can apply for protection of a Variety are breeder, farmers and university or publicly funded agriculture institutions.

The Owner of the Variety has a right to produce, sell, market, distribute, import and export the variety.

Anyone who sells, exports or imports a registered variety constitute infringement although use of a variety for research and experimental purpose is allowed.

A Farmer has the same privilege like that of a breeder. The farmer can save, sow, resow, exchange, share or sell his farm produce including seed of a protected variety. The farmer cannot sell branded seeds of a protected variety.

# SECTION II

# RESEARCH PAPERS



## STUDY OF ISOLATION OF STRESS RESISTANT GENES FROM *Aloe vera*

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### ABSTRACT:

*Aloe vera* has played a prominent role as a contemporary folk remedy and, over the years, many optimistic claims have been made for its medicinal properties. The plant has been studied to withstand stress conditions such as drought, cold and high salt concentrations. Few Genes widely known for stress tolerance are isolated from *Aloe vera* genome like DREB gene. These genes may be helping plant to give it a sturdy and stress-resistant growth pattern. With the understanding of molecular mechanisms such genes can be isolated cloned and can be used to transform other susceptible plant. The objectives proposed for this study were to isolate stress-tolerant gene/s of *Aloe vera*, and to transform the isolated gene/s into stress sensitive plant. Our understanding after completion of the work gives us great satisfaction to convey to the UGC and the entire scientific community that novel *Aloe vera* genes are isolated by PCR cloning method and one of them which is HVA22 was used in transforming stress sensitive Tulsi plant successfully. Other genes isolated are part of retrotransposon and Glycerol kinase homolog from *Aloe vera*.

**Key words:** Aloe vera, plant stress response genes, abiotic stress

### INTRODUCTION:

*Aloe vera* (L.) (*Aloe barbadensis* Miller) is a perennial succulent xerophyte, which develops water storage tissue in the leaves to survive in dry environmental conditions. It is a monocot and belongs to grass family. *Aloe vera* is most famously known for its medicinal properties. The plant has been studied to withstand stress conditions such as drought, cold and high salt concentrations. Few Genes widely known for stress tolerance was isolated from *Aloe vera* genome and shown to have expressed as well. For eg: DREB1 (Yang- Meng Wang, 2007).

According to a research done in other plants, Abscisic Acid (ABA) is said to induce the expression of a battery of genes mediating plant responses to environmental stresses. The early reported gene is of the ABA-inducible genes in barley is HVA22. HVA22 encodes a small peptide of 130 amino acids without an obvious functional motive. Expression of HVA22 is dramatically up-regulated by ABA not only in aleurone cells, but also in vegetative tissues. The expression of HVA22

in vegetative tissues can be induced by ABA and environmental stresses, such as cold and drought (Shen *et al.*, 2001).

One more remarkable characteristic of *Aloe* is its stress stability. Aloe as we all know grows in dry, arid and at extreme temperatures and salt conditions. It is very likely that ABA-induced genes like HVA22 homologues are governing this stress stability in *Aloe*. Since, HVA22 gene is well characterized, in *Hordeum vulgare* (Barley), using the primer sequences the homologous gene in *Aloe* can be cloned. In this study, this property of *Aloe vera* was analysed. If this HVA22 homologue is responsible for heat stability it can then be transferred to a stress-sensitive plant that could render heat or stress tolerance to the plant

Out of other genes involved in stress response mechanisms, transcription of retrotransposons were also found to be increased in several plants. Retrotransposons are ubiquitous in plant kingdom and are present in high copy numbers in most plants (Krishna Karajol,



2011). Especially Liliaceae, >90% of their genome is composed of LTR Retrotransposons (Feschotte C, 2002). Whenever plants experienced the type of stresses such as chilling, infection, mechanical damage, in vitro regeneration retrotransposons were shown to be transcriptionally highly active. All known active plant retrotransposons are largely quiescent during development but activated by stresses, including wounding, pathogen attack and cell culture (Wessler, 1996). Ty3 gypsy is known to be transcriptionally active, along with Ty1 and Ty2 in yeast. The part of retrotransposon isolated would be tested for its stress tolerance property.

Glycerol kinase is one of the key enzymes involved in G3P biosynthesis (Lai *et al.*, 2015). It catalyzes the rate-limiting step in glycerol utilization by transferring a phosphate from ATP to glycerol, yielding Glycerol- 3-Phosphate. Its gene has been conserved structurally and functionally during evolution (Cheng *et al.*, 2013). It is also known to play an active role in stress response in plants and animals. In one study conducted in Arabidopsis, it was observed that a mutant GLY gene showed better response to salt stress as compared to wild type; as the glycerol accumulated in the plant it protected the cells against osmotic shock (Bahieldin *et al.*, 2013). Therefore, it could be one of the potential genes that may be responsible for the stress resistance property in *Aloe vera*. The isolated gene from *Aloe vera* using the primers showed high homology with glycerol kinase gene from *Gluconobacter oxydans*. Glycerol kinase usually is present in NBD domain Sugar Kinase\_HSP70\_Actin, which when targeted, not only may provide us the fragment containing Glycerol kinase, but also HSP70, heat shock molecular chaperone. The purpose of producing Transgenic plant would be to understand the function of the isolated genes in a plant system, under normal and stress conditions.

## **MATERIALS AND METHODS:**

### **Extraction of genomic DNA from *Aloe vera*:**

Four grams of young leaf of *Aloe vera* plant were used for genomic DNA extraction using CTAB technique (Doyle J.J, 1987) with modification. The genomic DNA sample was purified by PCR Purelink Purification Kit (K-310001).

### **Extraction of RNA from *Aloe vera* and cDNA pool synthesis:**

One gram of stressed *Aloe vera* plant tissue was used for Total RNA extraction by TRIzol technique (Sigma's TRI Reagent). Quality and quantity of RNA was determined by standard methods. cDNA pool was generated using Invitrogen's Superscript III kit.

### **Primer Designing and Synthesis:**

Primers for the PCR and RT-PCR were designed using Primer-BLAST program from NCBI, and the primers were synthesized by Sigma-Aldrich Pvt. Ltd. Sequencing of RT-PCR and PCR products was outsourced.

### **Insilico analysis:**

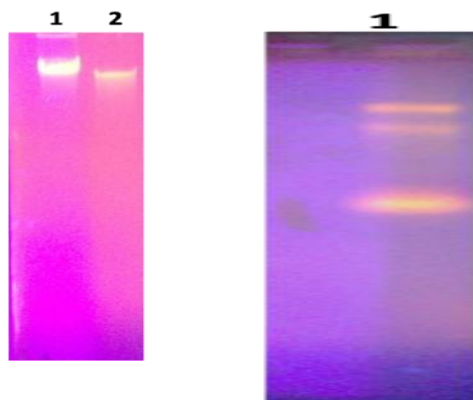
PCR and RT-PCR Product sequences obtained were subjected to NCBI BLAST (Sense & Antisense strands). BLASTn, BLASTx (Gish & States, 1993) and CDD (Conserved Domain Database) search was performed. ORF was found using NCBI's ORF finder program. The potential exons present within the fragment were analysed using FGESH 2.0 version Softberry Program (Solovyev *et al.*, 2006). Protein prediction was performed at Predict Protein website (Yachdav *et al.*, 2014).

## **RESULTS:**

### **Genomic DNA and RNA extraction results:**

Concentration of Genomic DNA was found to be 0.53µg/µl and of good purity. AGE analysis of unpurified and purified DNA revealed a single band with minimum smear and no RNA contamination (Figure 1A). Total RNA extracted from *Aloe vera* was estimated

to be 0.1µg/µl and was found to be of good purity. AGE analysis revealed 2 bands which were intact and degradation was minimum (Figure 1B).

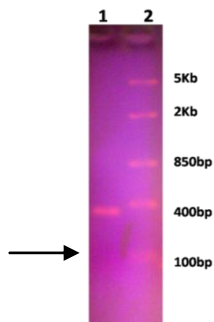


**Figure 1A: Lane 1- Genomic DNA isolated from young *Aloe vera* leaves; Lane 2- Spin- column purified Genomic DNA of *Aloe*. Figure 1B: Lane 1- RNA isolated from *Aloe vera* plant**

**PCR, RT-PCR and Sequencing results:** The PCR and RT PCR products were analysed by AGE and was run along with DNA ladder. The amplifications obtained with the Gene specific primers are as follows:

Fig	Gene Specific Primers	Tem-Plate	Amplif-ication
2	Ty3_Gypsy_Gag (RT PCR)	cDNA	350bp
3	HVA22 (RT PCR)	cDNA	368bp
4	Glycerol Kinase_Hsp70_NBD	gDNA	200bp

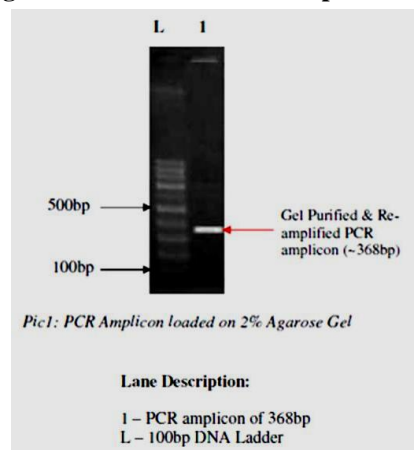
**Figure 2: Ty3\_Gypsy\_Gag RT PCR**



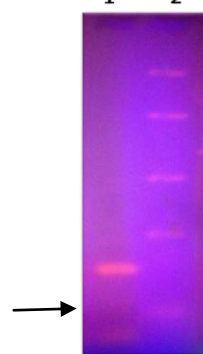
**Lane 1: ~350bp long PCR product amplified**

using c\_FP&c\_RP and cDNA pool obtained from *Aloe* total RNA as template; Lane 2: Mid Range Molecular weight marker ladder (from top: 5Kb, 2Kb, 850bp, 400bp, 100bp)

**Figure 3: HVA22 RT PCR Amplicon**



**Figure 4: Glycerol kinase<sub>2</sub> Hsp70\_NBD Domain**



**Lane 1: PCR product obtained from amplification using glycerol\_kinase primers. Lane 2: Low range molecular weight ladder in the order 5Kb, 2Kb, 800bp, 400bp and 100bp.**

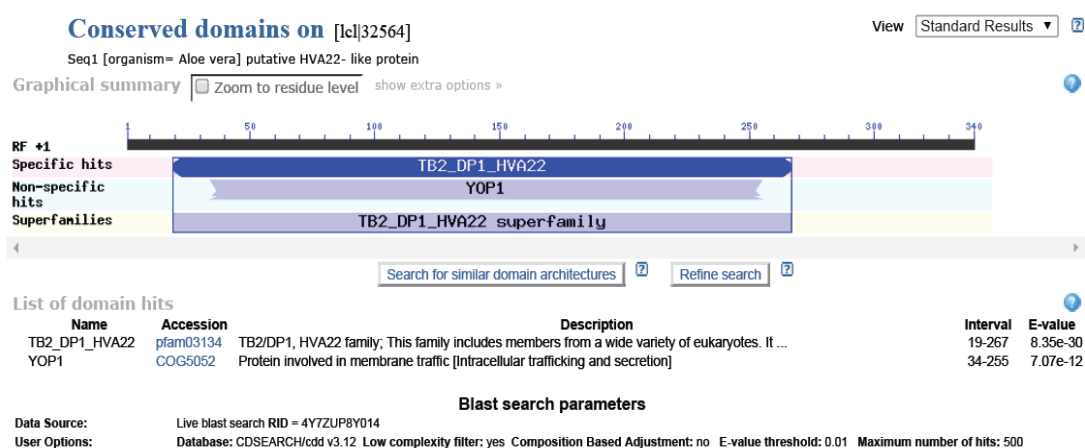
**Insilico Analysis:**

**Ty3\_Gypsy\_Gag (RT PCR)**

BLASTx results of the 318bp sequence showed highest identity (88%) with gag protease polyprotein of *Aloe vera* with a score of 196 and query coverage of 99%. Maximum hits were observed in the range of 50-80 on the scorecard and showing identity with gag protease polyprotein region of same plants as with “gag\_protease gene”.

### HVA22 (RT PCR):

From Sequencing, 330bp long sequence information was obtained which was subjected to Bioinformatics analysis, whose results were as follows: BLASTn showed 100% identity and 100% Query coverage with the original HVA22 coding gene of *Hordeum vulgare*, L19119. Other hits showed 98-95% identity with the HVA22 homologs from other monocots like *Triticum aestivum*, *Oryza sativa*, *Zea mays*, *Aegilops tauschii*, *Brachypodium distachyon* etc. BLASTx also showed results similar to that of BLASTn and additionally it showed the presence of TB2/DP1 domain, which is the conserved domain of all HVA22 homologs. CDD (Conserved Domain Database) confirmed the presence of the above mentioned domain and also showed the length of the same.



The Sequence shows an ORF from +18 to +329, a reading frame of +3. The translated sequence shows complete protein product as analysed by TranSeq, BLASTp, ProteinPredict and Swiss Model. Thus, it was found to be a potential coding DNA which could be further used for cloning into Plant expression vectors. This sequence was then submitted to NCBI via GenBank'sBankit Submission tool. The sequence is now available on NCBI, EMBL and DDBJ under the accession ID KP076225.

### Glycerol kinase\_Hsp70\_NBD domain

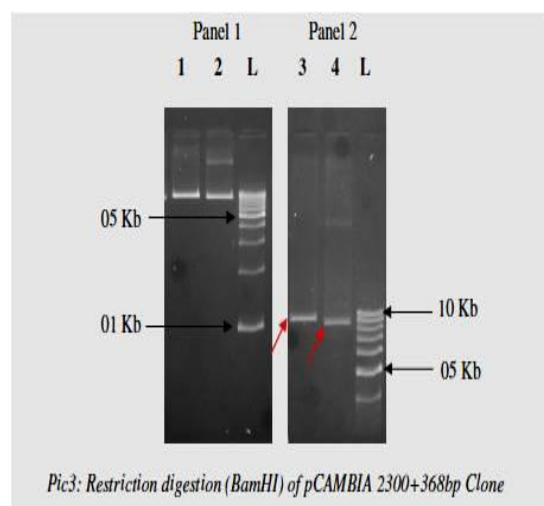
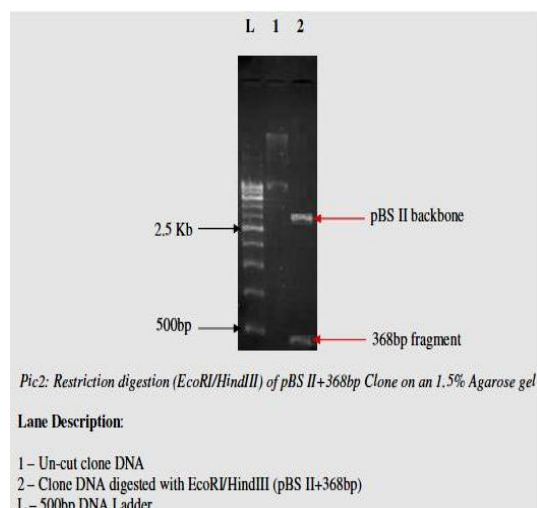
Sequencing of this PCR product revealed an approximately 200bp long DNA. BLASTn analysis of this sequence showed 98% identity with glycerol kinase gene from *Gluconobacteroxydans*. BLASTx results of this sequence showed highest identity (98%) with glycerol kinase protein from *Gluconobacter oxydans* with a score of 124 and query coverage of 96%. Maximum hits were observed in the range of 80- 200 on the scorecard and showing identity with glycerol kinase gene from different organisms such as *Sphingomonassps*, *Acetobacternitrogenifigens*, *Caulobactersp.*, etc. The percentage identity ranged between 96% to 55%; query coverage ranged between 93% and 74%. The CDD search revealed that this fragment harbored an NBD\_sugar-kinase\_HSP70\_actin domain

superfamily. It shows a complete ORF in the +1 Reading frame. Protein modeling of the translated sequence resulted in many hits for Glycerol Kinase and Heat shock cognate 71kDa protein.

### Cloning of HVA22 into pCAMBIA 2300:

HVA22 homolog of *Aloe vera* was first cloned into pBS II vector at EcoRI and HindIII restriction sites. This homolog was then sub cloned into pCAMBIA 2300 vector at the same restriction sites. The clones were confirmed by restriction fragments, as

observed on AGE.



## DISCUSSION:

HVA22 is known to be most conserved stress inducible gene across all kingdoms except Prokaryotes. Bioinformatics analysis of the amplification product obtained using GSP (Gene Sequence Protein) from *Aloe vera* cDNA confirmed the presence of the same in *Aloe vera*.

The transcript analysis of the coding region within the gene gave approximately 350bp RT-PCR product, which confirms the insilico information on the coding sequence of gag domain of aretrotransposon and also its active transcription. This data shows presence of a gene fragment from *Aloe vera* which is homologous to plant retrotransposon,

specifically TY3/Gypsy class. The percentage identity ranged between 48% and 30%; query coverage ranged between 93% and 70%. This amplified cDNA is a transcript of the gene fragment that was investigated before in other paper, gag protease polyprotein of *Aloe vera*, thus confirming its expression.

The glycerol kinase protein encoded by this gene GK belongs to the FGGY kinase family. It is the key enzyme in the regulation of glycerol uptake and metabolism. As mutations in the metabolic enzymes is related to breakdown of glycerol in human results in diseases and syndromes which lead to death, it may be conceivable that glycerol or G3P might also participate in disease physiology of plants and this role is yet to be discovered (Venugopal et.al., 2009). It was observed in a study conducted by Venugopal et.al., the following observation and hypothesis was made: A pathogenic fungi relies on glycerol for its growth and establishment, even in the presence of sucrose or glucose, from plants; it is these pathogen-mediated changes in glycerol levels and its utilization that signals the plant to initiate defense.

## CONCLUSION:

For this study, molecular cloning of three genes was carried out using PCR method. Three different sets of primers were used for these specific genes. The genes which targeted were HVA22 gene from Barley (*Hordeum vulgare*), multi-subunit Glycerol kinase gene and Ty3 Gypsy Retrotransposon; all of these genes are involved in stress-response mechanism in plants and animals. This work done here is capable and assures the strong presence of Aloe genes which can definitely transform its stress-tolerant characters to the other plants.

## ACKNOWLEDGEMENTS:

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## PROXIMATE ANALYSIS AND PHYTOCHEMICAL SCREENING OF RHIZOMES OF *Hedychium coronarium* J.Koenig, AN IMPORTANT MEDICINAL PLANT BELONGING TO ZINGIBERACEAE FAMILY

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### ABSTRACT:

Medicinal plants have been used in virtually all cultures as a source of medicine since ages. Plant parts such as rhizomes, leaves or barks etc. are used as crude drugs to treat illnesses. In herbal medicine, plant extracts are used as crude drugs for the treatment of various diseases including infectious diseases. Beneficial effects are attributed to plant phytochemicals.

*Hedychium coronarium*, also known as Butterfly lily, belonging to the Zingiberaceae family is an endangered and red-listed plant of high medicinal value. The rhizome of this plant is used for the treatment of various diseases. Crude extracts prepared from the rhizomes show antifungal property. Essential oils, obtained from *Hedychium coronarium* are found to be rich in terpenes, such as  $\alpha$ -pinene,  $\beta$ -pinene. The essential oils are used for bactericidal, fungicidal, medicinal and cosmetic applications.

Our study shows the quality of crude drugs and proximate analysis in plants gives valuable information and may help to assess the quality of the crude drug. Proximate analysis and phytochemical screening of rhizomes of *Hedychium coronarium*, which is frequently consumed as food and as medicine, were carried out. These analyses revealed that ash content was low ( $3.616 \pm 0.840$ ) as compared to the moisture content in *Hedychium coronarium*. The water extractive value was more than alcohol extractive. Preliminary tests carried out on phytochemicals revealed the presence of terpenoids.

**Key words:** phytochemicals, proximate analysis

### INTRODUCTION:

Medicinal plants have been used in virtually all cultures as a source of medicine since ages. The wide-spread use of herbal remedies and healthcare preparations as those described in ancient texts such as the Vedas and the Bible and obtained from commonly used traditional herbs and medicinal plants has been traced to the occurrence of natural products with medicinal properties. Medicinal plants play a key role in world healthcare systems. Approximately 75-90% of the world's rural population depends on herbal medicines for their primary health-care.

Many plant species, possessing medicinally important compounds are disappearing at an alarming rate due to the destruction of its

natural habitats, owing to rapid agricultural development, urbanization, indiscriminate deforestation and uncontrolled collection of plant materials (Bapat *et al.*, 2004; Kala *et al.*, 2007).

*Hedychium coronarium* is an endangered and red-listed plant of high medicinal value. *Hedychium coronarium* or Gulbakawali or Butterfly lily (Molur & Walker, 1998; Chadha, 2005; Singh & Singh, 2009) belongs to the family Zingiberaceae. The Himalayas are the probable centre of origin of this plant (Soares & Baretto, 2008). The plant is found in tropical and sub-tropical regions such as India, Brazil, Japan and South China (Lu *et al.*; 2009). This

plant is a host plant for the Grass demon butterfly.

This plant has tremendous medicinal properties and its various parts are used in traditional as well as modern medicine. The rhizome of the plant is used in the treatment of diabetes, cold, body aches, headache, lancinating pain, contusion, inflammation and rheumatic pain. The rhizome has anti-cancerous, antioxidant, anti-hypertensive, diuretic, leishmanicidal, anti-malarial activities and also used in irregular menstruation, piles bleeding and stone in urinary tract. Recently, antifungal activity of *Hedychium coronarium* crude extracts has been reported. Cancer chemoprevention activity is also reported recently of labdane diterpenes from rhizomes of *Hedychium coronarium*. Diterpenes showed anti-inflammatory and cytotoxic activities. In pharmacological studies of this natural medicine, it was reported that sesquiterpenes of *Hedychium coronarium* showed inhibitory effects on the release of beta-hexosaminidase. Terpenoids from *Hedychium* oil showed antioxidant and antimicrobial properties. Coronarin A, a terpenoid of this plant, is used to inhibit the proliferation of human umbilical vein. The rhizomes of this plant are also used in Chinese natural medicine. The medicinal value of this plant in the treatment of a large number of human ailments is mentioned in Ayurveda, Charaka Samhita and Sushruta Samhita.

## **MATERIALS AND METHODS:**

### **Collection of plant material:**

Fresh rhizomes of *Hedychium coronarium* were obtained from the greenhouse of Ramniranjan Jhunjhunwala College, Ghatkopar. They were washed in continuous running water for 1 hour. They were again washed with Tween 20 and a soft brush to remove all sticking soil particles, to obtain clean rhizomes. The cleaned rhizomes were cut into small pieces with a knife. The pieces

were then kept in a tray, and kept in the oven for 24 hours at a temperature of 50°C. The dried rhizome pieces were now put in a mixer (high volts) and ground to a dry, fine powder. They are then sieved through a sieve (180 µm), (Jayant Scientific India, Mumbai) to obtain a fine powder of uniform size. The process is repeated with the remaining powder, which has not ground finely. The fine powder is stored in an airtight container until further use.

The fine, ground powder obtained above was used for proximate analysis. The parameters studied were total moisture content, water extractive, alcohol extractive, total ash, water soluble ash and acid insoluble ash. Determination of all parameters was carried out in triplicates.

### **Preparation of plant extracts:**

#### **Cold extraction:**

A solution of 2 g rhizome powder in 100 mL petroleum ether was prepared. The flask was covered tightly with a cotton plug and refrigerated for 24 hours. The extract was filtered and then used for preliminary phytochemical tests.

#### **Sonication:**

A solution of 2 g rhizome powder was prepared each in 100 mL water, methanol and hexane. The solutions were sonicated and the respective extracts obtained were filtered before use for preliminary phytochemical tests.

### **Qualitative phytochemical analysis:**

The above extracts were tested for the presence of bioactive compounds by using the following standard methods:

#### **A] Test for terpenoids:**

##### **a) Vanillin-sulphuric acid reagent:**

To 1 mL of extract, 1 mL of Vanillin-sulphuric acid reagent was added. Colour formation is seen after heating the solution. Production of purple-blue colour indicates the presence of terpenoids.

**b) Anisaldehyde reagent:**

To 1 mL of extract, 1 mL of Anisaldehyde reagent was added. Colour formation is seen after heating the solution. Presence of terpenoids is indicated by the formation of pink-purple colour.

**B] Test for alkaloids:**

**a) Dragendorff reagent:**

To 1 ml of extract, 1 ml of Dragendorff reagent is added. Red-Brown colour is obtained if alkaloids are present.

**b) Wagner's reagent:**

To 1 ml of extract, 1 ml of Wagner's reagent is added. Alkaloids are present if an orange-red colour is produced.

**C] Test for flavonoids:**

**a) Pew's test:**

Add a pinch of zinc dust to the plant extract, followed by addition of concentrated hydrochloric acid. A cherry-red colour is developed in the presence of flavonoids.

**b) Sodium hydroxide test:**

Extract is combined with equal volumes of 10% aqueous sodium hydroxide and 5% aqueous hydrochloric acid. A canary yellow colour is formed if flavonoids are presents.

**D] Test for glycosides:**

**a) Kedde's test:**

1 mL of plant extract is mixed 2% ethanolic 2,3-dinitrobenzoic acid, followed by addition of 2-3 drops of 20% aqueous sodium hydroxide. Presence of glycosides is confirmed by the production of a pink-purple or blue-violet colour.

**b) Keller-Kiliani test:**

Mix 1 mL of plant extract with a solution of glacial acetic acid to which 2-3 drops of 5% aqueous ferric chloride have been added. Then, along the sides of the test tube, add concentrated sulphuric acid without mixing the solutions. If glycosides with 2-deoxysugar moieties are present, a brown-red colour ring is formed at the

junction of the liquids and the upper phase will turn blue-green.

**Test for cardiac glycosides:**

**Baljet's reagent:**

To 1 mL of extract, 1 mL Baljet's reagent is added. Production of an orange-red colour indicates the presence of cardiac glycosides.

**RESULTS AND DISCUSSION:**

Pharmacognosy is the study of crude drugs prepared from plants. Crude drugs are dried, unprepared material of plant, animal or mineral origin, used for medicine. Proximate analysis is done to determine the quality of crude drugs. The moisture content was found to be 15%. The determination of ash content is useful for detecting adulterants such as sand and earth, adhering to the crude drugs while collecting the sample. Inorganic elements may also be present in trace amounts in ash as impurities. The total ash content was found to be 3% only, of which 1.6% was water soluble ash. The acid-insoluble ash was found to be 0.69%. Water extractive was more than alcohol extractive indicating that water is able to extract more phytochemicals than alcohol.

The preliminary phytochemical tests show the presence of terpenoids. Terpenoids, which are known to be the ingredients of the essential oil obtained from *Hedychium coronarium* were not present in the aqueous extract but were present in methanol, hexane and petroleum ether extracts, indicating that they are extracted with non-polar solvents.

**CONCLUSION:**

Medicinal plants have been used in virtually all cultures as a source of medicine since ages. Beneficial effects of crude drugs are believed to be attributed to plant phytochemicals. The preliminary tests on rhizomes of *Hedychium coronarium*, an important medicinal plant, were found to be rich in terpenoids, which are



the main ingredients in essential oils obtained from this plant.

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PARAMETERS	OBSERVED VALUES (%)
Moisture	15.80±0.163
Water extractive	15.080±0.865
Alcohol extractive	4.0426±0.424
Total ash	3.616±0.840
Water soluble ash	1.676±0.966
Acid insoluble ash	0.690±0.0282

**Table 1: Proximate analysis of *Hedychium coronarium* rhizome**

Tests	Water Extract	Methanol Extract	Hexane Extract	Petroleum Ether Extract (cold extract)
<b>Terpenoids</b>				
Vanillin-sulphuric acid reagent	-	+	+	+
Anisaldehyde-sulphuric acid reagent	-	+	+	+
<b>Alkaloids</b>				
Dragendroff's reagent test	-	-	-	-
Wagner's reagent test	-	-	-	-
<b>Flavonoids</b>				
Pew's test	-	-	-	-
Sodium Hydroxide test	-	-	-	-
<b>Glycosides</b>				
Kedde's test	-	-	-	-
Keller-Kelliani test	-	-	-	-
<b>Cardiac Glycosides</b>	-	-	-	-

Note: '+'= detected, '-'= not detected

**Table 2: Preliminary phytochemical tests**

## EFFECT OF VERMIWASH AND *Azotobacter spp.* ON GROWTH OF *Triticum aestivum*

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### ABSTRACT:

Vermiwash and *Azotobacter spp.* are biofertilizers which are used extensively in agriculture. This study focuses on determining the synergistic effects of vermiwash and *Azotobacter spp.* on growth of *Triticum aestivum* (Wheat). Seed germination studies were carried out in a sterile setup using plate culture assay having different growth media such as vermiwash, saline suspension of *Azotobacter spp.* and a mixture of the two. Each system was layered with filter paper padding soaked with growth media. One surface sterilized seed was placed in each petri plate. Addition of biofertilizers (i.e. *Azotobacter* and vermiwash) was done only in the beginning and then length of root and shoot was measured on each day, up to 6 days in laminar flow hood. The results revealed that maximum average root length was obtained in the system where vermiwash was added, followed by the mixture (vermiwash + *Azotobacter spp.* suspension) and lowest for *Azotobacter spp.* suspension. Maximum average shoot length, however, was observed for the mixture of the two biofertilizers.

**Keywords:** Biofertilizers, *Azotobacter*, Vermiwash, Wheat.

### INTRODUCTION:

During the era of Green revolution, chemical fertilizers had played a very important role to boost agriculture. However, it was realized later that although chemical fertilizers provided plants with extra nutrients, it also had a variety of adverse effects on humans, as well as it caused soil and water pollution. Also, indiscriminate use of chemical fertilizer disturbed soil ecosystem and in turn caused loss in soil productivity. Owing to this, more emphasis is now given to the use of biofertilizers, which can be a good supplement/ alternative to chemical fertilizers (Aggani, 2013).

Biofertilizers are substances containing microbial cells which when applied to the seed, root or soil, stimulate plant growth. Biofertilizers keep the soil environment rich in all kinds of micro- and macro-nutrients via nitrogen fixation, phosphate and potassium

solubilization or mineralization, release of plant growth regulating substances, production of antibiotics and biodegradation of organic matter in the soil (Bhradwaj *et al.*, 2014).

While *Rhizobium*, Blue Green Algae (BGA) and *Azolla* are crop specific, bio-inoculants like *Azotobacter*, *Azospirillum*, Phosphate Solubilizing Bacteria (PSB), Vesicular Arbuscular *Mycorrhiza* (VAM) can be regarded as broad spectrum biofertilizers (Gupta, 2004).

*Azotobacter spp.* is Gram negative, free-living, nitrogen fixing aerobic soil dwelling, large blunt rods, oval cells or cocci that forms thick-walled cysts. The genus *Azotobacter* belongs to the subclass of the *Proteobacteria* and comprises seven species: *A. chroococcum*, *A. vinelandii*, *A. beijerinckii*, *A. paspali*, *A. armeniacus*, *A. nigricans* and *A. salinestri* (Jnawali *et al.*, 2015).

Besides playing role in nitrogen fixation, *Azotobacter* has the capacity to produce vitamins such as thiamine and riboflavin, and plant hormones viz., indole acetic acid (IAA), gibberellins (GA) and cytokinins (CK). *A. chroococcum* improves the plant growth by enhancing seed germination and advancing the root architecture by inhibiting pathogenic microorganisms around the root systems of crop plants (Bhradwaj *et al.*, 2014).

Vermiwash, a liquid extract obtained from vermicomposting beds, is used as an organic fertilizer for crop plants. It is composed of excretory products and mucus secretions from earthworms and micronutrients from the organic molecules in the soil. These nutrients are absorbed and then transported to the leaves, shoots, and other parts of a plant (Khan *et al.*, 2014). Vermiwash can be either be directly applied to soil or used as a foliar spray. Vermiwash contains several enzymes, sugars, amino acids and phenols, plant growth promoting hormones such as in indole acetic acid and humic acid, vitamins along with micro and macronutrients which increases the resistance power of crops against various diseases and enhances the growth and productivity of crops (Zambare *et al.*, 2008). Vermiwash is also known to contain plant growth enhancing bacteria like *Azotobacter*, phosphate solubilizing bacteria etc. These plant growth enhancing bacteria act as bioprotectants (i.e protect the plant from abiotic and biotic stress) and also provide resistance against pathogens by producing metabolites (Bhradwaj *et al.*, 2014).

Present study aims at comparing the effect of two previously known biofertilizers i.e., *Azotobacter* and Vermiwash, individually and in combination, on the growth of Wheat plant which is a major cereal crop in India.

## **MATERIALS AND METHODS:**

### **1. Isolation of *Azotobacter spp.* from soil:**

1g soil from rhizosphere was collected and

inoculated in 100 ml of sterile Ashby's mannitol broth and incubated for one week at room temperature. After 1 week of incubation, loopful of surface scum formed was isolated on Sterile Ashby's Mannitol agar systems and these incubated at room temperature. After 4 days, dew drop like colonies, characteristic of *Azotobacter spp.* were observed. The colony was Gram stained and found to be Gram negative rods. These colonies were maintained in pure culture on Sterile Ashby's Mannitol agar slants for further use.

### **2. Seed Sterilization:**

Wheat grains were procured from local market, Thane (Maharashtra, India). These seeds were washed with sterile distilled water once and soaked in 0.1% HgCl<sub>2</sub> for 4-5 minutes. The seeds were then washed thrice in sterile distilled water and soaked in it for 15 minutes. These seeds were then used for plate culture assay.

### **3. Collection of Vermiwash:**

Vermiwash was collected from the active vermicompost setup. Vermiwash was filtered using Whatman filter paper and stored at 4°C till further use.

### **4. Plate Culture Assay:**

System of sterile petri plate containing circular filter paper padding was used for the plate culture assay. One surface sterilized seed was placed in each petri plate. In one system, 5 ml of vermiwash was added (V), in second system, 5 ml of saline suspension of *Azotobacter spp.* was added (A), and in the third system, 2.5 ml saline suspension of *Azotobacter spp.* and 2.5 ml vermiwash was added (A+V). Addition of biofertilizers (i.e. *Azotobacter spp.* and vermiwash) was done only in the beginning and then length of root and shoot was measured using 'Vernier caliper' on each day up to 6 days in laminar flow hood. The assay was carried out in triplicates. In the present study, effect of each

of these was analyzed by measuring the length of root and shoot of the wheat seedling.

## **RESULTS AND DISCUSSION:**

Biofertilizers can help to solve the problem of feeding an increasing global population at a time when agriculture is facing various environmental issues. It is important to realize the useful aspects of biofertilizers and implement their application to modern agricultural practices. Biofertilizers would aid in sustainable development of agriculture with respect to cost effectiveness, easy availability, reproducibility, reliability and eco-friendliness.

### **1. Effect of *Azotobacter* (A), Vermiwash (V) and their combination (A+V) on the shoot growth of wheat seedling.**

Shangguan *et al.*, (2004) have reported that the shoot length is directly proportional to the nitrogen content of the soil. In current study, shoot length was considered as one criterion to determine the effect of (A), (V), and (A+V) on the wheat seedling.

During the initial period, for the first 4 days, better shoot development was observed in vermiwash system (V), as compared to the (A) and (A+V) systems. At the end of 6 days, however, maximum average shoot length was observed in the (A+V) system, followed by (V) and (A) systems.

Vermiwash is known to contain macro and micro nutrients along with plant growth promoting hormones. This might be responsible for maximum shooting during the initial period. On the contrary, when combination of A+V (1:1) was used, it resulted in effective dilution of vermiwash, and also its nutrients and phytohormones. This could be the reason for slower shooting observed for the (A+V) system during the initial period.

However, after day 4, the shoot length observed in (A+V) system surpassed the

length that was observed in (V) system. It can be speculated that *Azotobacter* thrived on the nutrients present in vermiwash. Upon flourishing, it carried out nitrogen fixation and production of certain metabolites, which resulted in availability of additional nutrients to the seedling and enhancement of shoot growth. This hypothesis is supported by the fact that Vermiwash is known to contain certain nutrients like phosphate, Mo, Mg and V that enhance nitrogen fixation. *Azotobacter* is also known to produce thiamine, riboflavin, nicotin, Indole Acetic Acid and Gibberalin, along with nitrogen fixation, all of which improve seed germination (Jnawali *et al.*, 2015).

As vermiwash contained enough nutrients for promoting the growth of *Azotobacter spp.* as well as the seed, no competition existed in the mixture (A+V). However, as saline suspension of *Azotobacter spp.* did not contain these nutrients, significant nitrogenase activity cannot be expected in this system. Also, as *Azotobacter spp.* has a very high respiratory rate, it is speculated to compete with the plant for available oxygen in a closed system. As a result, no further shoot growth was observed post Day 4 in the system containing saline suspension of *Azotobacter* (Fig. 1).

### **2. Effect of *Azotobacter* (A), Vermiwash (V) and their combination (A+V) on the root of wheat seedling.**

Nitrogen has significant effect on root and shoot development. Passioura (1983) reported that nitrogen deficiency increased root surface area and decreased shoot growth. However, extra nitrogen caused an excessive shoot growth and reduced root/shoot ratio. Therefore, the amount of nitrogen nutrition applied to plants must be optimal for proper growth. At higher concentration of nitrogen, cells present in the shoot region divide at faster rate and thus large amount of nutrients are

transported to the shoot region to fulfill its nutrient requirement, leaving root cells with comparatively less nutrients and thus affecting its growth.

In present study, average root length was found to be maximum in (V) system, followed by the (A+V) and lowest for (A).

Seeds placed in the system having neat vermiwash as media had access to optimum amount of nutrients and thus did not show inhibitory effect on root development, thus giving maximum average root length (Fig. 2).

### CONCLUSION:

Present study involved comparison of the effect of *Azotobacter* and Vermiwash, two well known biofertilizers, individually and in combination, on the growth of *Triticum aestivum*.

Vermiwash alone was observed to have positive effect on the root system, while synergistic effect of *Azotobacter* and vermiwash was observed on the shoot system of wheat seedling but not on the root system.

For a crop like wheat which is a major cereal crop in India, and a crop planted extensively in semi-arid and semi-moist areas, a well developed root system is essential. A well-developed root system as a constitutive trait is favorable in many environments. It enables the plant to make better use of water and minerals and is an important component of drought tolerance at different growth stages (Karim and Rahman, 2015). Thus for enhancing the growth of wheat in regions with limited water supply, vermiwash can be effectively used as a biofertilizer which will nourish the plant and promote the development of efficient root system.

In regions with ample water supply, 'combination biofertilizer' consisting of vermiwash and *Azotobacter* culture might be used, which will enhance shoot development

and thus increase plant yield. Also, the time of application of the 'combination biofertilizer' might be adjusted after appropriate rooting has occurred.

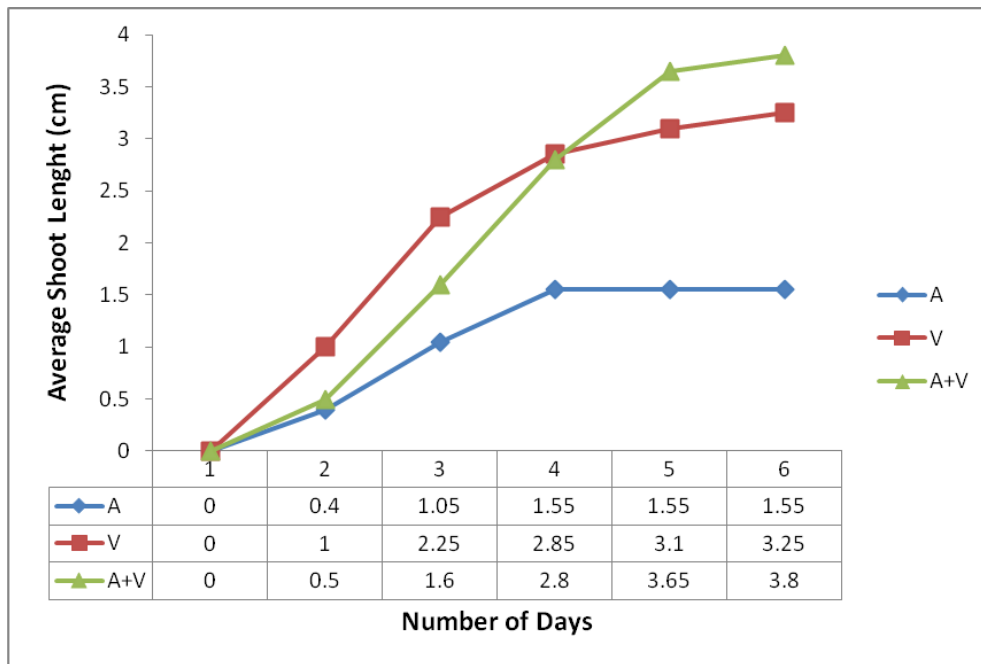
Present study has helped in understanding appropriate use of these biofertilizers for optimum plant growth. Based on the observations of this study it can be hypothesized that for efficient growth of plants like root tubers, vermiwash which enhances root growth might be used. In contrast, for fruiting plants and plants with economically important shoot, 'combination biofertilizer' might be preferred. However this assumption should be further investigated with field trials.

Vermiwash can also be used as a foliar spray which produces an almost immediate effect on the plants. Based on the observations of this study we propose the use of the 'combination biofertilizer' as foliar spray as it would have faster effect on plant growth and its dosage and time of application might be regulated depending upon the nutritional requirements of plant.

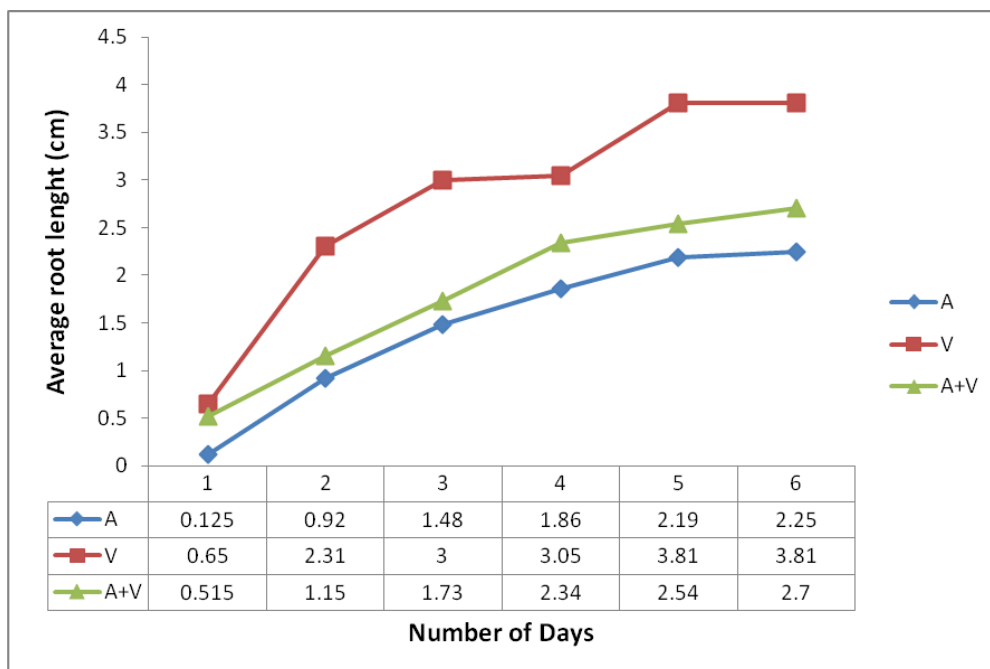
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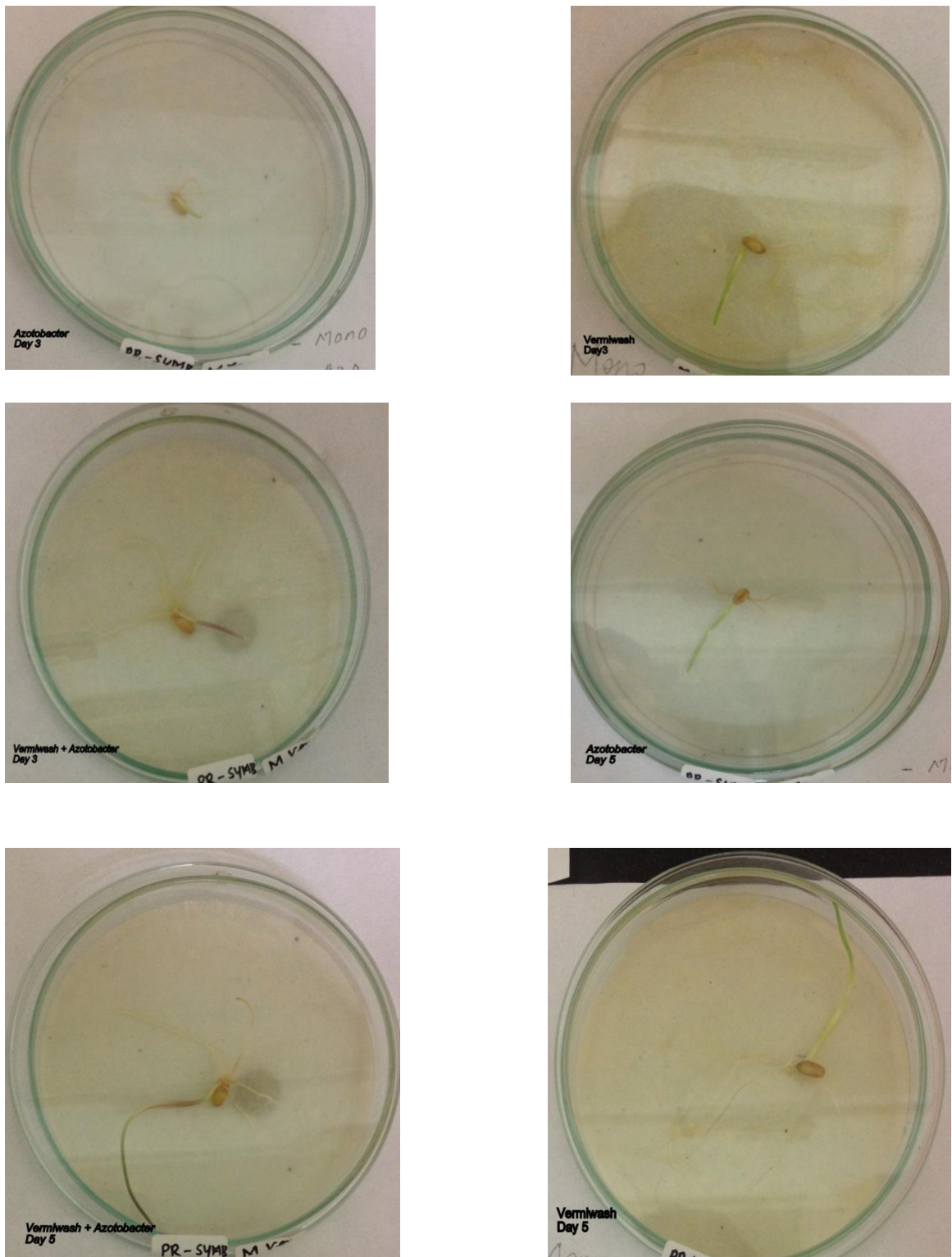


**Fig. 1: Effect of *Azotobacter* (A), Vermiwash (V) and their combination (A+V) on the shoot of wheat seedling.**



**Fig. 2: Effect of *Azotobacter* (A), Vermiwash (V) and their combination (A+V) on the root of wheat seedling.**





**Fig. 3: Effect of *Azotobacter* (A), Vermiwash (V) and their combination (A+V) on growth of wheat plant observed on day 4 and day 5.**

# DECOLOURIZATION OF MALACHITE GREEN BY BACTERIA AND THEIR POTENTIAL APPLICATION IN REDUCTION OF PHYTOTOXICITY OF MALACHITE GREEN

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## ABSTRACT:

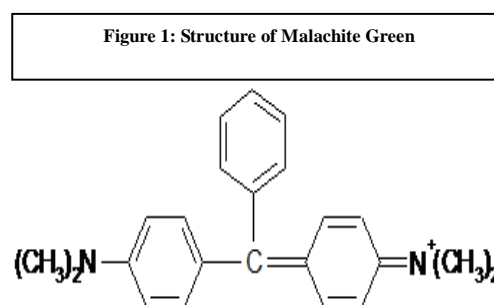
Dyes in different forms are being used in every aspect of day to day life. In the current study, bacteria capable of decolourizing malachite green were isolated from sample collected from Ulhasnagar water body near industrial area where industries release their effluent. Malachite green is an azo dye, used in a number of industries like textile, dyeing, food, cosmetics, etc. It is reported to be carcinogenic and mutagenic in nature. It is also reported to have negative effect on germination and growth of plants. Higher concentrations, up to 350 ppm of dye were used for enrichment of the organism and their efficiency of decolourization was checked by colorimetric analysis. Phytotoxicity of malachite green was checked by using *Sinapis arvensis* (Mustard) seeds. Two bacterial species were isolated capable of decolorizing malachite green. The decolorized product was obtained from individual bacterium and was used for the study. Effect of malachite green, its decolourized product and the isolated organism was checked on the germination and further growth of plant. Distilled water was used as control. The phytotoxicity studies indicated negative effect of malachite green on the germination of the seeds and growth of plant. It was observed that the decolourized product of malachite green for plant was less toxic since it showed better growth of plant as compared to growth of plant when treated with malachite green. Malachite green decolourization will help in bioremediation of agricultural land near water bodies polluted with malachite green.

**Key words:** Malachite Green, Decolourization, Phytotoxicity

## INTRODUCTION:

Malachite green (MG), N-methylated diaminotriphenylmethane, is one of the most common dyes used in textile industry, healthcare industry as medical disinfectant effective against protozoal, fungal and helminthic infections, food industry as food additives, coloring agents, paper & acrylic industries and in laboratories as a stain. (Srivastava 2004, Bayoumi, 2014).

The use of malachite green is controversial due to its hazardous effects at cellular as well as genetic level. (Srinivasan et.al, 2014) It has been reported that malachite green has various toxic effects on the animal tissue and also poses many environmental concerns.



(Ramezani et.al, 2013) Both clinical and experimental observations reported so far reveal that malachite green is a multi-organ toxin. (Culp et. al, 1999 Sudha, et.al, 2014) It decreases food intake, growth and fertility rates; causes damage to liver, spleen, kidney and heart; inflicts lesions on skin, eyes, lungs

and bones; and produces teratogenic effects in animals. (Culp et.al, 1999, Kasem et. al, 2016, Srivastava 2004)

The greatest environmental concern with dyes is their absorption and reflection of sunlight entering water. Light reflection diminishes photosynthetic activity of algae and affects the food chain. (Jana 2015, Kumar Vet.al, 2014) However, there has been very little research on the phytotoxic effects of malachite green. Negative effect of MG on seed germination rate and growth of plantlets have been reported. (Ho et. al., 2012, Sneha 2014, Sudha et.al, 2014) Various physical and chemical methods are available for treatment of effluent containing dyes. However, such treatment methods may be expensive.( Bhavani R et.al, 2016,Hameed, et al, 2009, Patel R, 2015, Pokharia et.al, 2013) Microbial degradation of malachite green can prove to be an effective and comparatively cheaper method that can be employed for treatment of effluents and fields containing malachite green. The current study focuses on the phytotoxic effects of malachite green and effect of its decolorized product on plant germination and growth.

## **MATERIALS AND METHODS:**

### **Sample collection:**

The effluent sample was collected from Ulhasnagar water body, at a site where effluents from nearby industries were disposed into the environment. The sample was obtained in the month of June 2016.

### **Enrichment of dye decolourizing bacteria:**

In order to obtain MG (Loba Chemie) decolourizers, it was important to grow the organism in presence of dye. 0.5 ml of the undiluted sample was inoculated in 1:10 diluted LB broth (HiMedia) containing 10 ppm of Malachite Green. This was incubated at 37°C for 48 hours. 0.1 ml aliquot of the above was then further inoculated in flasks containing 1:10 diluted LB broth with higher concentrations of Malachite Green up to 400

ppm.

### **Isolation of dye decolourizing bacteria:**

The dye decolourizing bacteria were isolated on LB agar plates supplemented with 350ppm MG. The colony characteristics of each organism were noted. The sample was also isolated on LB agar without MG for checking the opacity of the colony.

### **Decolourization efficiency of isolates:**

The decolourization efficiency of individual organism and consortium was determined by colorimetric analysis. 0.5 ml of the culture suspension adjusted to O.D. 0.1 at 530 nm was added in the LB broth containing 350 ppm of MG. Incubation conditions of 37°C for 48 hrs were provided. This was followed by centrifugation to obtain the supernatant. Absorbance of the collected supernatant at 530 nm was checked using colorimeter. The decolourization efficiencies of both the isolates were compared. Uninoculated sterile LB broth was used to set blank. The consortium was checked for decolourization efficiency to check the synergy between the organisms for decolourization. The formula used to determine the decolourization efficiency was as follows. (Sneha, et.al, 2014, Lal et.al, 2011, Raja, et.al, 2013)

$$\% \text{ decolourization} = \frac{[A_0] - [A_t]}{[A_0]} \times 100$$

Where,  $A_0$ = Initial absorbance

$A_t$ = Absorbance after incubation

### **Analysis of growth conditions of the isolate:**

#### **a. Optimum pH:**

The optimum pH for each organism was found out by inoculating 0.1 ml of its suspension in 1: 10 diluted LB broth having varying pH. The pH was checked using pH paper (Fisher Scientific). The tubes were incubated at 37°C for 24 hours.

**b. Optimum temperature:**

The optimum temperature of each organism was found out by inoculating 0.1 ml of suspension in 1:10 diluted LB broth and incubating them at different temperature conditions viz. 10°C, 27°C, 37°C and 55° C for 24 hours.

**c. Generation time:**

Growth curve studies of the isolated organisms were carried out using 25 ml of 1:10 diluted sterile LB broth in side-arm flask and their generation time was calculated.

**Phytotoxic analysis:**

The plant selected for phytotoxic analysis was *Sinapis arvensis* (Mustard). 5 pots with soil weighing 250 grams were subjected to different watering conditions as mentioned in Table 1. Five seeds were sown in each pot. The suspension of organism was adjusted to O. D. 0.1 at 530 nm when used for watering conditions. The germination, root length, shoot length, were observed at the end of 5 days. All the pots in each set were provided with equal and natural light and dark period. To check whether there were any other dye decolourizing organisms present in the soil, 0.5 ml of the soil suspension was added in Luria Bertani broth containing 350 ppm of Malachite green. The study was carried out in triplicates for each organism and consortium to ensure reproducibility of the result. The condition of MG 350 and control were kept common in all.

**RESULTS AND DISCUSSION:****Sample analysis:**

The sample obtained was black in colour with suspended solids. The temperature of the sample was 35°C.

**Enrichment and Isolation of dye decolourizing bacteria:**

The highest concentration showing

decolourization of malachite green was 350ppm. Two bacterial isolates obtained from the effluent sample were found to decolourize MG. The colony characteristics of both the organisms obtained on 1:10 diluted LB agar were observed and are recorded in table 2

**Decolourization:**

Both the organisms were able to decolourize MG. The organism from colony 1 was found to decolourize the dye more efficiently than the one from colony 2. The consortium was also checked for synergistic decolourization of 350 ppm of MG.

**Analysis of growth conditions of the isolate:**

The optimum pH for both the organisms was found to be 7. However they were also able to tolerate alkaline conditions. The optimum temperature for both the organisms was found to be 37°C indicating their mesophilic nature as shown in table below. From the growth curve analysis, generation time of organism 1 & 2 was found to be 60 minutes and 67.5 minutes respectively.

**Phytotoxic analysis:**

The germination, radical length and plumule length for both the organisms was recorded. The consortium did not show synergistic effect; hence was not used for the phytotoxicity studies. MG of 350ppm concentration had adverse effects on the germination of seeds as well as on growth of the plantlet. Their roots and shoots were found to be stunted, when compared with the growth of the plantlet in the control. In contrast, germination of the seeds provided with LMG and MG which was not decolourized was found to be less affected than MG. On comparison of the effects of two organisms, it was seen that colony 1 gave best result to reduce the phytotoxicity of the dye.

**CONCLUSION:**

The current study has given a natural and effective solution to reduce the phytotoxicity

of the azo dye MG. Treating the various industrial effluents with the organisms before disposing them to the environment will reduce the phytotoxicity of the dye. The differences in the decolourization efficiencies of the two organisms could be because of the different strategies used by the organisms for dye decolourization. The two organisms capable of decolorizing MG can be used in In-situ bioremediation as the radical and plumule growth is good in presence of organisms. Ex – situ methods can also be employed where the effluent containing malachite green is treated and then released in agricultural land for irrigation purposes ensuring sustainability of ground water. The increased attention to such studies will help in improving agricultural factors of a sustainably developing community.(Senthil et.al., 2013)

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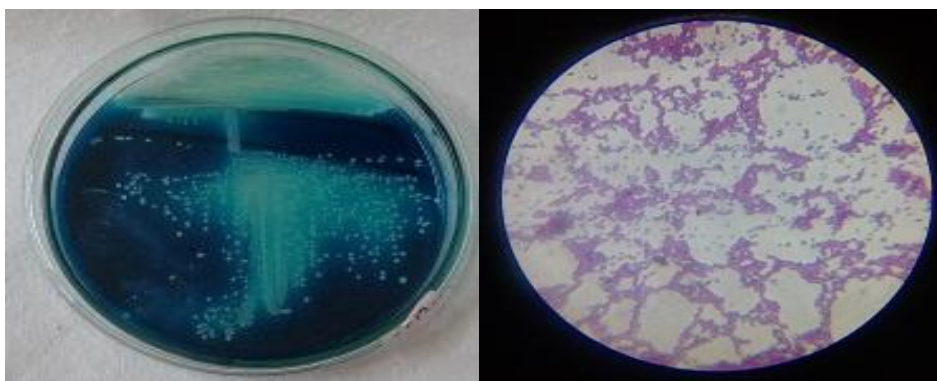
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<b>Sr. No</b>	<b>Watering conditions (5ml / day)</b>	<b>Significance</b>
1	MG 350	To check Phytotoxicity of MG
2	LMG	To check effect of decolourized product on germination & Plant growth
3	MG + ORG	To Check the efficiency of decolourization of organism in soil
4	ORG	To check the effect of Organism on growth of plant
5	DW	Control

MG – Malachite Green, LMG – Leuco-Malachite Green and undecolourized Malachite green, ORG – Organism, DW - Distilled Water

**Table 1: Conditions Provided for Seed Germination & Plant Growth**



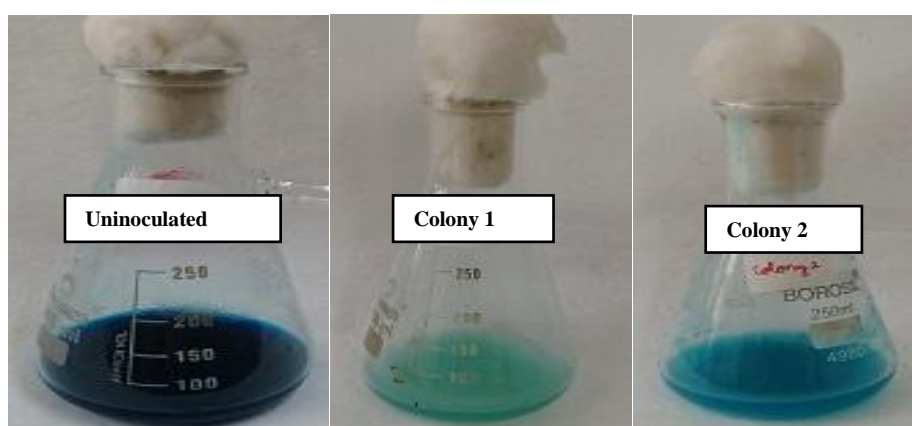
**Figure 2: Isolated colony 1 & Gram staining**

Characteristics	Colony 1	Colony 2
Size	Small	Small
Shape	Circular	Circular
Elevation	Convex	Flat
Colour	White	Transparent
Opacity	Opaque	transparent
Margin	Entire	Entire
Consistency	Sticky	Butyrous
Appearance	Mucoid	Dry
Gram Staining	Gram -ve coccobacilli	Gram +ve cocci

Organism	% decolourization at 530nm on Day 5
Colony 1	90
Colony 2	68.5
Consortium	86.4

**Table 3: Decolourization Efficiency**

**Table 2: Colony Characteristics of the Isolates**



**Figure 3: Decolourization by the Isolates**

Sr. No.	Watering conditions	Organism 1			Organism 2		
		Germination (%)	Radical (mm)	Plumule (mm)	Germination	radical (mm)	Plumule (mm)
1	MG 350	60	60	56.5	60	60	56.5
2	LMG	100	80	82	100	95	70
3	MG + ORG	100	97.5	85.3	100	80	85
4	ORG	100	65	66	100	75	75
5	DW	100	98.8	84.4	100	98.8	84.4

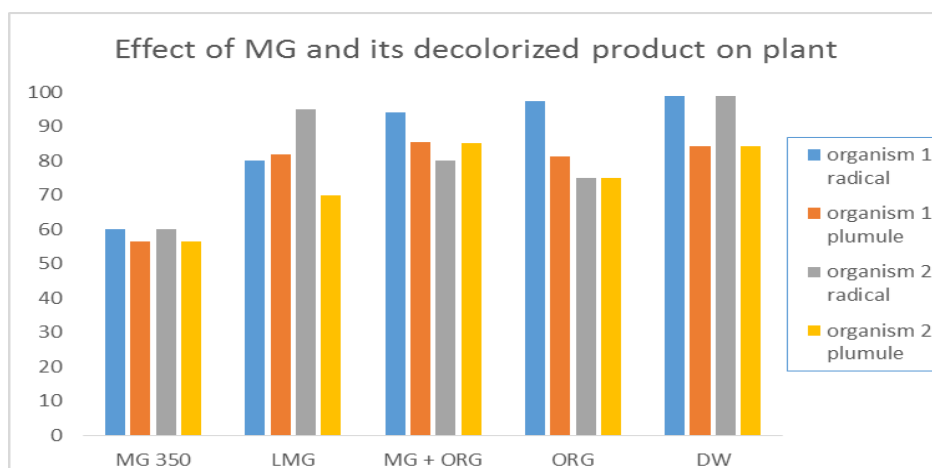
Key: MG – Malachite Green, LMG – Leuco-Malachite Green + Malchite green which was not decolorized, ORG – Organism, DW - Distilled Water,

**Table 4: Phytotoxicity analysis of different watering conditions**

pH	5	6	7	8	9	Temperature	10°C	27°C	37°C	55°C
Colony 1	-	-	++	+	+	Colony 1	-	+	++	-
Colony 2	-	-	++	+	+	Colony 2	-	+	++	-

- : No growth +: Growth ++ : Luxurious growth

**Table 5: The optimum pH and temperature conditions of the isolates**



**Figure 5: Graphical representation of Comparative Analysis of Organism 1 & 2**



# POTENTIALITY OF FERTILIZERS ON SUSTAINABLE GROUNDNUT (*Arachis Hypogaea* L.) YIELD

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## ABSTRACT:

Groundnut (*Arachis hypogaea* L.) is an essential annual oil seed crop cultivated all over the world for extracting edible oil. Groundnut also known as peanut is rich in its protein content. It produces high-protein forage that is used as ruminant feed. Critical assessment in India has revealed decrease in groundnut productivity even though area under production has gradually increased. Improved management practice is required for increasing the productivity of groundnut crop.

In the present study field experiments were carried out for improving groundnut (variety SB XI) productivity under the influence of various concentration of foliar spray of humaur, seed treatment (Beej sanjeevani), Bio-organic myceemeal and inorganic Nitrogen and Phosphate fertilizers on vegetative growth and yield of groundnut. Productivity was studied in terms of plant height, number of branches, per plant pod weight, kernel weight, dry matter of per plant and over all yields.

Response of humaur and seed treatment along with NP fertilizers showed significant increase in height of the plant. Application of seed treatment along with NP fertilizers produced maximum number of branches and per plant pod weight. Maximum kernel weight and dry matter yield was seen with treatment of humaur and NP fertilizers.

**Key words:** Groundnut (*Arachis hypogaea* L.), SB XI variety, Humaur foliar spray, NP fertilizers

## INTRODUCTION

The new world groundnut (*Arachis hypogaea* L.) has been accepted by Indians as a vegetable oilseed crop during the middle to late nineteenth century, and has since then occupied the first place among the oilseed crops grown in the country. Groundnut is an essential annual oilseed crop (Farag, and Zahran, 2014). It is cultivated all over the world for extracting edible oil. Groundnut also known as peanut is rich in its protein content. It produces high-protein forage that is used as ruminant feed (Ozyigit and Bilgen, 2013)

Groundnut (*Arachis hypogaea* L.) is one of the principal economic crops of the world that ranks 13<sup>th</sup> among the food crops. To make the country self-sufficient in edible oil, it is extremely necessary to increase the total production of oilseed crops including groundnut either by increasing their yield per hectare or by increasing their acreage of

cultivation or by a combination of both (Subrahmanyam *et al.*, 2000).

The productivity of groundnut depends on proper selection of variety, fertilizer management and other management practices. Proper fertilizer doses of nitrogen, phosphorus, calcium and boron have vital effect on the yield of groundnut (Lourduraj, 1999).

Critical assessment in India has revealed decrease in groundnut productivity even though area under production has gradually increased. Improved management practice is required for increasing the productivity of groundnut crop. Thus, the present experiment was designed to see the effect of different fertilizers on growth and yield of the crop.

## MATERIALS AND METHODS

A field experiment was carried out during the

summer season for assessing the “potentiality of fertilizers on sustainable groundnut (*Arachis hypogaea* L.) yield of groundnut variety SB XI”. Composite soil sample was taken for determination of physical and chemical properties of the soil before conducting the experiment. Seed variety SB XI were purchased from the local market. Seeds were sown at the rate of two seeds per hill.

The experiment was conducted in randomized block design. All together nine treatments were given with three replicates, gross plot size was 3 m X 3 m, net plot size was 2.60 m X 2.60 m. Fertilizers used were mycemeal, humaur, urea and superphosphate. Seed treatment of beej sanjeeveni was given before sowing to seed as per Bapat and Umale (1973) method. Mycemeal was given 250 kg/acre at seedling stage and remaining dose was given one month after sowing of the crop. Humaur a liquid fertilizer was sprayed at a rate of 1 ml/litre, 2 ml/litre and 3 ml/litre with five split doses along with recommended dose of NP fertilizers.

Statistical analysis was done as per method given by Panse and Sukhatame (1978).

**Experimental details:** The research experiment was conducted with following treatments.

T1 – Control (Recommended dose of fertilizer)

T2 – Humaur 1ml/lit + 25 kg N + 50 kg P<sub>2</sub>O<sub>5</sub>/ha.

T3 – Humaur 2ml/lit + 25 kg N + 50 kg P<sub>2</sub>O<sub>5</sub>/ha.

T4 – Humaur 3ml/lit + 25 kg N + 50 kg P<sub>2</sub>O<sub>5</sub>/ha.

T5 – Humaur 2ml/lit + Mycemeal 500 kg/acre.

T5 – Humaur 2ml/lit + Mycemeal 500 kg/acre.

T6 – Seed treatment + 25 kg N + 50 kg P<sub>2</sub>O<sub>5</sub>/ha.

T7 – Mycemeal 500 kg/acre.

T8 – Seed treatment + Humaur 2ml/lit + Mycemeal 500 kg/acre.

T9 – Humaur 2ml/lit + Humaur 2ml/lit.

**Growth observations:** For recording growth observations five plants were randomly selected from each plot. These five plants in each treatment were marked on labels by fixing long bamboo pegs. Observations were recorded at harvesting stage.

**Average height per plant:** Five observational plants were selected. Height in these plants was recorded from ground level to the base of the fully opened leaf.

**Average number of branches / plant:** Numbers of branches in five observational plants were recorded at harvesting.

**Average dry matter per plants:** To study the dry matter accumulation and distribution the plant samples were collected at harvest. At each sampling, five well sampled plants were randomly selected from each treatment, and were packed in labeled bags and dried in oven at 90<sup>0</sup> C for first one hour and then at 60<sup>0</sup> C till the samples were completely dried. The dry weight of individual plant was recorded separately for each treatment.

**Average number of pods per plant:** The number of pods of five plants was recorded in each treatment.

**Average weight of pods per plant:** Pods obtained from five plants were dried and dry pod weight was recorded.

**Average 100 pods weight in grams:** 100 pods were randomly selected from each plot and their dry weight was recorded.

**Average pod yield q/ha:** The pod yield was calculated on the basis of per plot yield.

## RESULTS AND DISCUSSION:

The observations recorded on various aspects of plant growth in the course of investigation carried out to find “potentiality of fertilizers on sustainable groundnut (*Arachis hypogaea* L.) yield of groundnut variety” SB XI is presented in Table no.1.

**Effect on plant height:** The observations recorded in plant height at harvest were statistically analysed and presented in table no.1.

It is observed from the data shown in table no.1 that the height of plant increased from 6.3cm to 32.1 cm in T3 treatment, T4 6.2 cm to 30.8 cm, T6 6.3cm to 30.6 cm and 6.1cm to 29.6 cm in T2. Increase in height of the plants in treated ones were significant over control. However, treatment T7, T8 and T9 were insignificant. Treatment T5 showed height 6.1cm to 29cm at harvest, similar to the height of control plant.

**Effect on number of branches:** The observations are recorded in number of branches per plant at harvest were statistically analysed and presented in table .no. 1.

It is observed from the data shown in table. no.1 that number of branches increased to 6.9 at harvest in T3 treatment and followed by T6 produced 6.8. However, treatment T2 and T8 produced 6.7 branches at the time of harvest which was lowest as compared to T2 treatment respectively. Treatment T4 produced 6.6 and T5 produced 6.5 branches per plant which were statistically significant over control. However, T9 produced same number of branches with that of control T9 6.4, treatment T7 was insignificant.

**Effect on Dry Matter / Plant:** The observations are recorded dry matter per plant on 21, 63 and at harvest time were statistically analysed and presented in Table No.1

It is observed from the data shown in table

no.1 that dry matter per plant was 37.9 gm at harvest in T3 treatment, T6 produced 35.2 gm, T4 34.4 gm and T2 34.2 gm dry matter per plant which were found to be statistically significant over control. However, treatment T5, T7, T8 and T9 were statistically insignificant.

**Effect on Pod Weight / Plant:** The data recorded per plant dry weight of Pod in grams were statistically analysed and presented in table No.1

The data shown in table no.1 revealed that per plant dry weight of pod significantly increased in T2, T3, T4 and T6 treatment i.e. 18.6 gm, 18.9gm, 18.4gm and 20.2 gm respectively over control. However, treatment T5, T7, T8 and T9 were found insignificant. Control produced 17.6 gm dry weight of pods per plant.

**Effect on 100 Kernel weights (gm):** The data recorded after harvesting dry weight of 100 kernel was taken in grams and presented in table .No. 1

The data shown in table no. 1 clearly indicated that treatment T2, T3, T4, and T6 produced more 100 kernel weight i.e. 39.4gm, 40.2gm, 39.0gm, 38.9 gm were found significant over control. However treatment T5, T7, T8 and T9 showed insignificant. Control produced 38.3 gm 100 kernel weight.

**Effect on Yield q/ha:** The pod yield was calculated on the basis of total yield obtained by every individual plant and not plots under each treatment. The yield q/ha is presented in table no.1

The data shown in table no.1 clearly indicated that various levels of fertilizers showed significant effect on pod yield treatment T2, T3, T5 and T6 i.e. 20.19, 20.69, 20.21 and 21.75 q/ha. However, treatment T5, T7, T8 and T9 were insignificant pod yield. Control produced 20.13 q/ha.

Similar types of finding were reported by (Chitale and Reddy 1991), reporting that groundnut crop responded well on application of N and P on the plant height and dry matter of the plant. (Kene *et al.*,1995) applied recommended fertilizers and foliar spray of IBA,GA and IAA at a rate of 15 ppm and 30 ppm concentrations which were given at bud stage (36 days after sowing).They have also recorded that plant height ,dry matter and total yield was significantly higher in 30 ppm treatment of IBA,GA and IAA. (Joshi and Nanir 1994) recorded similar types of observations on bhindi where in treatment with Humaur recorded significantly highest plant height and yield over control plants.

### CONCLUSION

Humaur foliar spray at a rate of 2 ml/litre along with recommended dose of NP fertilizers per hectares gave marked effect on height of the plant, dry matter per plant and pod yield q/ha.over the other treatment.Beej sanjeevani along with recommended dose of NP fertilizers per hectare also showed significant effect on height of the plant, dry matter per plant and pod yield q/ha.over the other treatment.Myceemeal recommended dose did not affect the height of the plant, dry matter per plant and pod yield q/ha.

### ACKNOWLEDGEMENTS:

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Treatment	At harvest					
	Avg. plant height in cm	Avg. no. of branches	Avg. dry matter/ plant	Dry weight of pod (gm)	100 Kernel weight (gm)	Avg. pod yield q/ha
T1	29.1	6.4	33.3	17.6	38.3	20.13
T2	29.6	6.7	34.2	18.6	39.4	20.19
T3	32.1	6.9	37.9	18.9	40.2	20.69
T4	30.8	6.6	34.4	18.4	39.0	20.21
T5	29.0	6.5	31.7	12.7	34.8	13.69
T6	30.6	6.8	35.2	20.2	38.9	21.75
T7	28.3	6.1	30.1	10.8	21.1	12.48
T8	28.5	6.7	32.3	16.6	34.7	18.79
T9	27.9	6.4	29.6	13.4	28.4	14.80
<b>S.E.</b>	<b>0.18</b>	<b>0.03</b>	<b>0.389</b>	<b>0.088</b>	<b>0.213</b>	<b>0.144</b>
<b>C.D.At 5%</b>	<b>0.37</b>	<b>0.07</b>	<b>0.795</b>	<b>0.183</b>	<b>0.440</b>	<b>0.297</b>
<b>Level F.(t)</b>	<b>7.50</b>	<b>5.80</b>	<b>4.77</b>	<b>310.19</b>	<b>197.18</b>	<b>127.62</b>

Table No. 1: Effect of fertilizers on various parameters

## EVALUATION OF SYNERGISTIC EFFECT OF PGPRs IN CONSORTIUM ON GROWTH OF *Vigna radiata*

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### ABSTRACT:

Vermiwash is a known biofertilizer obtained after the passage of water through vermicompost which collects the excretory products and micronutrients from soil. Plant growth-promoting rhizobacteria (PGPRs) live in association with plants and improve growth. Many PGPRs live in the form of microcolonies in soil and on the roots. PGPR bioformulations minimize the use of mineral fertilizers and maximize plant growth. Microbial consortia are much more efficient than individual organisms with diverse metabolic capabilities.

NPK estimation of neat vermiwash revealed low percentages of available NPK and organic carbon. To account for the growth stimulatory properties of vermiwash despite a low inherent NPK index, the composition of the microflora of different concentrations of vermiwash (10%, 20%, 50% and neat) was monitored using bacteriological culture analysis. Diluted (1:100) Nutrient Agar media supplemented with 10% sterile vermiwash was used to allow detection of slow-growing probable PGPRs. The plates were incubated at room temperature for a period of 7-10 days. The results obtained showed a high count of probable PGPR microcolonies in plates inoculated with 20% vermiwash. Peculiar, dew-drop bacterial colonies were obtained in the 50% and neat vermiwash inoculated plates. Although microcolony counts in these concentrations were low, they were observed only in the proximity of these peculiar colonies. Synergy between the microcolonies and the peculiar isolate for PGP activity was investigated using chamber-pot culture assay for *Vigna radiata* (green gram). Distilled water, 20% vermiwash; and culture suspensions of the microcolony isolate, the peculiar isolate and a consortium of the two, respectively, in 20% vermiwash were used as growth media in each pot with a filter paper and cotton substratum. Results revealed maximum growth characteristics for the consortium of the two isolates. The positive effect of a single microbial species on plant growth can thus be improved by the application of PGPR consortia.

**Keywords:** Vermiwash, PGPR, microcolony, consortium

### INTRODUCTION:

Vermicomposting is a process involving biological oxidation and stabilization which, in contrast to composting, is accelerated by the action of earthworms and micro-organisms without employment of a thermophilic stage. During this process turning, fragmentation and aeration by earthworms, transforms complex organic matter into biofertilizer.

Vermiwash is a known biofertilizer obtained after the passage of water through a vermicompost setup. It contains several

phytohormones and vitamins along with micro and macronutrients which increases the resistance power of crops against various diseases enhancing the growth and productivity of crops. The most important and well-established application of vermiwash is in the form of a foliar spray. When applied, these nutrients are absorbed and transported to the leaves, shoots, and other parts of a plant (Khan *et al.*, 2014).

Plant growth promoting rhizobacteria (PGPR)

are an important constituent of the soil microflora. They enhance plant growth by forming biofilms in the rhizosphere, which has advantages over planktonic existence (Seneviratne G. *et al.*, 2010). Vermiwash is also known to be rich in PGPRs such as *Azotobacter spp.*, *Nitrosomonas spp.*, *Nitrobacter spp.*, mycorrhizal fungi, phosphate solubilizing bacteria, etc. Microscopy-based studies of bacterial colonization in the rhizosphere indicate that PGPRs generally form microcolonies or aggregates on root surfaces. However, most attempts to exploit pure cultures of PGPRs as biocontrol inoculants, biofertilizers, phytostimulants or inoculants for bioremediation have had limited success on a large scale. In the soil, PGPRs are known to interact with various biotic and abiotic factors and so far the research has only focused on the latter. One of the most important biotic factors is the interaction of a PGPR with other beneficial or pathogenic microorganisms which may impact its PGP ability.

A microbial consortium is a group of species of microorganisms that act synergistically, either by cooperation or mutualism. The positive effect of a single microbial species on plant growth in low-fertility soils can be improved by the application of mixtures of different microbial species. Microbial consortia are much more efficient than individual strains of organisms with diverse metabolic capabilities (Sudharani M. *et al.*, 2014).

## **MATERIALS AND METHODS:**

### **Collection of Vermiwash**

Vermiwash was collected from the active vermicompost set up. It was then filtered using Whatman filter paper No. 1 and stored at 4°C for further use.

### **Determination of N, P, K:**

The pH and approximate percentages of oxidizable organic carbon, available nitrogen

(nitrous and ammoniacal), phosphorous (P<sub>2</sub>O<sub>5</sub>) and potassium (K<sub>2</sub>O) were determined using HIMEDIA Soil Testing Kit. Values were obtained in kg/ha.

### **Microbiological analysis**

To analyse the oligotrophic microflora of different concentrations of vermiwash (10%, 20%, 50% and neat), 1:100 diluted Nutrient Agar medium was used. 10 % vermiwash was added to this nutrient limiting medium to simulate the natural environment where the bacteria are thriving. Each of the prepared concentrations of vermiwash was used for isolation of bacteria using spread plate and streak plate techniques. The experiment was carried out in duplicates. The plates were incubated at room temperature till visible microcolonies developed. The plates were examined daily for late-appearing colonies. The select colonies were Gram stained and further used for the chamber culture assay.

### **Seed sterilization**

Seeds of *Vigna radiata* (green gram) were procured from the local market, (Maharashtra, India). These seeds were washed with sterile distilled water once and then treated with 0.1% HgCl<sub>2</sub> for 4-5 minutes. The seeds were then washed thrice in sterile distilled water and soaked in it for 15 minutes.

### **Chamber culture assay**

Twenty percent vermiwash was prepared using sterile distilled water and sterilized. Culture suspensions of two isolates (isolates 1 and 2) obtained in the microbiological assay were prepared using sterile saline. These were used for inoculation in 100 ml of sterile 20% vermiwash each. The O.D. of each vermiwash suspension was set at 0.1.

Glass chambers were disinfected using absolute alcohol. Filter paper and cotton were used as substratum. Ten surface-disinfected seeds of *Vigna radiata* were placed aseptically in each pot. 100ml of the different solutions,

viz. sterile distilled water, sterile 20% vermiwash, 20% vermiwash inoculated with Isolate 1, 20% vermiwash inoculated with Isolate 2; and 20% vermiwash inoculated with both isolates 1 and 2, were used as growth media in different chambers.

The chambers were incubated at room temperature with a photo-period of 16 hours. Addition of growth media was done only in the beginning and then length of root and shoot was measured using Vernier calliper each day up to 5 days.

## RESULTS & DISCUSSION:

The aim of the present study was to determine the positive effect of a consortium of two probable PGPR isolates from vermiwash on the growth of *Vigna radiata*.

### Determination of NPK:

Neat vermiwash was analysed for pH, oxidizable organic carbon (OC), phosphorus (P) and potassium (K), ammoniacal nitrogen (A/N) and nitrate nitrogen (N/N). The pH at the time of collection was found to be  $7.5 \pm 0.2$  and decreased to  $7.0 \pm 0.2$  over the time of storage. The percentage of OC was found to be 0.3-0.5 kg/ha (medium), available P was 22-56 kg/ha (medium), available K was 112-280 kg/ha (medium), A/N was  $\leq 15$  kg/ha (very low) and N/N was  $\approx 10$  kg/ha (very low).

### Microbiological analysis

Fertilizers are generally applied in a diluted form as over-application of any nutrient (particularly N, P and K) can cause leaf burn, increase risk of disease, delay maturation and even cause plant death. It is well-known that germinating seeds are much more sensitive to over-fertilization as the young root system is very sensitive to fertilizer burns. On-field application of fertilizers is also carried out using dilutions. In our previous study we used 4 concentrations, viz., 10%, 20%, 50% and neat to check the germinative properties of each (Data not shown). 20% vermiwash was

found to have maximum PGP abilities. Therefore, this study was carried out to account for these PGP activities at 20% concentration.

Straw-coloured microcolonies (referred as Isolate 1) developed on the plates after 3-4 days of incubation and were found to be most abundant on plates spread/streaked with 20% vermiwash. Probable *Azotobacter spp.* colonies (having typical colony characteristics of *Azotobacter* i.e., a dirty-white, dew-drop appearance) were also obtained on all the plates. Clear, dew-drop colonies (referred as Isolate 2) having a gummy consistency were found on plates spread and streaked with neat vermiwash. These colonies showed the presence of a slight halo, where maximum microcolonies occurred. Additionally, plates inoculated with neat vermiwash showed maximum total count of microcolonies and probable *Azotobacter* colonies.

Microcolonies obtained in 20% vermiwash inoculated plates were chosen as Isolate 1. The peculiar dew-drop colonies obtained only in 50% and 100% concentrations were chosen as Isolate 2.

On performing Gram staining, Isolate 1 was found to be pleomorphic coccobacilli with no defined arrangement. Isolate 2 occurred in the form of Gram positive coccobacilli arranged in chains and clusters.

Motility was checked using agar-butt inoculation. Both isolates were found to be non-motile.

### Chamber culture assay

Fig. 2 shows the effects of sterile distilled water (D/w), sterile 20% vermiwash (V), 20% vermiwash inoculated with Isolate 1 (1), 20% vermiwash inoculated with Isolate 2(2) and 20% vermiwash inoculated with both isolates 1 and 2(1+2) respectively on germination parameters of *V. radiata*.

Inoculated vermiwash significantly improved germination of the seeds, however the rate of



germination varied with each inoculum. Maximum seed vigour and number of leaves per shoot were observed for the mixed inoculum. Minimum values were obtained for the inoculum of Isolate 1.

Low values for available N, P, K and oxidizable organic carbon in neat vermiwash led to the observation that vermiwash has an inherently low to medium NPK content which alone cannot account for its plant growth promoting activity. Therefore, microbiological analysis was carried out for detection and isolation of probable PGPRs.

Abundance of microcolonies at the 20% concentration was presumed to be the reason for 20% vermiwash being obtained as the ideal concentration for growth of *V. radiata* in our previous study. Therefore, these microcolonies were considered to be probable PGPRs. The high occurrence of microcolonies around the peculiar colonies was considered to be a form of non-obligate symbiosis.

The gummy consistency of Isolate 2 is suggestive of Exopolysaccharides (EPS) production. EPS and certain outer membrane proteins are needed for effective root colonization by PGPRs (Barahona E. *et al.*, 2010). Motility of these isolates was determined as it is an important prerequisite of certain PGPRs for effective root colonization. Non-motile mutants are reported to be among the most impaired in competitive root colonization (Barahona E. *et al.*, 2010).

In our previous study, maximum plant growth parameters for *V. radiata* were obtained in the 20% vermiwash trial. This result is in accordance with trials on other plants (Ansari A. A. 2008). The positive effect of vermiwash on plant growth has been attributed by contemporaries to factors such as the presence of phytohormones (Suthar S. 2010) e.g.: IAA, cytokinins, gibberilic acid, etc., and micronutrients (Varghese S. M. and Prabha M. L., 2014) e.g.: Ca, Zn, Fe, Mn, Vd and Mo. The greater plant growth promoting capacity

of 20% vermiwash than neat vermiwash was thus attributed to the presence of certain rhizobacteria that flourish at this concentration. However, the low germination parameters obtained for the Isolate 1 suggest poor PGP activity. Isolate 2 showed some amount of PGP activity but was lower than that obtained for 20% vermiwash and D/w control.

The inability of Isolate 1 to produce EPS, promote plant growth and show motility could be attributed to the hypothesis of evolution of multispecies cooperation, which considers cooperation in complex bacterial communities as being a consequence of each species adapting to the presence of the other (Morris J. J. *et al.*, 2012). According to this hypothesis, in the presence of Isolate 2, which showed EPS production and PGP ability, Isolate 1 may delete vital functions or pathways that are provided by Isolate 2, so as to boost individual fitness. This leads to a communal dependency, which has been studied in case of biofilm production as the function that is lost by some species (Ren D. *et al.*, 2015). Additionally, bridging bacteria (EPS producers) may facilitate the association of other species that do not aggregate directly with each other. Thus, species that do not form biofilms as single strains (here, Isolate 1) may benefit from the advantages associated with biofilm formation, including enhanced protection from external stress and expanded niche availability, through engagement with multispecies communities (Ren D. *et al.*, 2015).

## CONCLUSION:

The present study confirmed the synergistic potential of two probable PGPRs isolated from particular vermiwash concentrations. The germinative parameters are clearly demonstrated to have been improved when the suspensions of both isolates were used in combination. Efficacy of this PGPR consortium is significantly higher than application of only vermiwash.

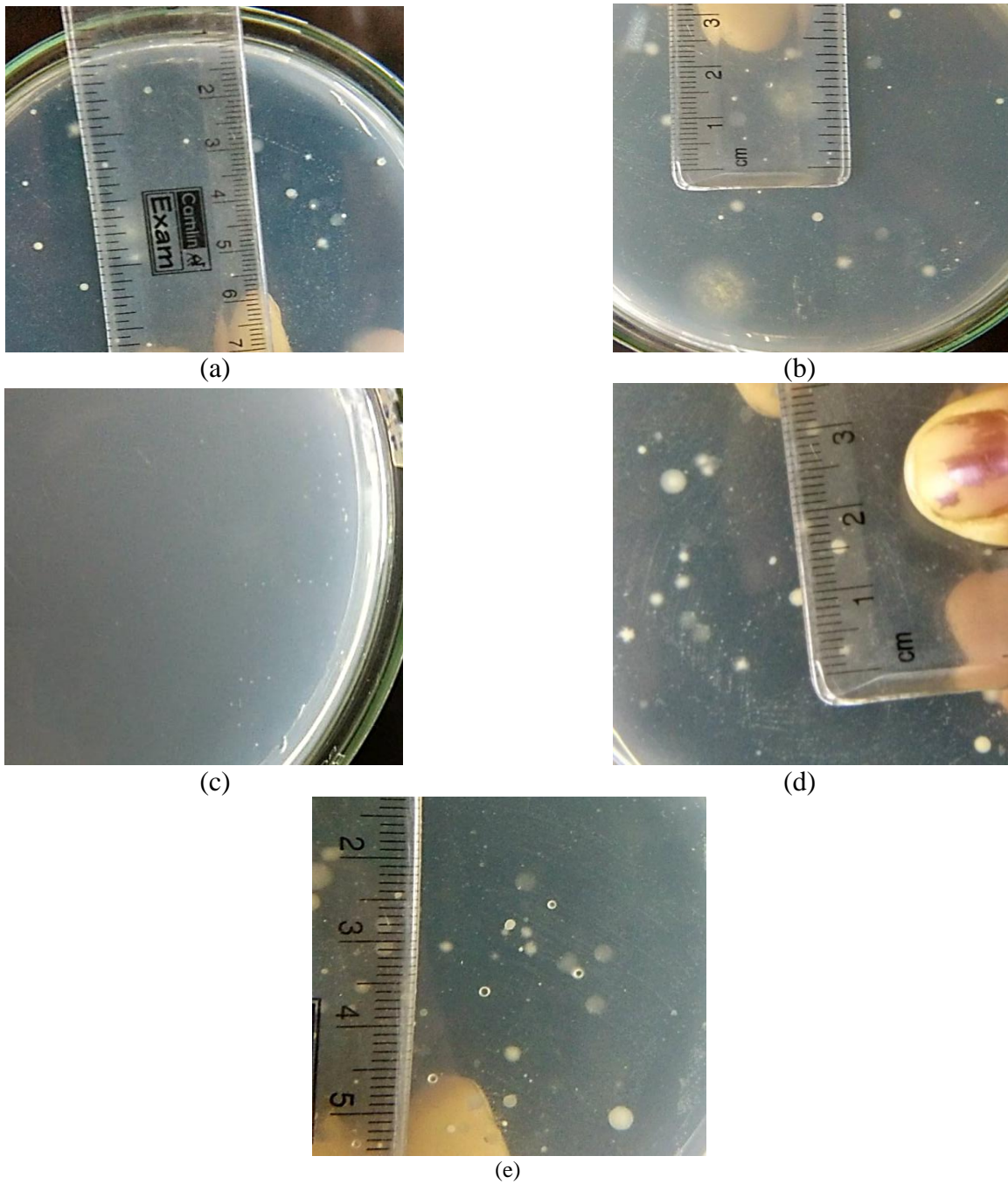
The present study noted the presence of varied bioactive bacterial flora when different concentrations of vermiwash were used for media preparation and as inoculum. Earlier studies have highlighted the importance of nutrient concentration for isolating novel bacteria. This is the first report according to our knowledge, where different concentrations of natural samples as inoculum also have led to isolation of varied microflora.

The study has also highlighted the significance of natural biofertilizers such as vermiwash along with the use of a PGPR consortium as an improved biofertilizer. Future studies would be performed on characterisation of these isolates to check if they are novel, yet uncultured bacteria; and on the EPS/biofilm production.

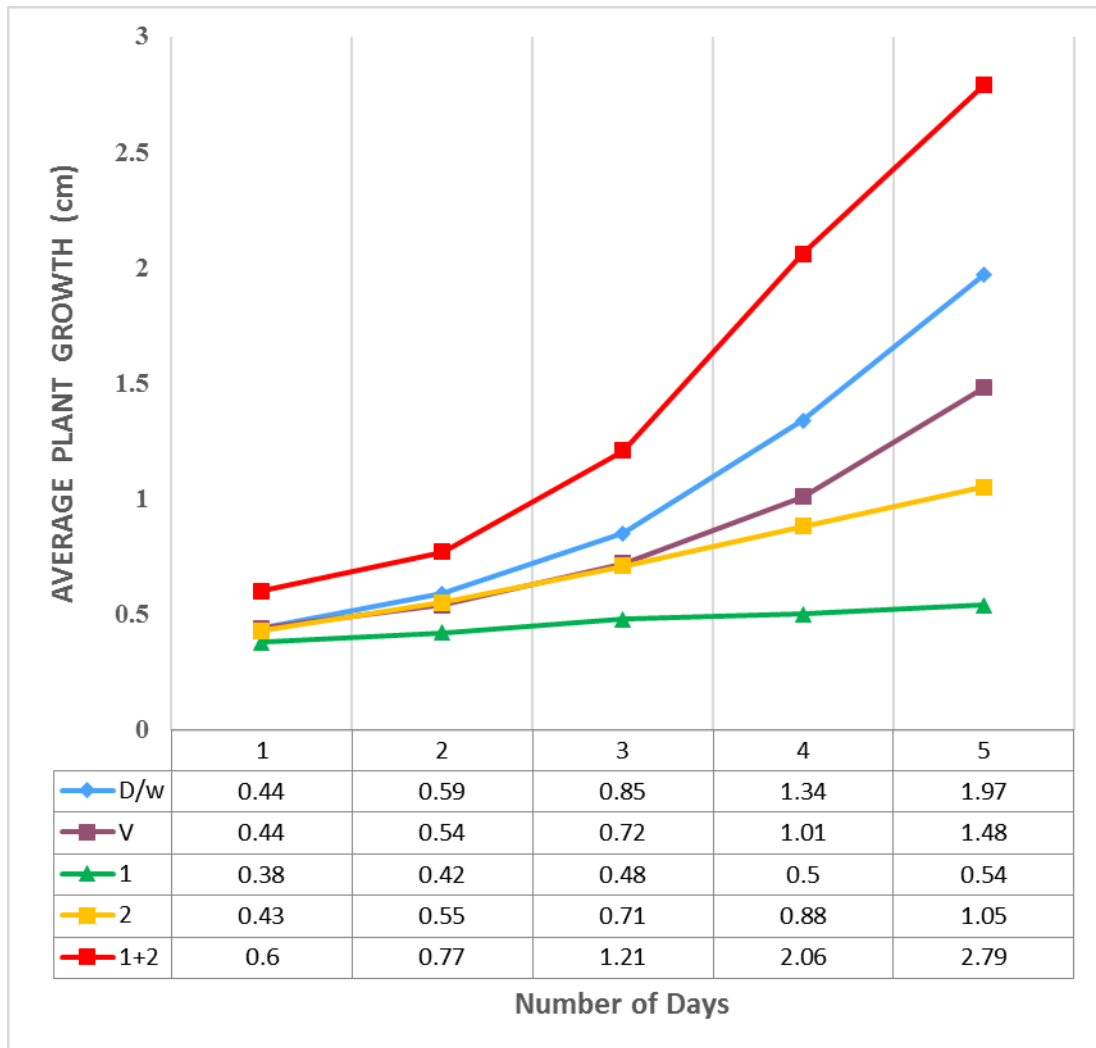
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**Fig 1.** Spread plates of all 4 concentrations showing the presence of microcolonies. (a) 10% vermivash: least number of microcolonies. (b) 20% vermivash (spread plate): Large number of microcolonies and probable *Azotobacter* colonies (c) 20% vermivash (streak plate): prominent straw-coloured microcolonies (Isolate 1), (d) 50% vermivash. (e) Neat vermivash: Presence of peculiar, clear dew-drop colonies (Isolate 2). Highest number of microcolonies and probable *Azotobacter* colonies.



**Fig 2**

## **COMPOSITION OF LIFE-FORMS AND BIOLOGICAL SPECTRUM AS INDICATOR FOR SUSTAINABLE AGRICULTURE-MUMBAI AND SUBURBS – A CASE STUDY.**

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### **ABSTRACT:**

In the advanced world of 21<sup>st</sup> century, the farmers are largely dependent on weather forecast in farming practices. The present study reveals intimate relationship between life-form classes and weather predictions leading to sustainable agriculture. Biological spectra based on it along the climatic gradient is a direct measure of phytoclimate of that region. Thus floristic composition classified according to Raunkiaerian life-forms or growth forms is a good ecological indicator of weather forecasting and subsequent microclimate of the region. In the current study different localities in Mumbai and suburbs were assessed for floristic composition. The study sites include Colaba, Malbar Hill, Vir Jijamata Udyan, Byculla, Patanpada, Yeoor and Kankeshwar, Alibaugh. The floristic composition listed was classified according to Raunkiaer's classification in five life-form classes. The percentage of Phanerophytes, Chamaephytes, Hemicryptophytes, Geophytes or Cryptophytes and Therophytes was calculated. The Biological spectra of study sites have been prepared and compared with Raunkiaer's normal Biological spectrum to know the phytoclimate of the region. The dominance of Phanerophytes appears to be due to high rainfall and temperature and low biotic pressure. In this region the vegetation was predominantly evergreen, although the tree flora has considerable elements of deciduous species. However the population of Therophytic community was increasing in highly grazed and eroded areas.

**Key words:** Life-forms or growth forms, Biological spectrum, phytoclimate, Phanerophytes.

### **INTRODUCTION:**

It is well known that plants are good bioindicators of the prevailing environmental conditions, in which they grow. Ellenberg (1974) was the first to provide theoretical and methodological basis for the evaluation and use of ecological indices of plants. Climate determines the type of plants that can exist in each ecosystem. Climate change and agriculture are interrelated processes, both of which take place on a global scale. Climate change affects agriculture in a number of ways, including changes in average temperatures, rainfall and climate extremes (e.g. heat waves), changes in pests and diseases, changes in atmospheric carbon dioxide and ground-level ozone concentrations, changes in the nutritional

quality of some foods and changes in sea level etc. The general appearance of vegetation is referred to as 'Physiognomy.' The individual species in a community can be grouped into various life-forms (Christopher Raunkiaer, 1934) on the basis of their Physiognomy appearance and growth performance. The life-form of the vegetation is the product of their genetic pool and tolerance towards the climatic variation. Moreover, biological spectra are important physiognomic attributes that have been widely used in vegetation analysis. The life-form spectra are said to be the indicators of micro and macroclimate (Asmus, 1990.) Climatic types can be characterized by the prevailing plant indicators in a particular plant community, under a given

climatic regime (Raunkiaer, 1934, Cain, 1950, Muller-Dombois and Ellenberg (1974). Very little work worldwide has been done on the vegetation analysis, life-form and leaf size spectra. The biological spectra of the Indian region have been related to specific climatic, edaphic and altitudinal factors. (Meher Homji, 1964, Pandey & Parmar 1993, Sharma & Dhakre, 1993, Reddy *et al*, 1999, Rana *et al* 2002, Reddy *et al* 2002, Pattanaik *et al* 2007). The current paper aims at evaluating biological spectra of five localities from Mumbai and suburbs with quantitative data of species that elucidate the relationship between vegetation and an elevational gradient in the study areas, that perhaps helps the farmers for early prediction of climatic gradient in their respective local areas in most economical and ecofriendly way.

#### **MATERIALS AND METHODS:**

Mumbai, the economical capital of India was chosen as study area along with its suburbs with great biodiversity of flora. The forests and cultivated land of the area is a fundamental and potentially sustainable source of many services including economically and ecologically important edible, medicinal and aromatic plants. The diversity of climate, altitude, edaphic and geographical attributes for plant growth signifies its predictive value for agricultural practices. Indian farmers are solely dependent upon accurate climatic predictions. The present evaluation on biological spectra of the flora for communities in Mumbai and suburbs and its analysis as per Raunkiaer's classification was carried out during spring and summer of 2016. Biological spectrum of the flora based on the life form classes was prepared according to Christopher Raunkiaer's (1934) classification as follows:

**Phanerophytes:-** They are shrubby and tree species whose perennating buds are borne on aerial shoot reaching a height of at least 25cm or more above the ground surface.

**Chamaephytes:-** Perennating buds located close to the ground surface (below the height of 25cm). They include herbaceous, low woody trailing, low stem succulents and cushion plants.

**Hemicryptophytes:-** Herbaceous perennial in which aerial portion of plant dies at the end of growing season, leaving a perennating bud at or just touching the ground surface.

**Cryptophytes or Geophytes:-** Perennating buds located below the surface of soil including plants with deep rhizomes, bulbs, tubers and corms, etc.

**Therophytes:-** Annual seed bearing plants which complete their life cycle in one year and over winter; the unfavourable season by means of seeds or spores.

After having assigned a life-form to all the plants species of plant communities from five different study areas from the regions of Mumbai and suburbs viz. Colaba, Malbar Hill, Vir Jijamata Udyan, Byculla, Patanpada, Yeoor and Kankeshwar, Alibaugh, the biological spectra was calculated as follows:

$$\text{Biological spectra} = \frac{\text{Number of species falling in a particular life-form classes}}{\text{Total number of all the species for that community/stand}} \times 100$$

#### **RESULTS AND DISCUSSION:**

The summary of percentage life-forms, as per Raunkiaer's classification in five study areas representing Mumbai and suburbs is as follows:

Life-form Class	Colaba (%)	Malabar Hills (%)	Byculla (%)	Yeoor (%)	Kankeshwar, Alibaug (%)	Average (%)	Normal Biological Spectrum (by Raunkiaer) (%)
Phanerophytes	74	77.04	63.63	68.57	54.73	67.59	46
Chamaephytes	10	4.95	16.78	14.29	28	14.80	26
Hemi-Cryptophytes	2.7	1.63	4.19	5.72	2	3.25	09
Cryptophytes or Geophytes	11	13.11	6.29	8.57	12	10.19	06
Therophytes	2.3	3.27	9.11	2.85	3.27	4.16	13

The forests in Mumbai and suburbs are predominated by Phanerophytes. It has its own floristic specificity and life-form proportions. Floristic composition is a good source of plant life, gene pool and diversity of plants and microclimate of any area. Occurrence of similar biological spectrum in different regions indicates similar climatic conditions.

### CONCLUSION:

Biological spectra are useful in comparing geographically widely separated plant communities and these Physiognomic features of ecosystem are regarded as indicators of biotic interaction, climate and habitat deterioration. Occurrence of similar biological spectrum in different regions indicates similar climatic conditions. According to Raunkiaer (1934), the climate of region is characterized by life-form. The overall vegetation of Mumbai and suburbs is dominated by Phanerophytes, followed by Chamaephytes, Cryptophytes, Therophytes and Hemicryptophytes. Shah *et al* (1991) and Sikarwar (1996), reported that Hemicryptophytes are indicators of high altitude while Therophytes are characteristic of desert climate and Cryptophytes or Geophytes are indicators of mediterranean climate. Accordingly it can be concluded that dominance of Phanerophytic communities are

indicators of tropical, wet and dry climate with high level of humidity, greatly influenced by proximity to Arabian sea, as only in such climate Phanerophytes can flourish well. Thus agricultural produce from Mumbai includes green leafy vegetables, cereals and pulses, fruits with requirement of such climate.

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## STUDY OF SIDEROPHORE PRODUCTION BY RICE GROWTH PROMOTING RHIZOBACTERIA

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### ABSTRACT:

The bacteria lodging around/in the plant roots (rhizobacteria) are more versatile in transforming, mobilizing, solubilizing the nutrients compared to those from bulk soils and they are crucial for soil fertility. Siderophore are relatively low molecular weight, ferric ion chelators synthesized by bacteria and fungi to scavenge iron from the environment to make it biologically available. Extensive screening for the siderophore producing bacteria from rice rhizosphere was carried out. The samples were collected from the agricultural fields of Goa, Maharashtra and Uttar Pradesh. 104 organisms were isolated from the collected samples. Amongst them 62 isolates were found to produce siderophore. The isolates G9.1 and R5.9 were selected on the basis of higher siderophore production. They were further characterized and identified as *Pseudomonas aeruginosa* and *Bacillus megaterium* respectively. Physico-chemical parameters were evaluated for optimum for production of siderophore by *Pseudomonas aeruginosa* and *Bacillus megaterium*. Both the isolates were found to produce maximum siderophore at pH 7 and 30°C. Glucose was found to stimulate siderophore production. Shake flask studies revealed that siderophore production increased with respect to time and cell mass. *Pseudomonas aeruginosa* showed positive results for phosphate solubilization, indole acetic acid production, hydrogen cyanide production and ammonia production. *Bacillus megaterium* showed positive results for phosphate solubilization, indole acetic acid production, hydrogen cyanide production, ammonia production and nitrogen fixation. Both the isolates produce catechol type of siderophore. The present study reveals *Pseudomonas aeruginosa* and *Bacillus megaterium* strains as promising candidates for crop improvement and protection due to its plant growth promoting activities.

**Keywords :-** siderophore, rhizosphere, catechol, rhizobacteria, chelators .

### INTRODUCTION:

Different bacterial genera are involved in various activities of the soil to make it dynamic for nutrient turn over and sustainable for crop production (Ahemad *et al.*, 2009; Chandler *et al.*, 2008). The bacteria lodging around/in the plant roots (rhizobacteria) are more versatile in transforming, mobilizing nutrients compared to those from bulk soils (Hayat *et al.*, 2010). Therefore, the rhizobacteria are the dominant driving forces in recycling the soil nutrients for soil fertility (Glick, 2012). In last few decades species of *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthrobacter*, *Bacillus* and *Serratia* have been

reported as PGPR (Vessy, 2003). Indole acetic acid production, phosphate solubilization, ammonia production, hydrogen cyanide production, siderophore production, etc. are the some activities of PGPRs which help in plant growth. Both microorganisms and plants require a high level of iron, and obtaining sufficient iron is problematic in the rhizosphere (Guerinot *et al.*, 1994). To survive bacteria acquire iron by secreting siderophore, either hydroxamate or catechol type. For example mung bean plants, inoculated with the siderophore-producing *Pseudomonas* strain GRP3 and grown under iron-limiting conditions, showed reduced chlorotic

symptoms and an enhanced chlorophyll level compared to uninoculated plants (Sharma, Johri *et al.*, 2003); the Fe-pyoverdine complex synthesized by *Pseudomonas fluorescens* C7 was taken up by *Arabidopsis thaliana* plants, leading to an increase of iron uptake and improved plant growth (Vansuyt *et al.*, 2007). Deshwal *et al.*, (2013) and Walia *et al.*, (2014) worked on bacterial isolates with plant growth promoting activities like P-solubilization, IAA production, siderophore production isolated from the rhizosphere soil of tomato seedlings.

The present study aimed to isolate rice plant rhizobacteria which show siderophore production, identification of the selected isolates, followed by its characterization and optimization.

## **MATERIALS AND METHODS:**

The soil samples were collected from the fields of Maharashtra (Ratnagiri, Chiplun, Nivali, Parshuram, Dahanu, Kolhapur, Walope), Goa and Uttar Pradesh. Isolation of siderophore producing bacteria was done by plating on Cetrimide agar and Yeast Mannitol agar. The plates were incubated at  $28 \pm 2^{\circ}\text{C}$  for 24h. Siderophore production of isolate was studied by CAS agar method (Alexander *et al.*, 1991). The selected isolates were identified on the basis of its morphological, cultural and biochemical characteristics.

Hydrogen cyanide production was tested by streaking the isolates on Nutrient agar plate containing 0.44 % glycine. A circular disk of Whatmann filter paper was soaked in 0.5 % picric acid solution + 0.2% sodium carbonate solution and disk was kept in lid of petriplates. Plates were then incubated at  $28 \pm 2^{\circ}\text{C}$  for 5 days. After incubation the plate was observed for development of brown color on filter paper. The isolates were spot inoculated on sterile Pikovaskaya agar plates to study phosphate solubilization. Plates were then incubated at  $28 \pm 2^{\circ}\text{C}$  for 72h. (Pikovaskay,

1948). Isolates were streaked on nitrogen free agar plate i.e. Norris agar medium, and incubated at  $28 \pm 2^{\circ}\text{C}$  for 48h. After incubation, plates were observed for growth (Vessy, 2003). Isolates were inoculated in 5mL sterile peptone broth, incubated at  $28 \pm 2^{\circ}\text{C}$  for 5 days. After incubation 0.5 mL Nessler's reagent was added to each tube and observed for the development of brown color (Cappuccino *et al.*, 1992). Isolates were inoculated in 5mL of sterile nutrient broth containing 0.1% of tryptophan, incubated at  $28 \pm 2^{\circ}\text{C}$  for 24h, centrifuged at 5000 rpm for 20 min. 0.5mL supernatant from each tube was mixed with 2mL of Salkowski reagent and the tubes were incubated at R.T. in dark for 30 min, development of pink color was observed for Indoleacetic acid production (Spaepen *et al.*, 2011).

King's B broth tubes were inoculated with isolates and incubated for 48h at different temperatures ( $20^{\circ}\text{C}$ ,  $28^{\circ}\text{C}$ ,  $30^{\circ}\text{C}$ ,  $37^{\circ}\text{C}$  and at  $40^{\circ}\text{C}$ ). King's B medium of pH 3,4,5,6,7,8 and 9 were used for optimal pH study. King's B media was prepared with the carbon sources glucose, fructose, sucrose, xylose, maltose, lactose, mannitol and starch was also used. Isolates were then inoculated in each tube and kept for incubation at  $28 \pm 2^{\circ}\text{C}$  for 48h. Then 2mL culture from each tube was centrifuged and supernatant from each tube was spot inoculated on sterile CAS agar plates. The plates were incubated at  $28 \pm 2^{\circ}\text{C}$  for 48h. After incubation the plates was observed orange color zone around the colony.

1mL sample was inoculated in 200 mL sterile king's B broth. Then the sample that was taken at "0" hours was spot inoculated on sterile CAS agar plates. After 2h again the sample was spot inoculated on sterile CAS agar plates. The procedure was repeated for 3h and 4h also. The plates were incubated at  $28 \pm 2^{\circ}\text{C}$  for 48h. The effect of siderophore with respect to growth was studied by measuring the orange color zones around the colony.

Hydroxamate or Catechol type of siderophore was determined chemically (Csaky, 1948 and Arnaw, 1937). Effect of siderophore on growth of plants was studied by pot method. For this 5gm of methi seeds were soaked in nutrient broth containing the isolate. 3 flasks were maintained, one containing broth inoculated with the isolate from Ratnagiri, second containing broth inoculated with the isolate from Goa, alongwith control. After overnight soaking the seeds from each flask were planted in 3 different pots. After one week of planting the plants were observed and compared to control pot for the results.

## RESULT AND DISCUSSION:

The randomly collected 9 soil samples and 104 rhizobacterial isolates were labeled as mentioned in Table 1. From these isolates, 62 isolates were siderophore producers were selected for further study. By comparing the orange color zone around the spot inoculated culture, best siderophore producers were further studied. The morphological characteristics of 9 selected isolates A1.1, K2.2, C3.6, P4.7, R5.9, N6.9, D7.8, W8.6, G9.1 were studied.

The 4 isolates selected were P4.7, R5.9, D7.8 and G9.1 based on growth and siderophore production and were identified using their biochemical and morphological characteristics (Table 2).

Isolate R5.9, G9.1 and D7.8 were found to produce HCN whereas isolate P4.7 did not produce HCN. Shobha *et al.*, (2012) also observed brown color on filter paper which indicates HCN production. Therefore, it can be said that isolates that gave positive result from present study are efficient producers of HCN and can be applied as a possible line of defense against soil borne plant pathogens.

Isolate R5.9 and G9.1 were able to solubilize phosphate whereas D7.8 and P4.7 were not able to solubilize phosphate. Yasmin *et al.*, (2011) tested phosphate solubilization of

rhizobacteria. The isolate that gave the positive result can solubilize phosphate hence can result in higher and better yield of crop. The nitrogen fixation in chickpea was studied in two species of genus *Mesorhizobium*, *M. ciceri* and *M. mediterraneum* (Nour *et al.*, 1995).

Isolate R5.9 and G9.1 showed positive ammonia production and Indoleacetic acid production test, which may indirectly influence the plant growth. The optimum temperature for siderophore production by the isolates is 30<sup>0</sup>C (Table 3). Tailor *et al.*, (2012) studied optimization of siderophore production from *Pseudomonas fluorescences*. In their study, they found that the optimum temperature for *Pseudomonas fluorescences* was 29<sup>0</sup>C.

The optimum pH for siderophore production by the isolates is 7 (Table 4). Bholay *et al.*, (2012) worked on fluorescent pseudomonads and their siderophoregenesis. They found the optimum pH for siderophoregenesis to be 7.

The maximum siderophore production was obtained with glucose and lowest with xylose as carbon source (Table 5). Tailor (2012) studied characterization and optimization of siderophore production from *Pseudomonas fluorescences*. They obtained maximum siderophore production when glucose was used as a carbon source.

Siderophore production with respect to growth of isolates showed that the siderophore production increased with time (Table 6). The siderophore produced by both the isolates was not of hydroxamate type, instead catechol type.

After one week of plantation it was observed that the pot with the isolate from Goa showed more growth and the plantlets were healthier, whereas the pot containing the isolate from Ratnagiri showed moderate growth.

## CONCLUSION:

Siderophore production was studied in rice plant rhizobacteria and the most efficient isolates were characterized and evaluated for optimum conditions for maximum siderophore production. A total of 104 organisms were isolated from the rhizospheric soil. The four isolates selected for the study: P4.7, R5.9, D7.8, G9.1, were further tested for their plant growth promotion activities. On the basis of the results of plant growth promoting traits isolate R5.9 and G9.1 which gave better results were used for further optimization procedures. Optimization studies on both the isolates (R5.9 and G9.1) showed maximum siderophore production at pH 7 and at temperature 30°C. Glucose as carbon source was found to stimulate siderophore production. The siderophore production started after 4h. From the morphological, biochemical and laboratory confirmation test revealed that the isolates were *Bacillus megaterium* (R5.9) and *Pseudomonas aeruginosa* (G9.1).

From the results obtained it can be concluded that the organisms isolated in this study produce efficient quantities of siderophore and all the other plant growth promoting traits. Hence these isolates can be used as inoculants in bio-fertilizer.

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<b>Sr. No.</b>	<b>Sample collected from</b>	<b>No. of isolates</b>	<b>Isolate no</b>
1.	Aligarh	14	1.1-1.14
2.	Kolhapur	10	2.1-2.10
3.	Chiplun	14	3.1-3.14
4.	Parshuram	10	4.1-4.10
5.	Ratnagiri	10	5.1-5.10
6.	Nivali	10	6.1-6.10
7.	Dahanu	14	7.1-14
8.	Walope	12	8.1-8.12
9.	Goa	10	9.1-9.10

**Table 1:** List of soil samples collected from various locations

Sr.no.	Test	R 5.9	G 9.1	P 4.7	D 7.8
<b>1. Sugar Fermentation</b>					
	Lactose	+	-	-	+
	Sucrose	+	-	+	+
	Maltose	+	-	-	+
	Glucose	+	+	-	+
	Fructose	+	-	+	-
	Mannitol	+	-	-	+
	Xylose	-	-	-	+
2.	Methyl red	-	-	+	-
3.	Voges Proskauer	-	-	-	-
4.	Indole	-	-	-	+
5.	Urease	-	-	-	-
6.	PPA	+	-	-	+
7.	Citrate utilization	+	+	+	+
8.	Lysine decarboxylase				
8.1	With lysine (a)	+	+	-	+
8.2	With lysine (an)	-	-	-	+
8.3	Without lysine (a)	+	+	+	+
8.5	Without lysine (an)	+	-	-	-
9.	TSI	Slant:-alkaline Butt:- alkaline H <sub>2</sub> S:- no Gas:- no	Slant:alkaline Butt:-alkaline H <sub>2</sub> S:- yes Gas:- no	Slant:alkaline Butt:- acidic H <sub>2</sub> S:- no Gas:- no	Slant:alkaline Butt:-alkaline H <sub>2</sub> S Gas:- no
10.	Oxidase	-	+	+	+
11.	Catalase	+	+	+	+
12.	Gram Character	Gram positive rods in chains	Gram negative rods	Gram negative coccobacilli	Gram negative rods

**Table 2:** Biochemical characteristics of the high amount of siderophore producing isolate

Sr. No.	Temperature	Zones (mm)	
		R5.9	G9.1
1.	20 <sup>0</sup> C	15	16
2.	28 <sup>0</sup> C	18	20
3.	30 <sup>0</sup> C	20	22
4.	37 <sup>0</sup> C	16	18
5	40 <sup>0</sup> C	-	-

**Table 3:** Effect of temperature on siderophore production



Sr. No.	Ph	Zones (mm)	
		R5.9	G9.1
1.	3	-	-
2.	4	-	-
3.	5	-	-
4.	6	9	-
5.	7	10	10
6.	8	9	11
7.	9	9	10

**Table 4:** Effect of pH on siderophore production

Sr. No.	Carbon source	Zone Diameter (mm)	
		R5.9	G9.1
1.	Glucose	20	20
2.	Sucrose	16	15
3.	Fructose	17	17
4.	Mannitol	17	19
5.	Lactose	19	16
6.	Xylose	-	-
7.	Maltose	17	15
8.	Starch	16	16

**Table 5:** Effect of carbon source on siderophore production

Sr.No.	Hours	Zone Diameter(mm)	
		R5.9	G9.1
1.	0	12	14
2.	2	13	15
3.	3	14	16
4.	4	15	17

**Table 6:** Siderophore production with respect to time of growth

## ISOLATION OF ENDOPHYTIC AND RHIZOSPHERIC PGPR USING NUTRIENT LIMITING PLANT BASED CULTURE MEDIA

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### ABSTRACT:

Indian soils have received negligence in terms of soil fertility since several years. This has led to one major issue of 21<sup>st</sup> century viz., sustainable and environmentally safe agriculture. Use of biofertilizers is one solution to this problem. Present study focuses on isolation of probable novel nitrogen fixing and phosphate solubilizing bacteria in order to formulate a bacterial biofertilizer with plant growth promoting characteristics. *Vetiver* was selected as a plant of choice.

Plant based dilute culture media used in current study simulate the natural environment of plant microflora. Twenty two endophytic and rhizospheric bacteria were isolated, all of which showed nitrogen fixing and phosphate solubilizing ability. These would be used in formulating a biofertilizer.

**Keywords:** PGPR, Biofertilizers, Plant based media, *Vetiver*.

### INTRODUCTION:

Agriculture plays a vital role in Indian economy. Majority of rural household depend on agriculture as their principal means of livelihood. Indian soils have been known for their varied nature allowing cultivation of diverse groups of crops. However, in due course of time, the fertility and productivity of Indian soil have got reduced because of excessive use of chemical fertilizers. Chemical fertilizers are "Agricultural Pollutants" (Savci, 2012). There is a dire need to explore solution for environmentally safe agriculture and meet the present day food demand of India.

Use of Plant Growth Promoting Rhizobacteria (PGPR) as biofertilizers is one approach for sustainable agriculture. PGPRs are the soil bacteria inhabiting on/around the root surface, and are directly or indirectly involved in promoting plant growth and development [12]. PGPR are known to promote plant growth directly by providing nutrient supply (nitrogen, phosphorus, potassium and essential minerals) or modulating plant hormone levels. They may influence indirectly by decreasing the inhibitory effects of various pathogens, thus acting as biocontrol agents and root colonizers (Govind *et al.*, 2015).

Availability of phosphorus and fixed nitrogen

play vital role in plant growth. Since 95-99% of the available phosphate is present in the insoluble, immobilized, and precipitated form, plants are unable to utilize the same. PGPR help in making phosphorus available for plants to absorb. Plants are also incapable of fixing atmospheric dinitrogen to ammonia. PGPR have been reported to bear ability to fix atmospheric nitrogen symbiotically or non-symbiotically.

Present study involved isolation of probable novel nitrogen fixing and phosphate solubilizing PGPR from the roots of *Vetiveria zizanioides* (commonly known as *Vetiver*, or "Khus"). *Vetiver* grass has massively thick root system that reinforces the soil and prevents soil erosion. It exhibits high degree of resistance to pests and diseases. It is tolerant to extreme climatic variations such as extreme temperatures, acidity, alkalinity, salinity, sodicity and magnesium (Truong & Baker, 1998). It is tolerant to heavy metals like Al, Mn, As, Cd, Cr, Ni, Pb, Hg, Se and Zn in the soil (Truong & Baker, 1998). It also shows tolerance to herbicides and pesticides and is efficient in absorbing dissolved N, P, Hg, Cd and Pb in polluted water. Bacterial flora associated with *Vetiver* roots might be

contributing to at least few of these unique properties of the plant.

A large proportion of microorganisms in any environment remain unculturable, which limits our understanding of microbial ecology and evolution. The bacteria that can be grown in the laboratory are only a small fraction of the total diversity that exists in nature. Uncultured bacteria that do not grow on standard media are playing critical roles in cycling carbon, nitrogen, and other elements, synthesizing novel natural products, and impacting the surrounding organisms and environment. Although molecular techniques, such as metagenomic sequencing, can provide some information independent of our ability to culture these organisms, it is essentially impossible to learn new gene and pathway functions solely from pure sequence data (Stewart, 2012).

Current study involved isolation of probable novel nitrogen fixing and phosphate solubilizing bacteria from *Vetiver* roots. The yet uncultured bacteria from *Vetiver* plant may exhibit unique characteristics in terms of nitrogen fixation and phosphate solubilization and could be further used in formulating an effective biofertilizer.

## **MATERIALS AND METHODS:**

### **1. Procurement of plant material:**

Samples of the full-grown *Vetiver* plants were obtained in plastic bags. The root system (intact roots with closely adhering soil) was carefully removed and transferred to plastic bags. Soil adhering to the roots was also collected to represent the rhizosphere. Samples were stored at 4°C until further analyses.

### **2. Preparation of *Vetiver* roots extract:**

*Vetiver* roots were trimmed from other plant parts and washed under tap water. Washed roots were surface sterilized with 95% ethanol for 10 seconds followed by washing with sterile distilled water 3 times. They were then transferred to 3% sodium hypochlorite with intermittent shaking for 15 minutes and

washed with sterile distilled water. The roots were then pat dried and blended using surface sterilized kitchen blender. The homogenate was filtered through muslin cloth to obtain the root juice.

### **3. Preparation of Plant Based Dilute Culture Media:**

Dilute plant-based culture media were prepared using 1:100 diluted nutrient broth containing different concentrations of root juice (1:10, 1:20, 1:40, 1:80, and 1:100 v/v) (pH 7.0).

### **4. Isolation of rhizospheric and endophytic bacteria on plant based dilute culture media:**

Saline suspension rhizospheric soil sample and undiluted *Vetiver* root juice were streaked on the dilute plant based media. Plates were incubated for 7 days at  $27 \pm 2$  °C. Colony characteristics of each of the isolates were noted down. The isolates were maintained on plant based agar media of similar composition.

### **5. Qualitative estimation of nitrogen fixing ability:**

Each of the rhizospheric and endophytic isolates was streaked on sterile Ashby's Mannitol Agar (Sharma *et al.*, 2011) to check its ability to fix atmospheric nitrogen. Plates were incubated for 7 days at  $27 \pm 2$  °C.

### **6. Qualitative estimation of phosphate solubilization ability:**

Isolates were spot inoculated on sterile Pikovskaya's agar. Plates were incubated for 7 days at  $27 \pm 2$  °C. Phosphate solubilization index (PSI) was calculated (Manal *et al.*, 2014).

## **RESULTS AND DISCUSSION:**

### **Isolation of rhizospheric and endophytic bacteria on plant based dilute culture media:**

Many different approaches like modification of

growth media, modification of growth conditions, community culture, coculture, use of optical tweezers and micromanipulator, multiwall microbial culture chip etc. have been used to cultivate the yet uncultured bacteria (Pham & Kim, 2012). Dilute media simulating the natural environment has been used in present study to cultivate probable novel nitrogen fixing and phosphate solubilizing bacteria associated with *Vetiver* roots.

Microorganisms can be divided into copiotrophic types and oligotrophic types by their ability to grow in conventional high-nutrient environments. The term “oligotrophic” is often used for those microorganisms that are incapable of growing in high-nutrient media such as a nutrient broth medium. Complex media contain high concentrations of nutrients such as sugars and amino acids, which implies that most cultivable bacterial species can grow easily on it. However, fast-growing, dominant bacterial strains (the r-selected bacteria) might overgrow slow-growing strains, which do not get the opportunity to develop. Dilute media, on the other hand, provide a limited concentration of nutrients, which leads to a slower and more selective growth and might also give slow growing strains (the K- selected bacteria), a chance to develop. Furthermore, the composition of these media might mimic the oligotrophic conditions actually present in natural environment, and therefore might allow the growth of these yet uncultured bacteria. Use of dilute media has helped culture some novel bacteria in earlier studies (Pham & Kim, 2012; Zubair A. *et al.*, 2010).

Moreover, ‘media simulation’ has been widely used to culture novel bacteria (Stewart, 2012). Inclusion of *Vetiver* root sap in the medium is expected to provide otherwise missing nutrients and/ or signaling molecules which would help in culturing the uncultured microorganisms. Also, in an environment, the bacteria exist as a community, with different kinds of interactions amongst them. Some bacteria might not grow on traditional culture

media because of lack of such signaling molecules or growth factors. Hanan *et al.* (2016) also have reported the efficient use of Plant-based culture media to support culturing of rhizobacteria.

Eleven isolates each of rhizospheric and endophytic bacteria were obtained in this study and were further used to check for nitrogen fixation and phosphate solubilization ability.

#### **Qualitative estimation of nitrogen fixing ability**

All the endophytic and rhizospheric isolates showed growth on Ashby’s Mannitol Agar. This indicated that all the isolates were able to fix atmospheric nitrogen nonsymbiotically.

#### **Qualitative estimation of phosphate solubilizing ability**

All the endophytic and rhizospheric isolates showed zone of clearance around the colony on Pikovskaya's agar (Fig. 1) indicating their ability to solubilize inorganic tricalciumphosphate.

$$\text{PSI} = [\text{Colony diameter (mm)} + \text{Halo zonediameter (mm)}] / \text{Colony diameter(mm)}$$

PSI was found to be highest for the endophytic isolate I2 obtained from plate containing 1:100 diluted plant sap and rhizospheric isolate I2 obtained from plate containing 1:80 diluted plant sap. No significant difference between PSI of endophytic and rhizospheric bacteria ( $p < 0.001$ ) was observed after carrying out t-test. Thus, endophytic as well as rhizospheric bacteria were found to be equally efficient in solubilizing phosphate.

PSI of endophytic and rhizospheric bacteria ranged between 2.50 to 5.40 and 2.25 to 6.67 respectively. Maximum PSI of upto 4.44 has been reported in previous studies (Karpagam & Nagalakshmi, 2014; Manal *et al.*, 2014; Mardad *et al.*, 2013; Sharma *et al.*, 2011). In

present study we report isolation of 3 endophytic and 6 rhizospheric bacteria with PSI above 4.44.

Also, very interestingly, all the endophytic and rhizospheric isolates exhibited both, nitrogen fixing as well as phosphate solubilizing activity. This is significant, since biofertilizer having better efficacy can be prepared using same single bacterial species.

### CONCLUSION:

Much information is available on the ecology of rhizobacteria based on the use of synthetic culture media. However, only few studies are available on the use of plant-based culture media to effectively mirror their community structure in the root spheres. Our study has used plant based 'dilute' cultivation media to better simulate the natural habitat for the growth of oligotrophic endophytic and rhizobacteria.

The isolates obtained in the current study that exhibited nitrogen fixing and phosphate solubilization activity might represent novel, yet uncultured bacteria, which would be confirmed by 16S rRNA gene analysis of the isolated cultures. Apart from nitrogen fixation and phosphate solubilization, evaluation of other plant growth promoting properties of the isolates is also required. Consortia of the obtained isolates might serve as an efficient biofertilizer and needs to be assessed.

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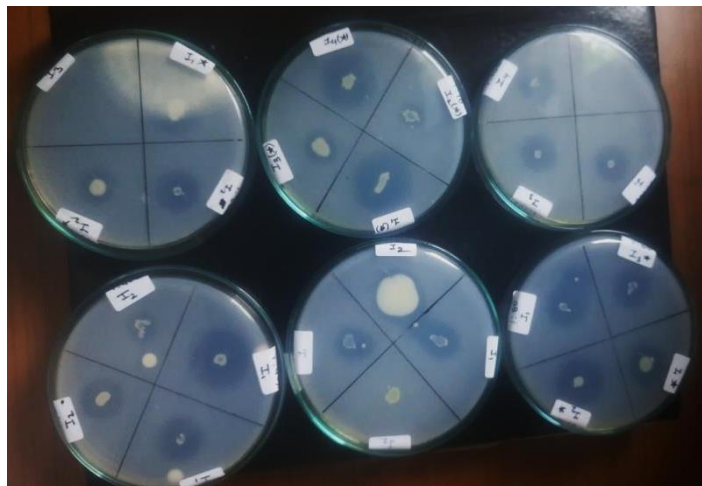
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**Fig. 1: Spot inoculation of rhizobacteria on Pikovskaya's agar**

Endophytic isolates	Dilution of root sap	PSI	Rhizospheric Isolates	Dilution of root sap	PSI
I1	1:10	4.71	I1	1:10	3.80
I2	1:10	3.00	I2	1:10	2.28
I3	1:10	2.50	I1	1:20	3.00
I4	1:10	4.17	I2	1:20	2.25
I1	1:40	5.33	I1	1:40	5.25
I2	1:40	3.20	I2	1:40	3.83
I1	1:80	3.11	I1	1:80	5.50
I2	1:80	4.17	I2	1:80	6.67
I3	1:80	4.40	I1	1:100	5.00
I1	1:100	3.50	I2	1:100	5.25
I2	1:100	5.40	I3	1:100	5.25

**Table 1: PSI of endophytic and rhizospheric bacteria**

## MASS PROPAGATION OF TWO DIOECIOUS DWARF VARIETIES OF *Carica papaya* L. FROM AXILLARY BUDS OF FIELD GROWN PLANTS

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### ABSTRACT:

*Carica papaya* L. is an important nutraceutical fruit plant. Plants can be dioecious or gynodioecious. There are three sex types in papaya: male, female and hermaphrodite. Identification of sex type of the plant prior to flowering in dioecious varieties is a major issue. This problem is heightened due to a long juvenile cycle of papaya. Uprooting excess male plants is labour intensive and expensive. Prior knowledge of the sex type of papaya can ensure improved production.

Two dioecious dwarf varieties of *Carica papaya* L. Co-6 and Pusa dwarf were selected for the present investigation. *Carica papaya* L. var. Co-6 is a selection from *Pusa majesty*. It is a dioecious variety with dwarf plants. It is suitable for papain and also for table purpose. *Carica papaya* L. var. Co-6 is an improved strain released from Tamil Nadu Agricultural University, Coimbatore. *Carica papaya* L. var. Pusa dwarf is a dioecious variety with dwarf plants and medium-sized oval fruits. It is an elite dwarf variety famous for high-density planting. It has been developed by the Regional Research Station at Pusa, Bihar. Axillary buds were collected from field grown plants. MS medium fortified with various concentrations of auxins and cytokinins singly or in combination were used for multiple shoot formation.

Multiple shoots developed from axillary buds were collected from field grown plants in *Carica papaya* L. var. Co-6 and Pusa dwarf in various combination of BAP+AS+NAA and BAP+AS+NAA+Sucrose (6%).

**Key words:** *Carica papaya* L., dioecious varieties, axillary buds, multiple shoots

### INTRODUCTION:

*Carica papaya* L. is valued for its nutritional qualities of its fruit as a source of vitamin A and calcium as well as for the pharmaceutical industry as a rich source of commercial papain, a proteolytic enzyme. Biologically papaya has three types such as male, female and bisexual but only female and bisexual types are productive. In commercial plantation it is very often found that male plants prevail as high as 30% and some times over 50% of the total (Jordan *et al.*, 1983).

Due to out breeding, plants are not true to type and exhibit significant variation in yield, fruit quality and disease susceptibility within cultivated populations. Additionally, as sex cannot be determined until the mid-

development stage, several seedlings are planted in each position and eventually thinned, retaining only the most vigorous female plants with one male to every 10 to 20 female as pollinators. A need to replant every three years to ensure quality fruit production, propagation by seed represents a significant cost to the producer (Saksena and Sharma, 2015).

The tremendous yielding potential of this crop is left economically unexploited due to several problems associated with its cultivation. Papaya is having dioecious nature therefore presence of equal number of unproductive male plants impediment its successful commercial cultivation. Conventional method

of propagation like cutting or grafting has not been found successful in papaya. In this regard clonal propagation represents the economic way of continuously producing new uniform true-to-parental type planting materials of known superior lines. The main objective of the present work was to develop a protocol for the clonal propagation of dioecious varieties of *Carica papaya* L. via multiple shoot from the axillary bud explants of the field grown plants. Two dioecious varieties of *Carica papaya* L. Co-6 and Pusa dwarf were selected for the present investigation. It is a dioecious variety with dwarf plants. Cloning of female papaya plants through *in vitro* axillary bud culture was an ideal approach.

#### **MATERIALS AND METHODS:**

In the present study field grown plants after flowering were selected. Axillary bud explants from male and female plants were maintained separately. Pretreated axillary bud explants were given pulses of GA<sub>3</sub> (500 mg/l) for 1 hour and then inoculated on MS medium (Murashige and Skoog, 1962) fortified with MgSO<sub>4</sub> (400 mg/l) for establishment. The established axillary bud explants for shoot bud initiation were inoculated on various induction media such as basal MS medium, MS medium fortified singly with IAA or NAA (0.05, 0.1, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/l), BAP, KIN or AS (0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/l) or AS at a higher range of (10, 20, 40, 60, 80 and 100 mg/l) or in different combinations. Axillary bud explants were then inoculated on various proliferation media i.e. MS medium (1-10% sucrose) fortified with BAP (5 mg/l), AS (10 mg/l) and NAA (2 mg/l) for proliferation of the shoot buds. Shoot buds (4-5 cm) with leaves (6-7 numbers) were later inoculated on MS medium fortified with various concentrations of NAA, IAA and IBA (1.0, 2.0, 3.0, 4.0 and 5.0 mg/l) singly or the base of the shoot was dipped in various concentrations of NAA, IAA or IBA (250, 500, 1500, 2000, 2500 and 3000 mg/l) for 10, 20 or 30 seconds and then inoculated on MS

basal medium for rooting. The plantlets were maintained in the hardening chamber and in green house on soil: vermiculite: cow-dung (1:1:1) for 4 weeks respectively. The plants were kept in shade house and gradually transferred to the field.

#### **RESULTS AND DISCUSSION:**

Initiation of the multiple shoot buds did not take place when axillary bud explants were inoculated on basal MS medium, MS medium fortified singly with various concentrations of IAA or NAA, BAP or KIN and AS; MS medium fortified with auxins (0.05- 0.5 mg/l) and cytokinins (0.5 - 3.0 mg/l) in various combinations. MS medium fortified with auxins (3.0 - 5.0 mg/l) and cytokinins (0.5 - 3.0 mg/l) in various combinations resulted in excessive callus formation. MS medium fortified with BAP, AS and IAA; KIN, AS and NAA or KIN, AS and IAA gave rise to the initiation of the shoot buds with excessive development of callus at the base of the axillary bud explants.

In *Carica papaya* L. variety Co-6, the axillary bud explants inoculated in the induction medium i.e. MS medium fortified with BAP (4.0 mg/l), AS (10 mg/l) and NAA (2.0 mg/l) gave rise to 1.73 and *Carica papaya* L. variety Pusa Dwarf axillary bud explants inoculated in the induction medium i.e. MS medium fortified with BAP (5.0 mg/l), AS (10 mg/l) and NAA (2.0 mg/l) gave rise to 1.38 shoot buds respectively. The axillary bud explants from *Carica papaya* L. variety Co-6 inoculated in the proliferation i.e. MS medium (6% sucrose) fortified with BAP (4.0 mg/l), AS (10 mg/l) and NAA (2.0 mg/l) gave rise to 5.28, whereas the axillary bud explants from *Carica papaya* L. variety Pusa Dwarf inoculated in the proliferation i.e. MS medium (6% sucrose) fortified with BAP (5.0 mg/l), AS (10 mg/l) and NAA (2.0 mg/l) gave rise to 4.14 shoot buds respectively. Proliferation medium supplemented with CM (10%) promoted elongation. In 8 weeks the length of



the shoots was 2.46 and 2.26 cm in *Carica papaya* L. varieties Co-6 and Pusa Dwarf respectively.

The shoots inoculated on MS medium fortified with IAA or IBA (1.0-5.0 mg/l) produced roots however percentage rooting was low and time taken for rooting was more, thus these rooting media were not used for further study. The base of the shoot dipped in IBA (2500 mg/l) for 30 seconds, inoculated on MS basal medium showed 89.60 and 64.60% rooting in 19 and 16 days in *Carica papaya* L. varieties Co-6 and Pusa Dwarf respectively. In the hardening chamber combination of soil: vermiculite: cowdung (1: 1: 1) used as a substratum could show 23 and 20 % survival of plantlets in *Carica papaya* L. varieties Co-6 and Pusa Dwarf respectively in 4 weeks (Table 1).

In the present study the axillary bud explants of *Carica papaya* L. varieties Co-6 and Pusa Dwarf inoculated on MS medium (6% sucrose) fortified with BAP (5.0 mg/l), AS (10 mg/l) and NAA (2.0 mg/l) showed shoot bud initiation and proliferation. Proliferation media supplemented with CM (10%) promoted the elongation of the multiple shoots. MS medium supplemented with sucrose 30.00 g/l and agar 6.50 g/l under light condition produced highest shoot number and longest shoot in papaya variety CO-5. Application of BA 0.50 mg/l along with NAA 0.10 mg/l was found to be better for initial culture establishment and proliferation of papaya variety CO-5. *In vitro* rooting was best in full strength MS medium supplemented with IBA 3.00 mg/l, sucrose 30.00 g/l and activated charcoal 0.05% (Bindu, 2015). A large number of shoots regenerated from lateral buds and young leaves of *Carica papaya* L. cv. Shahi on MS supplemented with 1.0 mg/l zeatin and 0.2 mg/l NAA. Addition of 200 mg/l casein hydrolysate (CH) to the medium increased the number of shoots per culture and incorporation of 2.0 g/l activated charcoal (AC) to the medium resulted effective

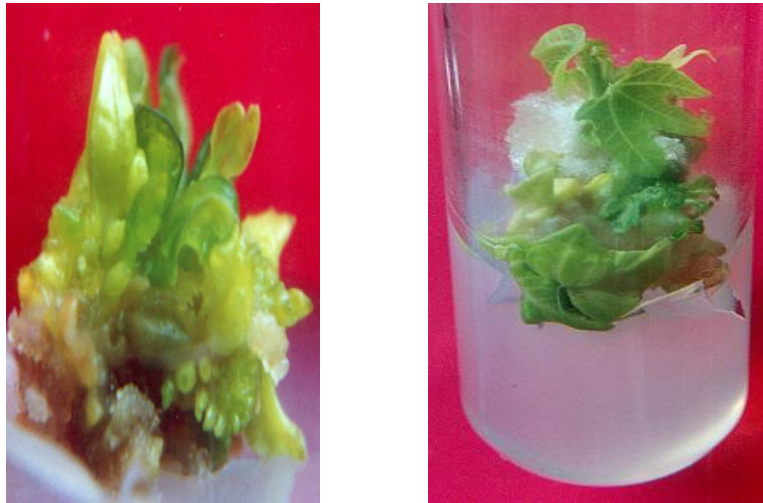
shoot growth with healthy leaf. While addition of 100 mg/l urea and 2.0 g/l activated charcoal to the medium showed proper shoot elongation. Best rooting was obtained from shoots cultured on half-strength of MS fortified with 4.0 mg/l IBA (Roy *et al.*, 2012). In the present study an attempt has been made to develop a protocol for the clonal propagation of dioecious dwarf varieties of *Carica papaya* L. through organogenesis by the direct multiple shoot formation from the axillary bud explants of the field grown plants.

#### ACKNOWLEDGEMENT:

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**Multiple shoots from axillary bud explants of *Carica papaya* L. var. Co6 and Pusa dwarf**

MS medium	(mg/l)	Varieties	
		Co-6	PD
		Shoot bud numbers	
<b>Induction media</b> BAP+AS+NAA	4.0+10+1.0	0.66 ± 0.03	0.56 ± 0.04
	4.0+10+2.0	0.80 ± 0.03	0.76 ± 0.05
	4.0+20+1.0	<b>1.73 ± 0.05</b>	0.86 ± 0.02
	4.0+20+2.0	0.53 ± 0.02	0.63 ± 0.02
	5.0+10+1.0	0.90 ± 0.03	0.86 ± 0.02
	5.0+10+2.0	0.80 ± 0.03	0.83 ± 0.08
	5.0+20+1.0	1.20 ± 0.06	<b>1.38 ± 0.05</b>
	5.0+20+2.0	0.73 ± 0.05	0.77 ± 0.06
<b>Proliferation media</b> BAP+AS+NAA+Sucrose (6%)	4.0+10+1.0	2.07 ± 0.003	1.18 ± 0.01
	4.0+10+2.0	2.13 ± 0.02	2.10 ± 0.04
	4.0+20+1.0	<b>5.28 ± 0.15</b>	2.42 ± 0.05
	4.0+20+2.0	0.85 ± 0.05	0.83 ± 0.02
	5.0+10+1.0	2.63 ± 0.03	1.22 ± 0.03
	5.0+10+2.0	2.48 ± 0.03	2.33 ± 0.07
	5.0+20+1.0	2.43 ± 0.01	<b>4.14 ± 0.03</b>
	5.0+20+2.0	0.92 ± 0.08	0.90 ± 0.07
<b>Elongation media</b> BAP+AS+NAA+CM (10%)		Length of shoot (cm) in 8 weeks	
	5.0+10+2.0	2.46 ± 0.12	2.26 ± 0.10

Values are mean of three sets of determinants. Each set containing 10 explants. Mean±SE

**Table 1: Response of axillary bud explants in *Carica papaya* L. varieties Co-6 and Pusa Dwarf Washington inoculated on different media for the initiation, proliferation and elongation of the shoot buds**

Rooting & Hardening		Varieties	
		Co-6	PD
Base of the shoot dipped in IBA (2500 mg/l) for 30 seconds, inoculated on MS basal medium	Rooting (%)	89.60 ± 0.51	64.60 ± 0.51
	Time taken (d)	19.0 ± 0.88	16.0 ± 0.88
Substratum - Soil: vermiculite: cow-dung (1:1:1) in green house in 4 weeks	Hardening success (%)	23.0 ± 0.71	20.0 ± 0.71

Values are mean of three sets of determinants. Each set containing 10 explants. Mean±SE

**Table 2: Rooting and hardening of the multiple shoots in different varieties of *Carica papaya* L**

## **CONVERSION OF *Tagetes Erecta* FLOWER A TEMPLE WASTE TO ORGANIC MANURE**

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### **ABSTRACT:**

*Tagetes erecta* Linn. belongs to family Compositae. The Marigold flower is commonly used in temple to worship God or for decoration purpose. These flowers after being used are collected and dumped in the open areas or in nearby water. It causes pollution. Hence it was felt necessary to convert this flower waste to organic manure.

Marigold flower was collected from the temple close to Mithibai College, Vile Parle (W). The flowers were separated from the capitulum inflorescence. An experimental pot was prepared by mixing the flower with the normal soil. This was compared with the control soil. After every two days the flowers were added in the experimental soil and mixed thoroughly. They were watered at intervals. After three weeks the organic manure was ready in the experimental pot. The soil from both the experimental and control pot was analysed for pH, organic carbon, Nitrogen content, Calcium and magnesium using standard methodology.

The marigold flower was converted to organic manure using simple techniques within three weeks. There was change in the colour of experimental soil as well as in granular size. The experimental soil also emitted a characteristic odour and colour change when dissolved in water as compared to control soil. The analysis of experimental soil revealed pH-6.44, N-940.8kg/ha, Calcium and Magnesium, high carbon content when compared with control.

The present study focuses on conversion of Marigold flower, a temple waste in to nutrient rich organic manure. The Marigold flower also exhibits fungicidal and insecticidal properties. Hence this organic flower manure will enrich the crops to grow well as well as protect them from insects and pests. On other hand the temple authorities can be trained to make such organic manure rather than polluting the water and land. Studies are in progress to grow plants in the treated soil and to see its effect on the plants. Thus a healthy soil is a key component of sustainability.

**Key words:** *Tagetes erecta*, Marigold, organic manure.

### **INTRODUCTION:**

India is a land of God. In India, religion is a path of life. It is an intrinsic element of the entire Indian culture. People worship God and are accustomed to go to the temples offering flowers, fruits, coconuts and sweets, etc. The bulk of the flowers, leaves of different plants, coconut shells, milk and curd are piled up. Every day, these flowers are offered by devotees in temples and are left unused and therefore become waste. (Yadav I. *et al.*, 2015) Because of our religious believes many of us avoid throwing flowers and other items that are used for prayers in the garbage and instead

of that they put them in the plastic bags and throw them directly in the water bodies or open land. (Pandey A. *et al.*, 2014). These flower wastes decay aerobically and anaerobically producing foul smell, toxic gases and solid liquid wastes causing severe health problem to the people around. (Barad G. and Upadhyay A., 2016) Every year, approximately 80, 00,000 tons of waste flowers are dumped into Indian rivers. Mostly marigold flower is used in abundance by the worshipers. (Makhania M. and Upadhyay A., 2015) In order to control water pollution

marigold flowers are collected and converted into organic compost. Marigold is botanically known as *Tagetes erecta* Linn. It belongs to family Compositae/Asteraceae. (Yadav S. R. and Sardesai M. M., 2002). The marigold flowers possess insecticidal properties too. (Dixit P. *et al.*, 2013)

### **MATERIAL AND METHODS:**

**Collection of Flower:** In the present study flower waste was collected from temple, Om Shri Ganesh Sai Seva Sangh, Vile Parle (W) near Mithibai College. The flower waste consists of *Hibiscus*, rose, jasmine, balsam, marigold, etc. Among these marigold flower was offered to the God in abundance. Hence marigold flower was selected for the current study. The flower was segregated from the others, separated from the capitulum inflorescence and was used for the experiments.

**Processing of flower into compost:** For the preparation of compost two pots were selected as follows: An experimental pot was prepared by mixing the flower with the normal soil and sprinkled with some cow dung. This was compared with the control soil without flower. After every two days the flowers were added in the experimental soil and mixed thoroughly. They were watered at intervals. After three weeks the organic manure was ready in the experimental pot.

**Analysis of soil:** The soil from both the experimental and control pot was analysed for the parameters like pH, organic carbon, Nitrogen content, Calcium and magnesium using standard methodology. (Anonymous, 2009)

### **RESULT:**

The result obtained are tabulated in Table: 1

### **DISCUSSION:**

The Marigold flower waste was collected from

the temple near Mithibai College, Vile Parle (W). The devotees offered marigold flowers more than any other flowers. These flowers were converted into organic manure using soil and very little cow dung. Cow dung was used to initiate decomposition of flowers. It was watered at intervals. The decomposition of flowers took place within a week. The experimental soil emitted a characteristic odour of marigold flowers. The soil showed colour change as compared to control soil. In the later third week the flower compost was ready for use.

The experimental soil and control was analyzed for the physicochemical parameters such as soil pH, nitrogen, magnesium, calcium and organic carbon. The experimental soil pH is slightly acidic. The nitrogen content of the experimental soil has increased (940.8 kg/ha) than the control soil (658.56 kg/ha). Calcium content in the flower compost is found to be 1.402 mg whereas the control soil had only 0.5608 mg of calcium. Thus the floral organic manure will improve microbial activity and enhance uptake of other nutrients. There is a decline in magnesium content in *Tagetes* manured soil (0.2 mg) while the control set revealed an increase in magnesium (0.6 mg). The excess magnesium will make the soil tight which will hamper the yield of crops. The smooth texture obtained by the experimental soil may be due to presence of less magnesium. The organic carbon is higher (0.12 %) in experimental soil and there is a decline in control soil (0.075%) respectively.

### **CONCLUSION:**

In nutshell, the current study is of utmost important as the organic manure obtained from marigold waste is rich in macro and micro nutrients. Converting flower waste in to organic manure will propose an alternative approach to waste management. It will throw light on reducing volume of temple waste. Floral waste utilization would eventually be beneficial to the society as people would get to

live in a cleaner and a healthier environment. The “green temple concept” can prove to be helpful in Government policy formulation for waste management. Thus the soil rich in marigold manure will be studied further by growing plants and to check their yield.

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Parameters	Soil without flower compost (Control pot)	Soil with flower compost (Experimental pot)
Soil pH	6.65	6.44
Nitrogen (kg/ha)	658.56	940.8
Magnesium (mg)	0.6	0.2
Calcium (mg)	0.5608	1.402
Organic carbon (%)	0.075	0.12

**Table:1 Physicochemical parameters of flower composted soil and control soil**

## STUDY OF SEASONAL WILD MONSOON VEGETABLES AS NUTRACEUTICALS

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### ABSTRACT:

Nutraceuticals are generally defined as parts or a whole food having medical or health benefit, including the prevention and treatment of disease. The present study aims at studying nutritional value, concentration of vitamins, minerals, calorific value as well as antinutritional factors of two wild edible seasonal vegetables namely *Bauhinia racemosa* (Family –Leguminosae, Sub family–Caesalpiniaie) and *Chlorophytum borivilianum* (Family –Asparagaceae). These leaves of these species are a part of the diet of the tribals, but the city dwellers are unaware of the health benefits of this seasonal flora. The determination of concentration of dietary fibres as well as calorific value helps us to consider these vegetables as potential nutraceuticals.

**Keywords** – Nutraceuticals, *Bauhinia racemosa*, *Chlorophytum borivilianum*, calorific value

### INTRODUCTION:

The term nutraceutical was originally defined by Dr. Stephen L. DeFelice, founder and chairman of the Foundation of Innovation Medicine (FIM), Crawford, New Jersey. Nutraceuticals are generally defined as parts or a whole food having medical or health benefit, including the prevention and treatment of disease. Nutraceuticals could be plants or plant products with nutritional and medicinal value. In other words, these are food (ingredients of diet) with pharmaceutical properties (bioactivities).

Increasing cost of health care, growing trend of self medication, increased awareness of the relationship between food and health with lifestyle changes in the society are the main reasons for popularity of plants in the form of nutraceutical.

Green leafy vegetables are considered as a rich source of carotenoids, vitamins and minerals. The dietary and medicinal value of the wild seasonal flora is still not completely known. Indian culture and festivals highlight the use of different wild edibles such as the ‘Bohag Bihu’, a festival in Assam in which it is customary to eat recipes prepared from rare

varieties of 101 herb species, on the first day of the festival. The tribals believe that these herbs protect them from all ailments throughout the year (Gogoi and Zaman, 2013). The leaves of the medicinal herb, *Chlorophytum borivilianum* or *Musali* are also consumed with a ceremony before the rice cultivation in tribal pockets of Maharashtra and Gujrat, with the belief of good yield. The traditional beliefs and customs are believed to be associated with certain nutritional or medicinal properties of the plants used.

A variety of seasonal herbs of rainy season having high nutritional value are used as food by the tribal communities living in and around the forests which are not the usual components of the diet of the masses.

The plants selected for the study are consumed by tribal communities and are known by local names in different parts of our country. Young leaves of *Bauhinia racemosa*, commonly called as *Kachnar* or *Koral* are used as a vegetable (Mandal and Nandi, 2013.Kumar, 2011).

Since the significance of these species as nutraceuticals is not studied so far, a

systematic analysis of these vegetables can throw light on their nutritional benefits as well as medicinal properties.

The present study aims at studying nutritional value of the following wild and edible species namely *Bauhinia racemosa* (Family – Leguminosae, Sub family–Caesalpiniae) and *Chlorophytum borivilianum* (Family – Asparagaceae) which are consumed as food by tribal communities in various parts of India (Dahikar, 2011).

Young shoots and leaves of *Bauhinia racemosa*, commonly called as *Kachnar* or *Koral* are used as a vegetable. *Musali* or *Chlorophytum borivilianum* is known for the medicinal uses of its roots, but its leaves are consumed as food by tribal communities of Maharashtra and Gujarat.

#### **MATERIALS AND METHODS:**

The vegetables were collected during rainy season and were shade dried. The estimation of vitamins was carried out using fresh sample to avoid damage due to drying. The estimation of vitamins was carried out using HPLC method (Diwedi, 2013; Karmi et al, 2010). The estimation of nutrients was carried out using standard methods.

#### **OBSERVATIONS:**

The vegetables were studied for the concentration of various nutrients. The concentration of the nutrients is listed in Table 1. Concentration of vitamins is listed in Table 2 and the concentration of minerals is listed in Table 3. The vegetables were found to contain very high amount of fibres, moderate concentration of carbohydrates and proteins. The calorific values suggest that these are good sources of energy.

The vegetables were observed to contain Nicotinic acid, Pyridoxin Hydrochloride and Vitamin C. The concentration of Nicotinic acid and Vitamin C was observed to be high in *Bauhinia* compared to *Chlorophytum*. The concentration of Calcium, Magnesium and

Iron was observed to be higher in *Chlorophytum borivilianum* than *Bauhinia racemosa*.

Qualitative test for saponin, the antinutritional factor was negative for both the vegetables selected.

The overall observation of these vegetables indicates that these are rich in nutrients and therefore could be considered to be potential nutraceuticals.

#### **CONCLUSION:**

The city dwellers are not familiar with the tribal and seasonal edibles from nature. This study indicated that the monsoon vegetables were studied for the level of various nutrients and calorific value. The levels of bio molecules, vitamins and minerals were observed to be high with large amount of dietary fibres. The seasonal flora is proved to be a healthy nutrient supplement and one must make those a part of the diet for their health benefits.

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Plant	Moisture %	Ash %	Carbohydrates %	Crude Fibre %	Proteins mg%	Fats mg%	Calorific value Kcal/100gm
<i>Bauhinia racemosa</i>	58.80	0.877	0.468	0.877	0.44	0.014	178.71
<i>Chlorophytum borivillianum</i>	65.18	0.76	0.45	0.76	0.530	0.003	292.36

**Table 1. Concentration of various nutrients in the vegetables:**

Vegetable	Nicotinic acid(B3) Ppm	Pyridoxin HCL (B6) ppm	Vitamin C Ppm
<i>Bauhinia racemosa</i>	433.255	114.24	769
<i>Chlorophytum borivillianum</i>	378.73	454.04	641

**Table 2. Concentration of vitamins in the vegetables:**

Vegetable	Ca (mg/gm)	Mg (mg/gm)	Fe (mg/gm)
<i>Bauhinia racemosa</i>	4.8096	2.918	0.007
<i>Chlorophytum borivillianum</i>	5.6112	3.4048	0.825

**Table 3. Concentration of vitamins in the vegetables:**

## STUDIES ON CROP PRODUCTIVITY OF LUCERNE (*Medicago sativa* L.) UNDER THE INFLUENCE OF VARIOUS CONCENTRATION OF FERTILIZER

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### ABSTRACT:

Lucerne (*Medicago sativa* L.) is flowering plant in the pea family Fabaceae used as green fodder. It is most popular forage crop as well as green manure of tropical as well as temperate climate. It is also called as Alfalfa. The green foliage is rich in protein and the use of this crop for regular production of leaf protein has been suggested by many workers. Alfalfa is rich in chlorophyll, carotene, vitamins B complex, vitamin C, vitamin D, vitamin E and vitamin K. The productivity of Lucerne (*Medicago sativa* L.). Var T-6 Chikalthana is studied under the influence of various concentrations of Humaur foliar spray. The harvest was analyzed and it was found that the yield and percent dry matter of Lucerne was increased by application of Humaur at the rate of 2ml/lit and 3ml/lit in five foliar spray.

**Keywords:** Lucerne (*Medicago sativa* L.), forage crop, Vitamin D, Humaur.

### INTRODUCTION:

Lucerne (*Medicago sativa* L.) is most popular forage crop of tropical as well as temperate climate. It is also called as "Alfalfa" in Arabic which means "the best". It is an oldest forage crop with 2000 years of documental history.

Lucerne is a perennial crop used as green fodder. It can maintain its productivity with cost benefit ratio of 1:3. In the month of October sowing is done broadcasting the seeds or by drilling them in rows. About 9-10 weeks are required to get the crop ready for harvesting. After subsequent regrowth of 20-30 days crop can be harvested. After fodder yield of Lucerne ranges from 850-900 quintals per hectare, this can be increased to 1200 to 5500 quintals per hectare in 9-10 cuttings by using high fertility, improved agronomic practices, superior germplasm and assured irrigation, (Relwani, 1979). Field trials at Research Laboratory of Botany Department Dr. Babasaheb Ambedkar Marathwada University, Aurangabad confirmed that Lucerne is highly productive crop with

consistent performance (Dev et al, 1974).

The green foliage of Lucerne is rich in protein and the use of this crop for regular production of Leaf Protein (LP) has been suggested by many workers.

The use of green leaves as source of protein in human nutrition has long been advocated (Pirie, 1971). By the popular techniques of "Green Crop Fractionation" (GCF) it is possible to extract the protein from green leaves and protein rich mineral vitamin rich concentrate referred as Leaf Protein Concentrate (LPC). In India it is also valued as green fodder especially for horses and its cultivation is confined to military farms. It is largely used by various pharmaceutical industries especially in homeopathic pharmacy. Updated market survey indicates that there is an increasing demand and use of this drug in preparation of health and vitality tonic i.e. Alfalfa tonic. It favorably influences nutrition, evidenced in 'tonic up' the appetite and digestion resulting improved mental and physical vigour with

gain in weight. It is used as tonic due to presence of high percentage of proteins (60.5%), minerals, enzymes, vitamins etc. Alfalfa is a valuable source of vitamins A and fresh is rich in Vitamin C (1.78 mg/g) but it loses 80% of vitamins on drying. (Rajat Rashmi *et al.*, .1997)

Humaur is a bio-organic foliar spray. It was manufactured by Hindustan Antibiotic Ltd. Pimpri, Pune. It is a nutritionally balanced food for plants. It is composed from microbially derived protein hydrolysates having enzymes, vitamins, organic acid precursors which are responsible for enhancing plant growth. It contains an adequate quantity of nitrogen along with Phosphorus, Potassium and bioactive forms of micro elements like Cc, Mg, B, Zn, Fe, etc. It enhances the fertility of soil. It is non-toxic, non-pollutant and safe to use. It ensures higher yield, good quality of produce and better price and profits.

In this present work, the productivity of Lucerne (*Medicago sativa* L.). Var T-6 Chikalthana is studied under the influence of various concentrations of Humaur foliar spray. The objective of the present study is to observe the effect of Humaur foliar spray which can be used to increase the vegetation of Lucerne.

#### **MATERIALS AND METHODS:**

The present investigation was undertaken to effect of various concentration of foliar spray on growth and yield of Lucerne. Var. T-6 Chikalthana. The experiment was conducted in randomized block design with plot size 6X5 meter.

**Treatment:** Experiment was conducted with the following treatment

1. T-1 Control
2. T-2 Humaur Spray (1 ml/L)
3. T-3 Humaur Spray (2 ml/L)
4. T-4 Humaur Spray (3 ml/L)

**Preparation of Land:** The research trial was laid out on black soil rich in organic matter

with well leveled field. The land was prepared by ploughing, harrowing and brought to fine tilt.

**Seed sowing:** The seeds were obtained from local market and sowing operation was done in November.

**Humaur Foliar Spray:** It is obtained from local market. The first spray was given at four leaf stage of crops followed by second spray after one week of interval.

**After Care:** The recommended practices of Lucerne cultivation and all the plant protection measures were timely carried out during the growth period of the crop.

Statistical analysis was carried out by method described by Panse and Sukhatame (1968).

#### **RESULTS AND DISCUSSIONS:**

The observations are recorded on per plot vegetation and percent dry matter were statistically analyzed and presented in Table No.1.

It was observed from the data shown in table no.1 that per plot vegetation increased from 2.980 kg in T4 treatment and T3 treatment 2.910 kg were statistically significant over control. Control produced 2.246 kg per plot vegetation.

As regards percent dry matter data shown in the table np.1 dry matter maximum increase from 21.1 % in T3 treatment and treatment T4 produced 20.7 % and T2 20.2 were statistically significant over control. Control showed 20.1 dry matter. Similar types of results recorded showed that dry weight of Soybean plant was promoted by triacontanol (Dong-Shu Fa *et al.*, 1987).

Growth regulator showed marked influence on average per plot vegetation. It was revealed that the vegetation increased in 2 ml/lit and 3

ml/lit concentrations. The maximum vegetation was recorded in T4 treatment 2.980 kg and T3 2.910 kg where growth regulator applied in five split doses. However increases of vegetation per plot due to easy availability of essential elements in it. Lowest per plot vegetation recorded in T2 treatment was 2.213 kg. Similar types of observation recorded, (Malik and Richa 1984) have shown 14 % increase in yield of rice by the foliar spray of Miztalol, (Reddy and Krishnappa 1984) recorded yield of Potato tuber were sprayed 2 ppm triacontanol over control. (Joshi and Nair 1994) recorded similar types of observations on bhindi where in treatment with Humaur recorded significantly highest plant height and yield over control plants.

#### CONCLUSION:

Investigation carried out and concluded that the application of Growth regulator at the rate of 2 ml/lit. and 3 ml/lit. in five foliar sprays was found to be suitable for more growth of the crop and percent dry matter of the crop. Humaur foliar spray enhances the fertility of soil. It is non-toxic, non-pollutant and safe to use. It ensures higher yield, good quality of produce, better price and profits.

#### ACKNOWLEDGEMENTS:

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Sr. No.	Treatment	Vegetation Per Plot (Kg)	Dry Matter %
	T-1 Control	2.246	20.1
	T-2 1 MI/Liter	2.213	20.2
	T-3 2MI/Liter	2.910	21.1
	T-4 3 MI/Liter	2.980	20.7
<b>S.E.</b>			
		<b>0.077</b>	<b>0.056</b>
<b>C.D. At 5 % level</b>			
		<b>0.189</b>	<b>0.135</b>
<b>F.(t)</b>			
		<b>14.21</b>	<b>35.30</b>

**Table No.1. Effect Humaur Foliar Spray on average number of per plot vegetation and percent dry matter of Lucerne.**

## EVALUATION OF SOME NUTRITIONAL ASPECTS OF UNCOMMON VEGETABLE: KORLA

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### ABSTRACT:

Korla (*Bauhinia malabarica* Roxb.) is a moderate sized deciduous tree belonging to family Caesalpiniaceae. Tender leaves of this plant are available in market as vegetable especially in monsoon season. The use of korla vegetable is limited to certain tribes and local people. The plant is not yet explored for nutritional aspects. In the present work estimation of certain important nutritional compounds such as carbohydrates, proteins, minerals, vitamin C and also crude fibers from leaves was done as per standard procedure. The dried powder of korla showed moderate amount of said nutrients. The generated data revealed the status of korla as nutritious leafy vegetable which can be added in diet.

**Key words:** *Korla, crude fibers, proteins, minerals*

### INTRODUCTION:

Since ancient times, wild edible plants are used as rich source of nutrients as well as therapeutic compounds. It is observed that the use of several edible wild plants is restricted to certain local tribal communities. Tribal people collect wild plants especially in monsoon period and make them available in market for limited period of time. Therefore many potential nutritional or medicinal wild plants are unpopular and unknown to common man. As most of the wild plants grow in natural conditions of forests without any cultivation practices, they may prove to be more nutritious or medicinal than domestic varieties (Atram, 2015). Considering these facts, the present work was undertaken. It involved nutritional analysis of one of the wild plants, i.e. Korla. *Bauhinia malabarica* Roxb. (Korla) grows as a wild deciduous tree in forests. The bark of tree is rough brown externally in form of linear flakes while red in colour internally. The leaves are 3 to 3.5 cm in length and 2 to 2.5 cm in breadth. They are typically heart-shaped. The young tender leaves are collected by tribal people in rainy season and sold as

vegetable in local markets. Leaves are sour in taste and used as flavouring agent in meat and fish preparations. As per the folklores, leaves, bark and roots are used medicinally mainly against stomach and liver ailments (Atram, 2015). However Korla is one of the uncommon vegetables and its nutritional benefits are not yet explored. The present work dealt with estimations of basic nutrients of leaves of Korla which may give an idea about nutritional status of this vegetable.

### MATERIAL AND METHODS:

Fresh leaves of *Bauhinia malabarica* (Korla) were collected from tribal women from Thane District. The leaves were cleaned and dried at 40°C in an oven. The dried sample of leaves was pounded and powder was stored in moisture free air tight containers with silica bags (Evans, 2001). Powdered leaf sample was used to find out contents of certain basic nutrients. The total carbohydrates were estimated by anthrone method using standard glucose as reference compound. The sample of leaf was treated sequentially with acetone to

remove interfering pigments, ethanol to extract soluble sugars and acid to extract starch. Determination of proteins was done by Folin-Lowry method using Bovine serum albumin as standard protein. Vitamin C content was found out by DNPH method. For proteins as well as vitamin C, water extract was used. Among minerals, iron and phosphate contents were estimated by colorimetric procedures. Minerals of plant sample were extracted in strong nitric acid. For all these methods standard graph was prepared using dilutions of respective standard compound. The value of unknown was found out by extrapolation of graphs. In case of crude fibres, leaf sample was treated with various solvents to remove fats, simple sugars, proteins and pigments, etc. Later the extracted fibre content was weighed and all values were expressed in terms of percent content (Sadasivam and Manickam, 2005; Elvehjem, 1930; Fiske and Subbarow, 1925; Nkafamiya, Ardo, Osemeahon, and Akinterinwa, 2016).

## RESULTS AND DISCUSSION

The leaves of *Bauhinia malabarica* were found to contain rich amount of proteins. The quantity of carbohydrates was significant. The basic structural and energy related bodily functions are fulfilled by them. Crude fibres of Korla was in considerable amount which may help in softening of stool (Nkafamiya et.al. 2016). Vitamin C acts as antioxidant agent and protects mainly connective tissues (Kuo, 2013). The satisfactory amount of Vitamin C of leaf sample may prove as potential redox and antioxidant agent. Similarly content of iron can also improve hemoglobin of red blood cells (Abbaspour, Hurrell, and Kelishadi, 2014). The substantial quantity of phosphates may act as supplement for bone development (Penido, and Alon, 2012).

## CONCLUSION

India has been known as rich repository of wild edible plants. Many times due to lack of

knowledge about nutritional benefits and cooking methods, several wild edible plants remain unpopular. The primary work on Korla i.e. *Bauhinia malabarica* revealed good nutritious status of the plant. The leaves of this plant can be included in diet as a new source of leafy vegetable. Its cooking method is similar to that of other common vegetables. Although Korla showed significant amounts of basic nutrients, the detailed nutraceutical profiling can be an extension of this work.

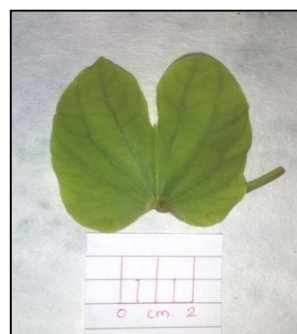
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**Picture No. 1 – Vegetable of Korla**



**Picture No. 2 – Heart shaped leaf of Korla**

Sr. No.	Nutrients	Amount in Dried Leaves
1.	Total Carbohydrates	2.75%±0.25
2.	Crude Fibres	0.62%±0.23
3.	Proteins	7.4%±0.20
4.	Vitamin C	0.16%±0.17
5.	Iron	0.16%±0.05
6.	Phosphates	1.46%±0.07

**Table No. 1 – Results of Nutritional Analysis**



## MASS PROPAGATION OF TWO GYNODIOECIOUS VARIETIES OF *Carica papaya* L. FROM SEEDLINGS REGENERATED *IN VITRO*

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### ABSTRACT:

*Carica papaya* L. is an economically important tropical plant, grown mainly for its fruits. Papaya fruits are of importance because of its high nutritive and medicinal value. Latex from the unripe fruit is the primary source of papain, a proteolytic enzyme. Conventional method of propagation of *Carica papaya* L. is by seeds. Elite varieties of papaya are highly priced. Dioecious varieties, seedless varieties, low seed germination, seed dormancy, vivipary, precocious germination etc. causes loss in papaya production. Vegetative propagation methods do not exist for large-scale production, thus need arises for the search of alternative method of propagation. Mass propagation of *Carica papaya* L. by tissue culture can serve as an important alternative to the conventional method of propagation. In the present investigation two gynodioecious varieties of *Carica papaya* L. Coorg Honey Dew (India) and Red Lady-786 (Taiwan) were selected for mass propagation from seedlings regenerated *in vitro*.

*Carica papaya* L. var. Coorg Honey Dew is a well-known cultivar, a selection from 'Honey Dew' at Chethalli Station of the Indian Institute of Horticultural Research Bangalore India. *Carica papaya* L. var. Red Lady 786 (Taiwan) is an early, vigorous and productive papaya that is tolerant to Papaya Ring Spot Virus. Both are gynodioecious varieties of *Carica papaya* L. which have plants bearing only female flowers or plants bearing hermaphrodite flowers, there are no male plants. Seeds of *Carica papaya* L. var. Coorg Honey Dew and Red Lady were inoculated on MS medium. Two month old seedlings were the source for shoot apex and node explants. MS medium fortified with various concentrations of auxins and cytokinins singly or in combination were used for multiple shoot formation from node and shoot apex explants from seedlings regenerated *in vitro*. The plantlets were maintained in the hardening chamber and in the green house.

Multiple shoots developed from shoot apex and node explants of seedlings regenerated *in vitro* in *Carica papaya* L. var. Coorg Honey Dew and Red Lady 786 in various combination of BAP+AS+NAA and BAP+AS+NAA+CM (10%).

**Key words:** *Carica papaya* L., gynodioecious varieties, *in vitro* seedlings, multiple shoots

### INTRODUCTION:

*Carica papaya* L. is a polygamous fruit crop. Unripe fruit contains latex which is a good source of proteolytic enzymes, papain and chymopapain (Setargie *et al.*, 2015). Papaya is propagated by seeds however due to heterozygosity, it is difficult to obtain true-to-type plants. Propagation by grafting (Allan *et al.*, 2010) and rooted cuttings (Rajan and Markose, 2007) exist, but these methods of propagation are too laborious to justify their

commercial use. Mass propagation of *Carica papaya* L. by tissue culture can thus serve as an important alternative to obtain true-to-type plants on a large scale compared to the conventional method of propagation. Papaya plant has three different sex types: staminate (male plant) producing staminate flower, female plant producing pistillate flower (female plants) and hermaphrodite plants producing bisexual flower (Bruce and Peter,

2008). In *Carica papaya* L. only female and the hermaphrodite plants produce fruits. In gynodioecious cultivar, there are no male plants; female and bisexual occur in equal proportions (Morton, 1987). The sex of dioecious papaya plants can be deduced only after they attain reproductive maturity (6 – 8 months) (Parasnis *et al.*, 1999). The variation in sex and detection of sex only after flowering makes *Carica papaya* L. an interesting system for study (Saha, *et al.*, 2004). In the present investigation two popular elite gynodioecious varieties of *Carica papaya* L. Coorg Honey Dew (India) and Red Lady-786 (Taiwan) were selected for mass propagation from seedlings regenerated *in vitro*.

## MATERIAL AND METHODS:

**Collection of plant material:** *Carica papaya* L. var. Coorg Honey Dew procured from 'Honey Dew' at Chethalli Station of the Indian Institute of Horticultural Research Bangalore India. *Carica papaya* L. var. Coorg Honey Dew plant is heavy bearing. This variety can be maintained pure by growing in isolation. This gynodioecious variety is used for hybridization purpose. *Carica papaya* L. var. Red Lady – 786 (Taiwan) was procured from Mumbai. *Carica papaya* L. var. Red Lady is popular in Maharashtra. Seeds are easily available, though very highly priced.

**Explants preparation:** Shoot apex and node explants from two months old seedlings regenerated *in vitro* were selected for present study.

**Media preparation:** Murashige and Skoog medium (1962) supplemented with NAA (0.5, 1.0 mg/l), BAP (2.0 mg/l), addenda's like AS – adenine sulphate (0.5, 1.0 mg/l) or CM – coconut milk (10%) in various combination were used as the induction medium or proliferation medium.

**Inoculation:** Shoot apex and node explants from two months old seedlings regenerated *in vitro* were inoculated on the induction medium or proliferation medium. The length of the shoots regenerated *in vitro* was measured.

**Rooting:** Regenerated shoots base were dipped in IBA (2500 mg/l) for 30 seconds and then inoculated on MS basal medium for rooting.

**Hardening:** The rooted plantlets regenerated *in vitro* were removed from rooting medium and transferred to small plastic cups with soil: vermiculite: cow-dung (1:1:1) and kept in hardening chamber having  $26\pm 1^{\circ}\text{C}$  temperature and 90% RH for 30 days. The temperature was gradually increased to room temperature and RH was reduced to 70%. The plantlets were then transferred to the green house.

## RESULTS AND DISCUSSIONS:

Node and shoot apex explants from seedlings regenerated *in vitro*, inoculated on induction medium showed initiation of shoot buds within 4 weeks. In the induction medium node explants from seedlings regenerated *in vitro* of *Carica papaya* L. var. Coorg Honey Dew inoculated on MS medium fortified with BAP (2.0 mg/l), AS (0.5 mg/l) and NAA (0.5 mg/l) gave rise to maximum shoot buds 3.32 and node explants from seedlings regenerated *in vitro* of *Carica papaya* L. var. Red Lady-786 inoculated on MS medium fortified with BAP (2.0 mg/l), AS (1.0 mg/l) and NAA (0.5 mg/l) gave rise to maximum 1.75 shoot buds (Table 1). In the induction medium shoot apex explants from seedlings regenerated *in vitro* of *Carica papaya* L. var. Coorg Honey Dew inoculated on MS medium fortified with BAP (2.0 mg/l), AS (0.5 mg/l) and NAA (0.5 mg/l) gave rise to maximum shoot buds 3.86 and shoot apex explants from seedlings regenerated *in vitro* of *Carica papaya* L. var. Red Lady-786 inoculated on MS medium

fortified with BAP (2.0 mg/l), AS (1.0 mg/l) and NAA (0.5 mg/l) gave rise to maximum 1.82 shoot buds (Table 1). A protocol was developed for *in vitro* propagation of hermaphroditic papaya (*Carica papaya* L.) from shoot buds. In this study, best initiated and proliferated shoots were obtained on MS medium with 1mg/l BAP and 0.5 mg/l NAA (Setargie *et al.*, 2015). In the present study induction medium included BAP, NAA and AS in various combinations.

In the proliferation medium node explants from seedlings regenerated *in vitro* of *Carica papaya* L. var. Coorg Honey Dew inoculated on MS medium fortified with BAP (2.0 mg/l), AS (0.5 mg/l), NAA (0.5 mg/l) and CM (10%) gave rise to maximum shoot buds 5.00 and node explants from seedlings regenerated *in vitro* of *Carica papaya* L. var. Red Lady-786 inoculated on MS medium fortified with BAP (2.0 mg/l), AS (1.0 mg/l) NAA (0.5 mg/l) and CM (10%) gave rise to maximum 1.84 shoot buds (Table 2). In the proliferation medium shoot apex explants from seedlings regenerated *in vitro* of *Carica papaya* L. var. Coorg Honey Dew inoculated on MS medium fortified with BAP (2.0 mg/l), AS (0.5 mg/l) NAA (0.5 mg/l) and CM (10%) gave rise to maximum shoot buds 5.32 and shoot apex explants from seedlings regenerated *in vitro* of *Carica papaya* L. var. Red Lady-786 inoculated on MS medium fortified with BAP (2.0 mg/l), AS (1.0 mg/l) NAA (0.5 mg/l) and CM (10%) gave rise to maximum 1.91 shoot buds (Table 2). *In vitro* propagation of *Carica papaya* L. has been reported using shoot tips and axillary buds (Rajeevan and Pandey, 1986). Supplementation of multiplication medium of papaya by 3% sucrose was reported by Mondal *et al.*, (1990). In the present study supplementing the induction medium with 10% coconut milk gave rise to an increase in the number of shoot buds. However responses are usually genotype dependent as seen in the present study.

The proliferation medium also promoted the elongation of the shoots regenerated *in vitro* in both varieties. In 8 weeks the length of the shoots was 2.06, and 2.33 cm in *Carica papaya* L. var. Coorg Honey Dew and Red Lady respectively (Table 3). Regenerated shoots were separated and the base was dipped in IBA (2500 mg/l) for 30 seconds inoculated on solid MS medium. It showed 64.3, and 62.6% rooting in 17.3 and 18.3 days in *Carica papaya* L. var. Coorg Honey Dew and Red Lady respectively (Table 3). The plants were kept in shade house and gradually transferred to the field. In the present work *in vitro* rooting of multiple shoots gave rise to plantlets. These plantlets were transferred to green house in substratum [soil: vermiculite: cowdung (1:1:1)] with a hardening success of 19 and 10% in *Carica papaya* L. var. Coorg Honey Dew and Red Lady respectively (Table 3). IBA (10.0  $\mu$  m) induced profuse rooting in *Carica papaya* L. (Pandey and Rajeevan, 1983). *In vitro* regeneration with shoot tip, excised from mature papaya plant, has been tried (Litz & Conover, 1978; Rajeevan & Pandey, 1986; Drew, 1992). Protocols are usually genotype dependent and are difficult to reproduce. In *Carica papaya* L. only female and the hermaphrodite plants produce fruits. Male plants do not bear fruits. Gynodioecious varieties of *Carica papaya* L. produce both female and the hermaphrodite plants. Thus in the present study, for mass propagation by tissue culture, seedlings regenerated *in vitro* of two gynodioecious varieties of *Carica papaya* L. var. Coorg Honey Dew (India) and var. Red Lady-786 (Taiwan) were selected.

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Multiple shoots from node and shoot apex explants of  
*Carica papaya* L. var. Coorg Honey Dew



Multiple shoots from node and shoot apex explants of  
*Carica papaya* L. var. Red Lady-786

BAP+AS+NAA (mg/l)	Varieties			
	CHD	RL	CHD	RL
	Number of shoot buds / node explants		Number of shoot buds / shoot apex explants	
2.0+0.5+0.05	1.62±0.23	1.19±0.11	1.79±0.09	1.30±0.10
2.0+0.5+0.1	1.57±0.06	1.15±0.11	1.64±0.12	1.28±0.17
2.0+0.5+0.5	<b>3.32±0.31</b>	1.10±0.00	<b>3.86±0.36</b>	1.18±0.11
2.0+0.5+1.0	1.30±0.10	1.05±0.05	1.23±0.16	1.10±0.00
2.0+1.0+0.05	1.54±0.12	1.17±0.10	1.73±0.18	1.22±0.10
2.0+1.0+0.1	1.53±0.10	1.13±0.07	1.59±0.12	1.13±0.07
2.0+1.0+0.5	1.30±0.10	<b>1.75±0.11</b>	1.44±0.09	<b>1.82±0.04</b>
2.0+1.0+1.0	1.30±0.10	1.02±0.08	1.33±0.08	1.10±0.00

Values are mean of three sets of determinants. Each set containing 10 explants.

CHD- *Carica papaya* L. var. Coorg Honey Dew, RL- *Carica papaya* L. var. Red Lady-786

**Table 1: Effect of MS medium fortified with various concentrations of BAP, AS and NAA (induction medium) on the number of shoot buds initiated on node and shoot apex explants of seedlings regenerated *in vitro* in different varieties of *Carica papaya* L.**

BAP+AS+ NAA (mg/l) +CM (10%)	Varieties			
	CHD	RL	CHD	RL
	Number of shoot buds / node explants		Number of shoot buds / shoot apex explants	
2.0+0.5+0.05	2.12±0.06	1.57±0.18	2.15±0.12	1.59±0.12
2.0+0.5+0.1	1.73±0.11	1.30±0.10	1.77±0.15	1.33±0.08
2.0+0.5+0.5	<b>5.00±0.10</b>	1.37±0.11	<b>5.32±0.04</b>	1.38±0.14
2.0+0.5+1.0	2.27±0.16	1.19±0.11	2.39±0.16	1.23±0.16
2.0+1.0+0.05	1.60±0.24	1.67±0.18	1.72±0.16	1.76±0.08
2.0+1.0+0.1	1.49±0.11	1.65±0.20	1.53±0.10	1.67±0.21
2.0+1.0+0.5	2.95±0.10	<b>1.84±0.15</b>	2.99±0.09	<b>1.91±0.17</b>

Values are mean of three sets of determinants. Each set containing 10 explants.

**Table 2: Effect of MS medium fortified with various concentrations of BAP, AS and NAA and CM (10%) (proliferation medium) on the number of shoot buds initiated on node and shoot apex explants of seedlings regenerated *in vitro* in different varieties of *Carica papaya* L.**

Rooting & Hardening		Varieties	
		CHD	RL
Length of the shoots in 8 weeks		<b>2.06±0.15</b>	<b>2.33±0.20</b>
Base of the shoot dipped in IBA (2500 mg/l) for 30 seconds, inoculated on MS basal medium	Rooting (%)	<b>64.3±2.08</b>	<b>62.60±2.08</b>
	Time taken (d)	<b>17.3±2.08</b>	<b>18.3±0.57</b>
Substratum - Soil: vermiculite: cow-dung (1:1:1) in green house in 4 weeks		<b>19.0 ±1.78</b>	<b>10.0 ±0.98</b>

Values are mean of three sets of determinants. Each set containing 10 explant.

**Table 3: Length of the multiple shoots in 8 weeks, rooting and hardening of the multiple shoots in different varieties of *Carica papaya* L.**

## PRELIMINARY PHYTOCHEMICAL SCREENING OF FENUGREEK LEAVES (AIR, OVEN AND MICROWAVE DRIED) AND DRY SEEDS

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(Under DBT Star College Scheme, Govt. Of India, New Delhi)

### ABSTRACT:

Fenugreek (*Trigonella foenum-graecum* L.), is also known as Methi. Fenugreek is an annual plant in the family Fabaceae. Fenugreek leaves (dried or fresh) and seeds are used as vegetable spices and condiments in Indian cuisine. Fenugreek being an important part of dietaries, it contributes substantially to nutrient intake. Fenugreek has considerable medicinal value and it serves as a significant source of energy, protein, dietary fiber, vitamins, minerals, and phytochemicals. Research strongly suggests that consuming foods rich in phytochemicals provides health benefits, but not enough information exists to make specific recommendations for phytochemical intake. Therefore, it remains a challenge for scientists to provide efficient, safe and cheap medications, especially for rural areas, with the available resources at the nearest. Phytochemicals in freshly harvested plant foods may be degraded by processing techniques, including cooking. The main cause of phytochemical loss from cooking is isothermal decomposition. In the present study preliminary phytochemical analysis of the air, oven and microwave dried samples of fenugreek leaves and dry seeds were extracted using different solvents.

Extraction process was carried out using selective solvents through standard procedures. Air, oven and microwave dried leaves of fenugreek and dry seeds were powdered and extracted in petroleum ether, methanol, chloroform, toluene, and distilled water. Phytochemical examinations were carried out for all the extracts as per the standard methods.

Qualitative phytochemical tests of different extract demonstrated the presence of various phytochemicals such as carbohydrates, proteins, glycosides, steroids, phenols, saponins, alkaloids and flavonoids as major active constituents in air, oven and microwave dried samples of fenugreek leaves and seeds.

**Key words:** Fenugreek, dietaries, phytochemicals

### INTRODUCTION:

Plants are potent biochemists. Plant based natural constituents can be derived from various plant parts. Most important of these bioactive constituents of plants are alkaloid, tannins, flavonoids and phenolic compounds (Nyananyo *et al.*, 2005). Fresh or dried plant materials can be used as a source for the extraction of secondary plant components. Plants are used in the dry form (or as an aqueous extract) by traditional healers and due to differences in water content within different plant tissues, plants are usually air dried to a

constant weight before extraction (Tiwari *et al.*, 2011). Fenugreek (*Trigonella foenum-graecum*) being rich in phytochemicals has traditionally been used as a food, forage and medicinal plant (Leung and Foster, 1996). *Trigonella foenum-graecum* L also is known as one of the oldest medicinal plants recognized in recorded history (Mawahib *et al.*, 2015). The plant reaches a height of 0.3 to 0.8 meters and has trifoliate leaves, while flowers appear in early summer and develop into long, slender, yellow brown pods containing the brown seeds, which are

hard, yellowish brown and angular or oblong or rhombic with a size of about 3 mm (Karna, 2013). Leaves and seeds of fenugreek have been used extensively to prepare extracts and powders for medicinal uses. Fenugreek is reported to have antioxidant properties (Naidu *et al.*, 2010), immunomodulatory effect (Meghwal and Goswami, 2012), digestive effect (Platel and Srinivasan, 2000), gastro and hepatoprotective effect (Blank, 1996), anticancer activity (Mathern, *et al.*, 2009), hypoglycemic activity (Roberts, 2011) and hypocholesterolaemic effects (Srivastava *et al.*, 2012) etc. The seeds of the *Trigonella foenum-graecum* L. herb possess toxic oils, volatile oils and alkaloids have been shown to be toxic to bacteria, parasites and fungi (Mawahib *et al.*, 2015). From ancient time spices are used to flavor and improve the taste of food recipes. Besides this they are used in cosmetics and medicinal preparation of Indians and Nepali systems such as Ayurveda and Unani (Karna, 2013).

Leafy vegetables occupy an important position in the Indian diet. Drying is the most commonly used method for enhancing shelf life of leafy vegetables. The utilization of dried leaves powder reduces the volume required for storage and easy to handle. During the drying process there is lot of losses that takes place, like nutritional, physical and chemical composition of leaves (Satwase *et al.*, 2013). In the present study preliminary phytochemical analysis of the air, oven and microwave dried samples of fenugreek leaves and dry seeds were carried out.

## **MATERIALS AND METHOD:**

**Collection of plant material-** Fenugreek leaves and seed sample was collected from the local market of Thane (Maharashtra). Leaves and seed samples were washed thoroughly 2-3 times with running tap water and once with sterile distilled water to remove the extraneous material and blotted dry.

**Preparation of Sample for solvent extraction-** Clean leaves and seeds were dried as follows-

- d. Fenugreek leaves were air dried under shade and used for further analysis.
- e. Fenugreek leaves were dried in preset oven at  $40 \pm 2^{\circ}\text{C}$  and then grounded into fine powder and used for further analysis.
- f. Fenugreek leaves were dried in microwave and grinded in a blender to fine particle and used for further analysis.

## **Determination of solvent soluble extractive-**

The solvent soluble extractive values of powders of fenugreek leaves (air dried, oven dried, microwave dried) and dry seeds in solvents (water, methanol, chloroform, toluene petroleum ether) were carried out. The extraction was carried out by plant tissue homogenization method (Tiwari *et al.*, 2011). 1 gram powder of fenugreek leaves (air dried, oven dried, microwave dried) and dry seeds was macerated separately with distilled water, methanol, chloroform, toluene, petroleum ether (100 ml) in a closed flask for twenty four hours, shaking frequently. Solutions were filtered and 25 ml of filtrates were evaporated in tarred flat bottom shallow dishes. They were further dried at  $100^{\circ}\text{C}$  and weighted when at room temperature. The percentage of water, methanol, chloroform, toluene petroleum ether soluble extractive was calculated with reference to the air dried, oven dried and microwave dried drugs and dried seeds.

## **Preliminary phytochemical analysis-**

Fenugreek leaves (air dried, oven dried, microwave dried) and dry seeds extracts were subjected to preliminary phytochemical screening to test the presence or absence of phytochemical constituents such as alkaloids, saponins, phenolic compound, tannins, flavonoids, steroids and terpenoid as per standard procedure given in Kokate *et al.*, 2005; Harborne, 2005.

## RESULT AND DISCUSSION:

The phytochemicals present in different plants are species specific thus they have different chemical profile. Phytochemicals present in plants are dissolved in different solvent for the purpose of further analysis. In the present study five solvents (water, methanol, chloroform, toluene, petroleum ether) were selected to determine the soluble substance in powders of *Trigonella foenum-graecum* L. leaves (air dried, oven dried, microwave dried) and dry seeds.

The air dried sample of *Trigonella foenum-graecum* L. leaves showed maximum water soluble extractive and minimum toluene soluble extractive. The extractive value in oven dried sample of fenugreek leaves was the highest in toluene. The microwave dried sample of fenugreek leaves showed maximum water soluble extractives and minimum petroleum ether soluble extractive. The extractive value in fenugreek seeds was the highest in chloroform (Table 1). The dried sample of fenugreek seeds showed maximum chloroform soluble extractive and minimum petroleum ether soluble extractive (Table 1). The air and microwave dried sample of leaves showed higher content of water soluble extractive as compared to oven dried sample which showed maximum toluene soluble extractive (Table 1). Extractive values play an important role in evaluation of crude drugs. Less extractive value indicates incorrect processing during drying or formulating, improper storage, adulteration or addition of exhausted material.

Qualitative phytochemical analysis of powders of fenugreek leaves (air dried, oven dried, microwave dried) revealed the presence of acid compounds, aleurone grains, alkaloids, proteins, carbohydrates, fats and fixed oils, glycosides, flavonoids, starch, steroids, tannins, resins, essential oils and saponins in various solvents (Table 2). Dry seed powder of

*Trigonella foenum-graecum* L. showed the presence of aleurone grains, carbohydrate, alkaloids, proteins, fats and fixed oils, tannins, saponin, glycosides, steroids and resins in various solvents (Table 2).

Preliminary qualitative phytochemical analysis made for the air, oven and microwave sample dried sample of fenugreek leaves revealed the presence of alkaloids, glycosides, flavonoids, glycosides, phenols, resins, saponins, steroids, tannins, terpenoids and triterpenoids. These secondary metabolites are reported to have many biological and therapeutic properties, so this species is expected to have many medicinal uses. The extraction yield calculated for water, methanol, chloroform, toluene, and petroleum ether extracts showed that water extract registered higher percentage of yield in air and oven dried sample of fenugreek leaves. It may be due to high polarity of water solvent which can draw high variety of plant constituents than the other solvents did. Pandhre *et al.*, 2011 studies that when fenugreek leaves were dried by using solar, infra-red and tray drier there was a loss of color pigments. Fenugreek leaves pretreated with 0.1% Sodium bicarbonate gave better results in chlorophyll-a, chlorophyll-b and carotene retention. So, to minimize drying losses various pretreatments are used.

Fenugreek is one of the important vegetable to have rich and balanced nutritional value. It is a cheap source of high amount of protein and amino acids. No significant difference in presence of phytochemicals was observed between the Air, oven and microwave sample dried sample of fenugreek leaves. It is recommended that the fenugreek could be used in daily human diet due to high nutritional value.

*Trigonella foenum-graecum* L. have great medicinal value and proved to serve as good hypoglycemic, hypocholesterolemic, galactagogue, laxative stimulant, carminative, stomachic, antacid, antiulcerative,



antibacterial, antihypertensive, antithrombotic, anticarcinogenic, antioxidant and diuretic. The previous pharmacological studies of various seed extracts of Fenugreek have also shown that it contains mucilage, volatile oils and alkaloids such as choline and trigonelline, sotalone and pyrazines,. Bitterness of fenugreek seeds is mainly due to the oil, steroidal saponins and alkaloids which are all non-toxic on consumption. Various ethnobotanical surveys observed fenugreek seeds can be used in simple remedies for treating a variety of ailments. A reference cited wrote, one teaspoon of fenugreek seeds cooked with rice and eaten regularly for 10 to 15 days shows remarkable rise in hemoglobin (Karna, 2013). Further research is necessary for better understanding of Methi so as to throw more light on its pharmacological efficiency.

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		Extractive value (%) of Solvents				
Plant Parts		Water	Methanol	Chloroform	Toluene	Petroleum ether
Leaves	A	32	31	12	7	21
	O	33	26	6	53	15
	M	49	17	22	22	13
Seeds		26	22	30	17	8

L=Leaves; S=seeds; A=air dried leaves; O=oven dried leaves; M=microwave dried leaves

**Table 1: Extractive values of powders of fenugreek leaves (air dried, oven dried, microwave dried) and dry seeds in various solvents.**

Plant constituents	Water				Methanol				Chloroform				Toluene				Petroleum ether			
	L			S	L			S	L			S	L			S	L			S
	A	O	M		A	O	M		A	O	M		A	O	M		A	O	M	
Acid compounds	-	+	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
Aleurone Grains	+	+	+	+	+	+	-	+	+	-	-	+	+	-	+	+	+	-	+	+
Alkaloids	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
Anthraquinone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Amino acids	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Proteins	+	-	+	+	-	-	+	+	+	-	+	+	-	-	-	+	-	-	+	+
Carbohydrates	-	-	+	-	-	-	+	-	-	-	+	+	-	-	+	+	-	-	+	+
Starch	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-
Fats and Fixed Oils	-	+	-	+	+	-	-	-	-	+	+	+	+	+	-	+	+	+	+	+
Glycosides	-	+	-	-	-	-	-	+	+	+	+	-	-	+	+	+	-	+	+	+
Mucilage	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phenols	-	-	-	-	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-
Flavonoids	-	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Steroids	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	-
Tannins	+	+	+	+	+	+	+	+	+	-	+	-	+	-	+	-	-	-	+	-
Saponins	+	+	+	+	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-
Essential Oils	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-
Resins	-	-	+	+	+	-	+	+	+	-	+	-	+	-	+	-	-	-	+	-

L=Leaves; S=seeds; A=air dried leaves; O=oven dried leaves; M=microwave dried leaves; +=present; -=absent

**Table 2: Qualitative phytochemical analysis of powders of fenugreek leaves (air dried, oven dried, microwave dried) and dry seeds.**

## PHYTOCHEMICAL SCREENING AND DETERMINATION OF ANTIOXIDANT POTENTIAL OF *Bauhinia malabarica* Roxb.

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### ABSTRACT:

The tender leaves of *Bauhinia malabarica* Roxb. are sold under the local name of korla. This leafy vegetable is available during monsoon season. The plant is a moderate sized deciduous tree belonging to family Caesalpiniaceae. The medicinal properties of korla are meagerly understood. The decoction of root bark is used for liver problems. Root and stem are used for the treatment of cholera.

The present work had been undertaken to know the phytochemical composition of leaves using different solvents. In addition to it, determination of antioxidant activity of aqueous extract of leaf was done by DPPH method.

Preliminary phytochemical analysis showed the presence of carbohydrates, proteins, amino acids, alkaloids, tannins, phenols, etc.

The generated data may serve as primary data for therapeutic analysis of the said plant.

### INTRODUCTION

Herbal formulations today are the symbols of safety in contrast to synthetic drugs. More over some plants are not only considered for nutrition and but also are recommended for certain therapeutic values. Recently WHO (World Health Organization) estimated that 80% of world's population rely on herbal medicines for their primary health care needs<sup>[1]</sup>. Although several ailments have been treated with herbal medicines throughout the history of mankind, newly emerging diseases pose upsurge of screening of new plants with medicinal potential. Considering the increasing demand of herbal drugs, the present work had been undertaken. It intended to explore the phytochemical and antioxidant potential of one of the uncommon vegetables, i.e Korla. The young leaves of a deciduous tree of *Bauhinia malabarica* Roxb. are used as vegetable in rainy season. As per the folklores, leaves are externally applied on forehead for headache. They are also used as flavouring agent in meat and fish preparations. Root extracts are effective in liver disorders. The plant has digestive, antibacterial and stomachic properties<sup>[2]</sup>. Present work dealt with preliminary phytochemical screening of leaf extracts in order to know phytochemical composition of leaves. In addition to this, determination of antioxidant capacity was also carried out.

### MATERIAL AND METHODS:

1. Collection of material – fresh leaves of *Bauhinia malabarica* (Korla) were collected from tribal women from Thane District. The leaves were dried at 40°C in an oven. The dried sample of leaves was pounded and powder was stored in moisture free air tight containers with silica bags<sup>[3]</sup>.
2. Preparation of leaf extracts – five different samples of extracts of leaves were prepared with various solvents, viz. water, methanol, petroleum ether, toluene and chloroform. 1.0 g of powdered sample was extracted with 100 ml respective solvent using cold extraction method<sup>[4]</sup>.
3. Phytochemical analysis – preliminary phytochemical screening was done using reconstituted solutions of respective extracts. Standard phytochemical tests were used to detect presence of various primary and secondary metabolites of leaf extracts<sup>[5,6]</sup>.
4. Antioxidant activity – the free radical scavenging activity of water extract was determined by DPPH (1,1-diphenyl-2-picrylhydrazyl) method using ascorbic acid as reference compound. The total antioxidant potential of water extract was evaluated by phosphormolybdate method. The total phenolic content was estimated using Folin-Ciocalteu reagent<sup>[7,8]</sup>.

**OBSERVATIONS:**

Table No. 1 – Results of Preliminary Phytochemical Tests

Table No. 2 – Results of Antioxidant Potential

**RESULTS AND DISCUSSION:**

The qualitative phytochemical analysis of leaf extracts of *Bauhinia malabarica* revealed presence of various primary and secondary metabolites. The results are mentioned in Table No. 1. Among all solvents, water exhibited best extractable capacity. In addition to basic metabolites, the secondary metabolites included important compounds such as flavonoids, glycosides, phenols and tannins. The antioxidant activity is due to flavonoids and biflavones<sup>[9]</sup>. Phenolics have potential of free radical scavenging and act against lipid peroxidation<sup>[10]</sup>. As these secondary metabolites are functional antioxidants, the antioxidant potential has been found out by various methods from water extract of *Bauhinia malabarica*. The results are depicted in Table No. 2. The total antioxidant capacity was moderate for water extract and expressed as number of equivalents of ascorbic acid. Considerable amount of total phenolics was also found in same extract. DPPH is a stable free radical and its absorption decreases with the increased antioxidants. Extract of Korla exhibited DPPH scavenging potential which is comparable to standard antioxidant i.e. Ascorbic acid (Picture No. 3).

**CONCLUSION:**

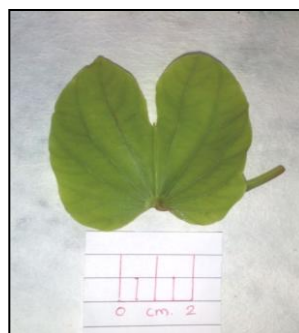
Korla i.e. *Bauhinia malabarica* is less known vegetable available for limited period of year. It contains rich combination of phytoconstituents which may give nutritional as well as medicinal benefits. The studies of leaf water extract revealed significant antioxidant potential of this vegetable. Thus this work may lead to popularize and introduce a novel source of antioxidants in our diet.

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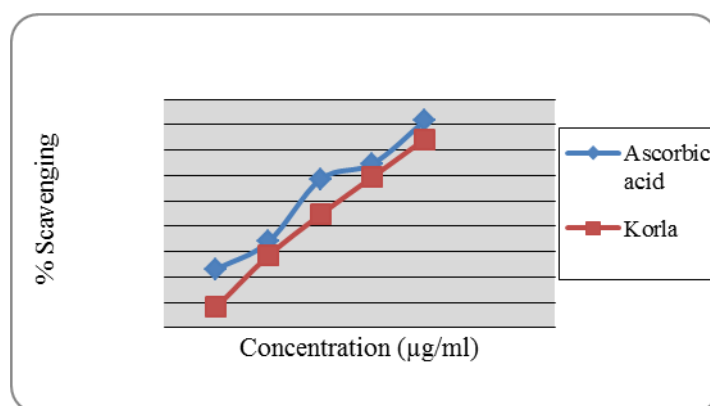
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Picture 1 – Vegetable of Korla



Picture 2 – Heart shaped leaf of Korla



Picture 3 – Comparative % scavenging of DPPH by Standard Antioxidant (Ascorbic Acid) and *Bauhinia malabarica* – Korla extract

Sr. No.	Phytochemical Tests	Leaf Extracts <i>Bauhinia malabarica</i> Roxb.				
		Petroleum ether	Methanol	Toluene	Chloroform	Distilled Water
1.	Amino acids	-	+	+	+	+
2.	Proteins	-	+	+	+	+
3.	Carbohydrates	+	+	+	+	+
4.	Starch	+	-	-	+	+
5.	Fats & Oils	+	+	+	+	+
6.	Mucilage	-	-	-	-	-
7.	Alkaloids	-	+	-	+	+
8.	Anthraquinones	-	-	-	-	-
9.	Glycosides	+	-	+	+	+
10.	Saponins	-	-	-	+	+
11.	Steroids	+	+	-	-	+
12.	Tannins	+	+	+	+	+
13.	Phenols	+	+	+	+	+
14.	Flavonoids	+	+	+	+	+
15.	Essential Oils	-	+	-	+	-
16.	Resins	+	+	+	+	+

Key: '+' Present and '-' Absent

**Table 1 – Results of Preliminary Phytochemical Tests**

Sr. No.	Parameter	Water extract of <i>Bauhinia malabarica</i> Roxb.
1.	Total Antioxidant Capacity	1.5gm/100gm
2.	Total Phenolic Content	1.74gm/100gm
3.	DPPH Scavenging Activity	68.60mcg/ml

**Table 2 – Results of Antioxidant Potential**

## CALCIUM ESTIMATION OF FENUGREEK LEAVES AND SEEDS BY VARIOUS ESTIMATION METHODS

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### ABSTRACT:

Calcium is an important mineral required for human growth and development. In our body, 99% calcium is found in bones and teeth. Mushrooms, broccoli, kale, dry fruits and meat are rich sources of calcium. However, calcium deficiency is more prominent in the poor masses. Hence, our investigation is based on calcium content in 'Fenugreek' as it is the most economical source of calcium. Fenugreek (*Trigonella foenum-graecum.L*) is an annual plant in the family Fabaceae. It is a rich source of energy, minerals (calcium, iron, phosphorus), dietary fibre, vitamins and phytonutrients. The calcium content in Fenugreek leaves and seeds were estimated by methods such as volumetric titration (Redox, complexometry), and colorimetry. The comparative study was carried out on Fenugreek leaves that were air dried, oven dried and microwave dried and seeds.

The calcium content in the air dried Fenugreek leaves was highest and that of oven dried and microwave dried Fenugreek leaves were similar. Seeds exhibited higher amount of calcium as compared to leaves. Thus, Fenugreek leaves and seeds can be good calcium source for the poor masses.

**Key words:** Fenugreek (*Trigonella foenum-graecum.L*), calcium, colorimetry, complexometry, volumetric titration.

### INTRODUCTION:

Fenugreek is an annual plant in the family Fabaceae. It is a rich source of minerals (calcium, iron, and phosphorus), vitamins and phytonutrients (Wani S. A. and Kumar P., 2016). Fenugreek is one of the well known spices in human food. Its seeds and green leaves are used in food as well as in medicinal application which is an old practice of human history. It provides natural food fibre and other nutrients required in human body (Meghwal M. and Goswami T. K., 2012). The plant, being a good source of calcium, is used to make the body stronger and to increase vitality. It has been observed that water forms the basis of all plant and animal material. Water acts as a medium for intercellular mechanisms and biological processes. It is said that around 65-70% of total plant weight constitutes water. Determination of moisture content is an

important factor in industrial preparations as preservation is the key factor. Considering the current need of the hour, an estimation of moisture content is done.

Calcium is a very important mineral required for human growth and development. In our body, 99% calcium is found in bones and teeth. Calcium has been documented in studies of nutrient composition of local foods since the early part of the century. One of the earliest reports was that of Morris and Oliveira (1933) who documented the content of this mineral in some 60 types of foods. In that study, calcium was precipitated as calcium oxalate, converted to calcium oxide, weighed and reported as such. Leong and Morris (1947) used a different procedure for determining this mineral. Calcium was again precipitated as oxalates, but instead of using the more



cumbersome gravimetric procedure, calcium present was next titrated with potassium permanganate and results expressed as milligrams of calcium. The quantitative estimation of micronutrients (Ca, Mg, Fe, Zn, Cu) was carried out using atomic absorption spectrophotometer (Pathak N. and Agrawal S., 2014). Also several mineral content in the Indian species get examined by ICP-OES method (Kumaravel S. and Alagusundaram K., 2014). There are many vegetarian and non-vegetarian sources of calcium. Mushrooms, broccoli, kale, dry fruits are some vegetarian sources whereas eggs, fish and meat are non-vegetarian sources of calcium (Weaver and Plawecki, 1994; Feskanich D. et al., 2003). However, calcium deficiency is more prominent in the poor masses. The current work is based on determination of calcium content in 'Fenugreek' (*Trigonella foenum-graecum*) as it is the most economical source of calcium; thus compensating for the calcium deficiency in the poor masses of the society.

## MATERIALS AND METHODS:

In the preparation of reagents, chemicals of analytical grade purity and distilled deionised water were used.

### Material collection and sample preparation of fenugreek leaves:

Fresh plant leaves were purchased from local markets of Thane. Whenever applicable, refuse in each food items was removed and its proportion in the food determined. The leaves were dried using air drying, oven drying and microwave drying. The microwave drying were carried out at Microwave oven for 8 minutes at 180 °C, the oven drying were carried out at 90 °C for 24 hours and air drying were carried out for 7 days in a sterilised laboratory at room temperature. The all dried sample was then homogenized into a powder.

### Material collection and preparation

#### fenugreek seeds:

Take 100 g of fenugreek seeds purchased from the local market. The seeds were dried at 90 °C for one hour and crushed into a fine powder.

#### Determination of loss of moisture content:

The moisture contents of fenugreek can be calculated as follows using various drying procedures (Bradley and Nielsen):

$$\% \text{Moisture (wt/wt)} = \frac{\text{weight of wet sample} - \text{weight of dry sample}}{\text{weight of wet sample}} \times 100$$

### General procedure for determination of Calcium in the fenugreek by using Potassium permanganate titration method:

The sample powder (10 g) was charred in a silica crucible for 4 hours. A weighed quantity (0.25g) of ash was dissolved in 8 ml of conc. HCl and 30 ml of distilled water. The resultant solution was heated to concentrate and it was filtered using filter paper. The glacial acetic acid was added to the resultant solution till pH of the solution reaches to 3. A saturated solution of ammonium oxalate was added in the beaker containing the sample in hot condition (70 °C) until the solution became crystal clear. After clearing, excess amount of saturated solution of ammonium oxalate was added until the solution turned white and hazy. The solution was allowed to stand at room temperature for 5 hours to precipitate as calcium oxalate. The precipitate was washed with hot water and filtered using Whatman filter paper No.1. The precipitate was dissolved in some of amount of 4N H<sub>2</sub>SO<sub>4</sub>. To dissolve the precipitate, the solution was heated and dilutes the sample upto 50 ml in Erlenmeyer flask. 10 ml of sample solution was pipette out and titrated against 0.01 N KMnO<sub>4</sub> solution in hot condition. The endpoint was colourless to light pink (Song T. E. et al., 1989).

**General procedure for determination of Calcium by using complexometric method:**

The sample powder (10 g) was charred in a silica crucible for 4 hours. A weighed quantity (0.25 g) of ash was dissolved in 5 ml of conc. HCl and 20 ml of distilled water. The solution was heated to concentrate and it was filtered using filter paper. The dil. NaOH solution was added to the resultant solution till pH of the solution reaches to 7. The solution was diluted to 50 ml in an Erlenmeyer flask. 25ml of diluted sample solution was pipette out in a conical flask and 25 ml distilled water was added to the solution. The solution was maintained at pH 12-14 by adding 8M NaOH solution. This was carried out to precipitate magnesium and thus, nullify its interference. The mixture was shaken vigorously. The precipitate was allowed to settle down for 10 minutes. 0.1g of Patton - Reeder indicator was added in the flask. The mixture was stirred to dissolve the indicator. The solution was titrated against 0.02N EDTA solution. The endpoint was wine red to blue (Patton and Reeder, 1956).

**General procedure for determination of Calcium in the fenugreek by using colorimetric method:**

The sample powder (10 g) was charred in a silica crucible for 4 hours. A weighed quantity (0.25g) of ash was dissolved in 8 ml of conc. HCl and 30 ml of distilled water. The resultant solution was heated to concentrate and it was filtered using filter paper. The glacial acetic acid was added to the resultant solution till pH of the solution reaches to 3. A saturated solution of ammonium oxalate (0.01 N) was added in the beaker containing the sample in hot condition (70°C) until the solution became crystal clear. After clearing, excess amount of saturated solution of ammonium oxalate was added until the solution turned white and hazy. The solution was allowed to stand at room temperature for 5 hours to precipitate as calcium oxalate. The precipitate was washed

with hot water and filtered using Whatman filter paper No.1. The precipitate was dissolved in some of amount of 4N H<sub>2</sub>SO<sub>4</sub>. To dissolve the precipitate, the solution was heated and diluted the sample upto 100 ml in Erlenmeyer flask. Take 6 volumetric flasks (100 ml), numbered with 1 to 6 and add 4, 8, 12, 16, 20 ml of 0.1N ammonium oxalate solution. In the 6<sup>th</sup> volumetric flask add 25 ml sample solution. In all the Erlenmeyer flasks add 5 ml dil. sulphuric acid and 30 ml of 0.1 N KMnO<sub>4</sub> solutions. Dilute all solutions upto 100 ml with distilled water. Using colorimeter measured absorbance of all solutions at 530 nm ( $\lambda_{max}$ ).

**RESULTS AND DISCUSSION:**

From the observations, it is clear that oven drying (90.9 %) effectively removes water content from the *Fenugreek* as compared to microwave (83.5 %) and air drying (83.1 %). Thus, oven drying was the best method for preservation as most of the moisture content removed. (Figure 1) During microwave and oven drying, rapid water loss occurs. Thus, the amount of powder prepared after microwave drying (29.7g) and oven drying (16.3g) is relatively less as compared to air drying (30.26g) using the same fresh weight (180 g) of edible portion of fenugreek.

The ash samples obtained from air, oven and microwave drying of Fenugreek leaves were used for the further determination of amount of calcium. The Potassium permanganate titration and complexometric titration method shows good agreement of results of calcium amount in oven and microwave dried samples. (Table 1, entries 2-3) Air dried sample shows the highest calcium amount among the three. (Table 1, entry 1) Further investigation is in progress to investigate the probable reason for the highest amount of calcium is the air dried sample. Instrumental method of colorimetric analysis shows higher amount of calcium as compared to potassium permanganate and

complexometric titrimetric methods.

As compared to leaves, seeds showed the highest amount of calcium. Both Potassium permanganate titration and complexometric titration method shows higher values of calcium, a significant difference in results was obtained. (Table 2, entry 1) As seeds contain negligible amount of moisture, the amount of dried powder was more. Thus, seeds exhibit higher levels of calcium.

### CONCLUSIONS:

In the present study, the potassium permanganate titration and complexometric titration methods did not give significantly different calcium concentrations for Fenugreek leaves and seeds. Air dried samples shows higher amount of calcium as compared to microwave and air dried samples. Either method can, therefore be used for this analysis. Colorimetric analysis shows high amount of calcium as compared to potassium permanganate and complexometric titrimetric methods. From the observations, it is clear that oven drying effectively removes water content as compared to microwave and air drying. Thus, oven drying is the best method for preservation as most of the moisture content is removed from the leaves. Seeds exhibit higher levels of calcium. Although microwave and oven drying are faster and better methods for drying, it is advisable to preserve plant material using air drying method to prevent calcium loss. Further investigation is in progress for to investigate the higher values of calcium by colorimetric method.

### ACKNOWLEDGMENT:

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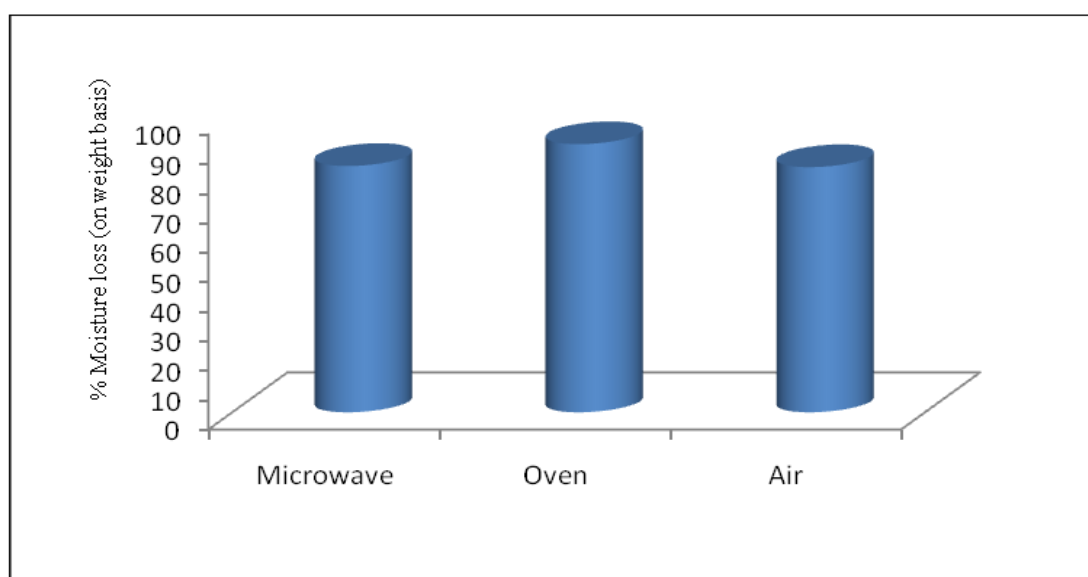


Figure 1: Moisture loss in Fenugreek (*Trigonellafoenum-graecum*) leaves.

No.	Sample (Drying method)	Potassium permanganate Titration (mg of Ca/100 g of edible portion)	Complexometric Titration (mg of Ca/100 g of edible portion)	Colorimetric method (mg of Ca/100 g of edible portion)
1	Air	132.6	164.0	336.5
2	Oven	77.2	63.1	207.0
3	Microwave	78.0	75.0	80.0

Table 1: Calcium estimation of Fenugreek (*Trigonellafoenum-graecum*) leaves.

No.	Sample (Oven dried)	Potassium permanganate Titration (mg of Ca/100 g)	Complexometric Titration (mg of Ca/100 g)
1	Ground seeds	208.6	159.0

Table 2 : Calcium estimation of Fenugreek (*Trigonellafoenum-graecum*) seeds.

## COMPARATIVE EFFECT OF VERMIWASH AND *Azotobacter spp* ON THE GROWTH OF *Vigna radiata*

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### ABSTRACT:

Vermiwash and *Azotobacter spp.* are biofertilizers which are used extensively in agriculture. In present study, comparative analysis of vermiwash and *Azotobacter spp.* on growth of *Vigna radiata* (green gram) was studied. Germination studies were carried out in a sterile setup using plate culture assay having different growth media such as vermiwash, suspension of *Azotobacter spp.* and a mixture of the two. Results showed maximum average plant growth with vermiwash alone, followed by the mixture of vermiwash and *Azotobacter spp.* suspension and the lowest for *Azotobacter spp.* suspension.

To investigate the most effective concentration of vermiwash, the maximum germination and plant growth parameters were investigated using chamber-pot culture assay. Neat vermiwash along with 10%, 20%, 50% concentrations were used as growth media in each pot with a filter paper and cotton substratum. Results revealed maximum growth characteristics for 20% vermiwash. Hence, 20% vermiwash is the ideal concentration at which the biofertilizer was found to have maximum effect on plant growth.

**Keywords:** Vermiwash, *Azotobacter*, Biofertilizer, *Vigna radiata*.

### INTRODUCTION:

Agriculture plays a vital role in India's economy. Over 58 per cent of the rural households depend on agriculture as their principal means of livelihood. Agriculture, along with fisheries and forestry, is one of the largest contributors to the Gross Domestic Product (GDP).

Also, in order to meet the growing requirements of food, maximum output from minimum available agricultural land is essential. To improve the yield and product quality, plants should be provided with optimum nutrients. Fertilization increases efficiency and obtains better quality of product recovery in agricultural activities.

Traditional farming was based on the use of natural fertilizers. However to meet the demand to supply ratio, chemical fertilizers were introduced as they caused tremendous increase in crop yield.

Chemical fertilizers are chemically

synthesized substances composed of known quantities of nitrogen, phosphorus and potassium, which if used indiscriminately causes air and ground water pollution (Bharadwaj *et al.*, 2014). They also destroyed beneficial rhizospheric microflora reduced soil fertility and making the crop more prone to diseases.

Upon comprehending the adverse effects of chemical fertilizers, the focus was shifted back to the use of natural/ biofertilizers. Now a days extensive research is going on to optimize the use of biofertilizers for the development of sustainable agriculture.

Bio-fertilizer consist of living micro-organisms that are agriculturally useful as they accelerate plant growth by converting nutritionally important elements (nitrogen, phosphorus) from unavailable to available form through biological process such as nitrogen fixation and solubilization of rock

phosphate (Rokhzadi *et al.*, 2008; Mohapatra *et al.*, 2013). Biofertilizer is a cost effective, eco-friendly, renewable source of land nutrient which helps in maintaining a long term soil fertility & sustainability (Aggani, 2013). Use of biofertilizers ensures food safety and helps in preserving soil diversity.

*Azotobacter* is a genus of usually motile, large blunt rods, oval cells or cocci that form thick-walled cysts and may produce large quantities of capsular slime. They are aerobic, free-living soil microbes which play an important role in the nitrogen cycle in nature, binding atmospheric nitrogen, which is inaccessible to plants, and releasing it in the form of ammonium ions into the soil. (Amutha *et al.*, 2014).

Vermiwash (VW), a foliar spray, is a liquid biofertilizer collected after the passage of water through a column of worm activation. It is a collection of excretory and secretory, products of earthworm, along with other macro and micronutrients (Elumalai *et al.*, 2015). Macronutrients include nitrogen, phosphorous and potassium whereas the micronutrients include boron, copper, iron, manganese, molybdenum and zinc. These micronutrients are also critically important in plants. Boron is required for cell wall formation, copper is essential for carbohydrate and nitrogen metabolism, iron is necessary for chlorophyll production (Manyuchi *et al.*, 2013)

Besides nutrients, vermiwash contains several beneficial microbes (PGPRs) which help to increase yield, impart resistance to diseases and insect pests, improve drought tolerance and enhance crop quality (Pillai and Sheela, 2015). Plant growth promoting rhizobacteria (PGPRs) can directly facilitate nutrient uptake or increase nutrient availability by nitrogen fixation, solubilization of mineral nutrients, mineralize organic compounds and production of phytohormones such as IAA, cytokinin or by inhibiting the effect of plant growth

inhibitors such as ethylene.

The present study aims at comparing the effect of two previously known biofertilizers i.e. *Azotobacter* and Vermiwash, individually and in combination on the growth of green gram and to determine an ideal concentration of vermiwash for the growth of the same.

## MATERIAL AND METHODS:

### 1. Isolation of *Azotobacter spp.* from soil:

1g soil from soil rhizosphere was collected and inoculated in 100 ml of sterile Ashby's mannitol broth and incubated for one week at room temperature. After 1 week of incubation, scum layer was formed on the surface of the broth. A loopful of this layer was isolated on Sterile Ashby's Mannitol agar plates and these plates were incubated at room temperature for 4 days. After 4 days, dew drop like colonies (characteristic of *Azotobacter spp.*) were observed. The colony was Gram stained and found to be Gram negative rods. These colonies were maintained in pure culture on Sterile Ashby's Mannitol agar slants for further use.

### 2. Seed Sterilization:

Green gram grains were procured from local market, Thane (Maharashtra, India). These seeds were washed with sterile distilled water once and then they were soaked in 0.1% HgCl<sub>2</sub> for 4-5 minutes. Then the seeds were washed thrice in sterile distilled water and soaked in it for 15 minutes. These seeds were then used for plate culture and chamber pot assay.

### 3. Collection of Vermiwash:

Vermiwash was collected from vermicompost setup, B. N. Bandodkar College of Science, Thane (Maharashtra, India). Vermiwash was then filtered using Whatman's filter paper no. 41 and stored at 4°C for further use.

#### 4. Plate culture assay:

System of sterile petri plate containing circular filter paper padding was used for the plate culture assay. One surface sterilized seed was placed in each petri plate. In one system, 5ml of vermiwash was added, in second system 5ml of saline suspension of *Azotobacter spp.* was added and in the third system, 2.5ml saline suspension of *Azotobacter spp.* and 2.5ml vermiwash was added. Addition of biofertilizers (i.e. *Azotobacter spp.* and vermiwash) was done only in the beginning. A system containing distilled water was kept as control. To analyze the effect of these biofertilizers, length of root and shoot was measured on each day up to 6 days in laminar flow. The assay was carried out in triplicates.

#### 5. Pot culture assay:

Different dilutions of vermiwash (10%, 20%, 50%, and 100%) were prepared using sterile distilled water. Glass chamber-pots were disinfected using absolute alcohol. Filter paper and cotton were used as substratum. Ten surface-disinfected seeds of *Vigna radiata* were placed aseptically in each pot. 100ml of the different dilutions of vermiwash were used as growth media in each pot. The pots were incubated at room temperature with a photo-period of 16 hours. Addition of growth media was done only in the beginning and then length of root and shoot was measured using Vernier calipers on each day up to 6 days. A system containing distilled water was kept as control.

### RESULT AND DISCUSSION:

#### 1. Effect of *Azotobacter* (A), Vermiwash (V) and their combination (A+V) on growth of *Vigna radiata* :

Maximum plant growth was observed for the vermiwash (V), followed by distilled water, mixture of the two biofertilizers (A+V) and least for saline suspension of *Azotobacter spp.* (A).

Vermiwash is known to contain phytohormones like IAA, micro and macro nutrients and PGPRs which promote the growth of plant thus seeds placed in vermiwash showed maximum growth.

Similar results were obtained by Varghese and Prabha, (2014) which demonstrated that treatment of *Capsium frutescens* with vermiwash showed increased root and shoot length as well as number of leaves than the vermiwash untreated plant. Also, Rajan and Murugesan (2012) observed that vermiwash increased the germination rate, shoot and root length of *Vigna unguiculata* (Cow Pea) and *Oryza sativa* (Rice).

As *Azotobacter spp.* has a very high respiratory rate, hence it is speculated to compete with the plant for nutrients and available oxygen in a closed system. Thus Plant growing in the medium containing mixture of *Azotobacter spp.* and vermiwash showed relatively lesser growth. In case of seed placed in the medium containing only saline suspension of *Azotobacter spp.* no significant plant growth was observed as this media do not contain any nutrient for plant growth.

Vermiwash can be used as foliar spray. On-field application of vermiwash is carried out using dilutions. Therefore, application of undiluted vermiwash might affect germination of seeds as it is well-known that germinating seeds are much more sensitive to over-fertilization as the young root system is very sensitive to fertilizer burns. Since undiluted vermiwash is used in this study, hence its PGP effect is only slightly higher than distilled water. Therefore several dilution of vermiwash was used for further study.

#### 2. Effect of different concentrations of vermiwash on the growth of *Vigna radiata*.

Maximum plant growth was observed for the 20% vermiwash followed by 100%, distilled water, 10% and least for 50%.

Plants require micronutrients always in low amounts while their moderate decrease causes the deficiency symptoms, there moderate increase causes toxicity. Thus there is a narrow range of concentration at which the elements are optimum. Eg. Toxic effects of Cr on plant growth and development include alterations in the germination process as well as in the growth of roots, stems and leaves, which may affect total dry matter production and yield. Cr also causes deleterious effects on plant physiological processes such as photosynthesis, water relations and mineral nutrition (Shanker *et al.*, 2005)

Similar to micronutrient plants require phytohormone (present in vermiwash) in optimum concentration and increase in their concentration lead to inhibitory effect in spite of their growth promoting activity. Eg. Auxin at higher concentration triggers biosynthesis of Abscissic acid thus show inhibitory effect (Hansen and Grossmann, 2000)

PGPRs are slow growing organisms when present in 100% vermiwash other undesired bacterial weeds overgrowth PGPRs. Thus suppresses the beneficial effect PGPRs however in diluted vermiwash the limiting nutrients conditions being unfavorable for the bacterial weeds, allow slow growing PGPRs to flourish and thus they might show plant growth promoting activity in diluted medium.

After 2 days of germination, it was observed that in the system containing distilled water (control), plant growth was less than that observed for 20% vermiwash. The cotyledon, which forms in the embryo of seed before germination, stores food for the embryo. Along with the endosperm, the cotyledon nourishes the growth of the new plant. Hence, germination occurred also in the presence of distilled water; however fertilizers would be required for growth enhancement once the leaves began to appear.

## CONCLUSION:

The aim of the present study was to compare the PGP activity of two known biofertilizer i.e. *Azotobacter* and vermiwash on the growth of *Vigna radiata* and to determine the ideal concentration of vermiwash for the same. It was found that vermiwash alone shows high PGP activity with its optimal concentration to be at 20% which is may be due to the presence of various macro and micronutrients and phytohormones in optimal concentration. Also, it can be speculated that 20% dilution of vermiwash might have enhanced PGPR growth and activity.

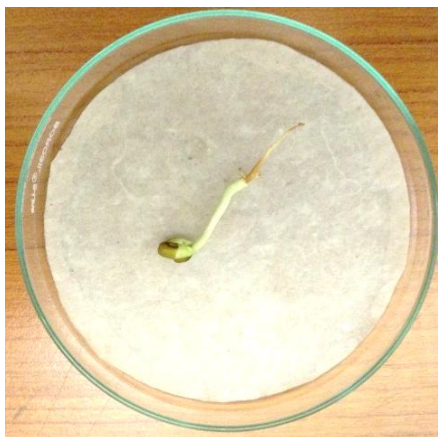
The major challenge lies in the isolation and identification of PGPRs along with the analysis of their growth enhancing properties. Isolation of these PGPRs will help in enhancing the efficiency of biofertilizers for the development of sustainable agriculture.

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(a) *Azotobacter* spp.



(b) Vermiwash



(c) *Azotobacter* spp. + Vermiwash

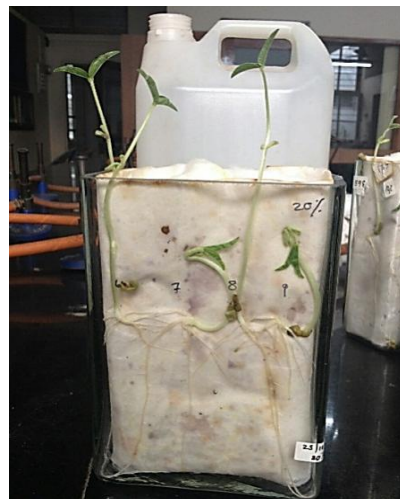


(d) Distilled water

**Fig.1: Plate culture assay.**



(a) 10% Vermiwash



(b) 20% Vermiwash



(c) 50% Vermiwash

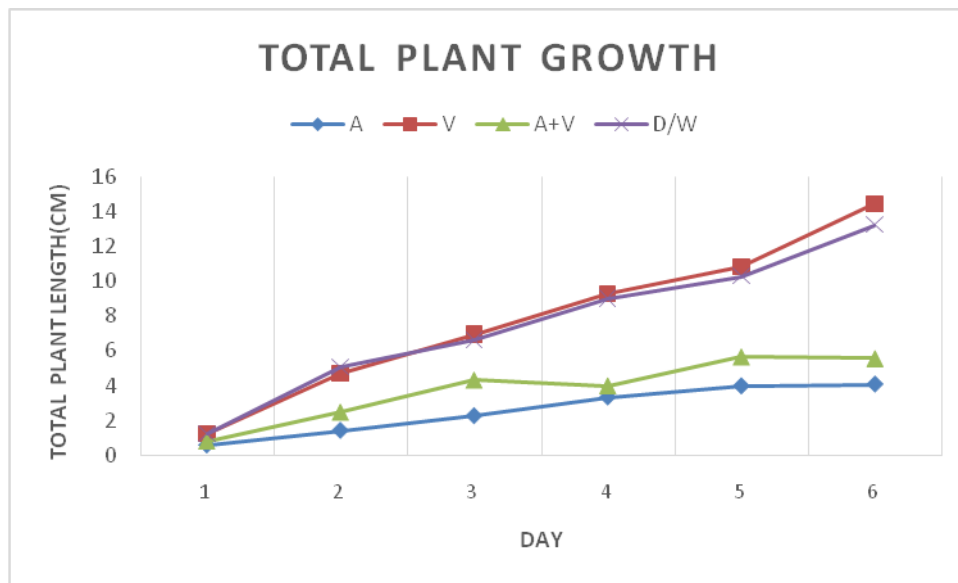


(d) 100% Vermiwash

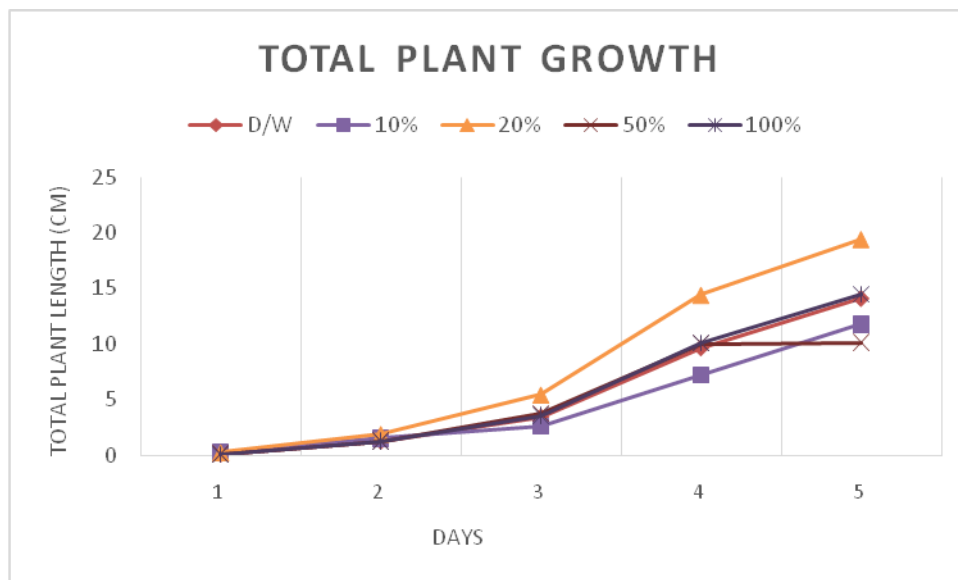


(e) Distilled Water

**Fig.2: Pot Culture assay.**



**Fig. 3: Effect of Azotobacter (A), Vermiwash (V) and their combination (A+V) on growth of *Vigna radiata*.**



**Fig. 4: Effect of different concentrations of vermiwash on the growth of *Vigna radiata*.**

## GREEN SYNTHESIS OF IRON NANOPARTICLES USING *Azadirachta indica* AND ITS APPLICATIONS

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### ABSTRACT:

Nanotechnology has immense potential in the fields of biotechnology and agricultural industries. Nanoparticles have unique physicochemical properties like high surface area and reactivity and thus are widely used to overcome the problems of environmental pollutions, caused by toxic metal ions, radionuclides, organic and inorganic solutes, nanocapsules for efficient delivery of pesticides, fertilizers and other agrichemicals, nanosensors for detection of animal and plant pathogens, etc. One of the major applications of nanotechnology is in enhancing the growth of plants.

This article presents an overview on the efficiency of iron nanoparticles [Fe NPs] prepared from *Azadirachta indica* (neem) in enhancing the growth of moong plant [*Vigna radiata*]. The plant extract was prepared from neem leaves and the above was treated with 0.008% ferric chloride solution. The formation of Fe NPs was confirmed by observing the colour change from yellow to greenish black under boiling conditions. Different concentrations of the Fe NPs solution [100, 200, 500, 1000 and 2000ppm] were prepared to check their efficiency in enhancing the growth of moong plant. The root lengths were measured and the growth of the plant was seen to be highest in case of 1000 ppm on comparison with the control group. The future prospects of this study include testing the efficiency of Fe NPs prepared by green synthesis in bioremediation of heavy metals in soil.

**Key words:** Green Synthesis, Iron nanoparticles, *Vigna radiata*, *Azadirachta indica*, Bioremediation.

### INTRODUCTION:

Nanotechnology opens a large scope of novel applications in the field of biotechnology and agricultural industries because NPs have unique physicochemical properties i.e. high surface area, high reactivity, tunable pore size and particle morphology (Manzer S., *et al.*, 2015). Nanotechnology a new emerging and fascinating field of science permits advanced research in many areas, and nanotechnological discoveries could open up novel applications in the field of biotechnology and agriculture. Although fertilizers are very important for plant growth and development most of the applied fertilisers are rendered unavailable to plants due to many factors such as leaching degradation by photolysis, hydrolysis and decomposition. Hence it is necessary to minimise nutrient losses in fertilisation and to

increase the crop yield through exploitation of new applications with the help of nanotechnology and nanomaterials. Nanotechnology has large potential to provide an opportunity for the researchers of plant science and other fields to develop new tools for incorporation of nanoparticles into plants that could supplement existing functions and add new ones. In the present study the recent developments in plant science that focuses on the role of nanoparticles in plant growth and development and also on plant metabolism. Nanoparticles interact with plants causing potent effect in the growth of the plants. Many researchers from their findings suggested both positive and negative effects on plant growth and development, and the impact of engineered nanoparticles (ENPs) on plants

depends on the composition, concentration, size, and physical and chemical properties of ENPs as well as plant species (Ma X. *et al.*, 2010).

In recent years, the development of efficient green chemistry methods for synthesis of metal nanoparticles has become a major focus of researchers. They have investigated in order to find an eco-friendly technique for production of well-characterized nanoparticles. Nanoparticles produced by plants are more stable and the rate of synthesis is faster than in the case of microorganisms. Moreover, the nanoparticles are more varied in shape and size in comparison with those produced by other organisms (Ramesh P. *et al.*, 2014), which can be studied by scanning electron microscopy. Extracts from plants act as both reducing and capping agents in nanoparticle synthesis (Iravani S., 2011), (Shakeel A. *et al.*, 2016). The advantages of using plant and plant-derived materials for biosynthesis of metal nanoparticles have interested researchers to investigate mechanisms of metal ions uptake and bioreduction by plants, and to understand the possible mechanism of metal nanoparticle formation in plants.

Various nanoparticles including gold, silver, silicon dioxide, iron, zinc (Devi S. & Gayathri R., 2014) synthesized by green method are utilised in the field of nanotechnology. Iron as a metal has many feasible effects in the field of nanotechnology. Iron's reactivity is important in macroscopic applications (particularly rusting), but is a dominant concern at the nanoscale. Finely divided iron has long been known to be pyrophoric, which is a major reason that iron nanoparticles have not been more fully studied to date. This extreme reactivity has traditionally made iron nanoparticles difficult to study and inconvenient for practical applications. Iron however has a great deal to offer at the nanoscale, including very potent magnetic and catalytic properties. (Huber D., 2014) The biological approach of synthesis of Fe NPs as

described in the present paper using plant extracts appears to be ecofriendly and cost effective alternative to conventional chemical and physical methods and would be suitable for large-scale production. Nanomaterials can be used in treatment of various contaminated media by chemically transforming contaminants or acting as a “super adsorbent” for many compounds (Khan I *et al.*, 2014).

Various chemical, physical and biological synthetic methods have been used in production of metal nanoparticles. Most of these methods are still in the development stage and the problems experienced are stability and aggregation of nanoparticles, control of crystal growth, morphology, size and size distribution. Moreover, separation of produced nanoparticles for further applications is still an important issue.

## **MATERIALS AND METHODS:**

Fresh *Azadirachta indica* (neem leaves) were purchased from a local market at Thane. The leaves were washed thrice using tap water and again washed thrice using distilled water to remove the adhering soil and dust. Further the leaves were exposed under sunlight until they dried completely. They were cut into fine pieces using sterile blade that was sterilised using alcohol and crushed into fine powder using mortar and pestle.

### **Preparation of plant extract using *Azadirachta indica***

100 grams of dried powder were heated at 80°C in 100ml distilled water. The following extract was cooled and filtered through Whatman No. 1 filter paper to obtain the broth. The pH of the extract was found to be 4.10 which was slightly acidic. The extract was stored at 4°C until use. (Shah S. *et al.*, 2014)

### **Preparation of Fe NPs using plant extract**

5 ml of the plant extract was added to 5 ml of 0.001M Ferric Chloride at a temperature of about 50°C - 60°C (Pattanayak M. and Nayak P., 2013). A change in colour from faint yellow

to brownish yellow and finally greenish black was observed which confirmed the formation of Fe NPs.

The crushed materials were then boiled with 100 mL distilled water at 80°C for 5- 10 minutes, cooled and filtered using Whatman No. 1 filter paper thrice. 20mL of the prepared plant extract was added to 20 ml of 0.001 M Ferric Chloride at a temperature of about 50-60°C (Pattanayak M. and Nayak P., 2013). A change in colour from faint yellow to greenish black after certain time period of time indicates the formation of Fe NPs. Further the preparation of different concentration of Fe NPs was carried out. There were a total of eight sets maintained. These included 100, 200, 500, 1000, 2000ppm solutions, a control group of plant extract, and a compound control of FeCl<sub>3</sub> (500 ppm) that were maintained. The compound control was prepared by dissolving 0.05 grams of ferric chloride in 100ml of distilled water. These sets were then then tested for its efficiency in enhancing the growth of moong plant.

### Moong Seed Sterilisation

The moong seeds were washed in 5 percent liquid detergent [Tween 20] for ten to fifteen minutes. Further these seeds were washed with sterile distilled water thrice. The distilled water was decanted and the seeds were further treated with 0.1 percent mercuric chloride and swirled intermittently. Further the seeds were again washed with sterile distilled water. The washing step was repeated three times to ensure sterilisation step to be effective. The seeds were further soaked in sterile distilled water for 30 minutes. These seeds were further inoculated into sterile petri plates containing Whatman filter paper, into which 7 ml of the prepared concentration of solutions were added, till the filter paper soaked completely.

### RESULTS:

The results obtained were recorded at an interval of twenty four hours.

Day 1: Table (1)

Day 2: Table (2)

### DISCUSSION:

The results were recorded in an interval of twenty four hours. There were two sets of petri plates and one set of bumper tube kept for each concentration. After addition of the nanoparticle solution into the respective petriplates and sterile bumper tube the efficiency of the nanoparticle solution in enhancing the growth of moong seeds were studied, measuring the root length. The results obtained revealed that the concentration of 1000ppm showed efficient enhancement in the growth of moong seeds compared to the control group and other sets. The results proved that leaves extract of *Azadirachta indica* were efficient in capping the Fe ions (Irvani S., 2011). Nanoparticle solution prepared from plant extract have various benefits as it is an easy, cost effective and safe green method in scale up and industrial production of metal nanoparticles. Thus plant extracts are considered certainly better than plant biomass or living plants and are valuable in environmental, biotechnological and biomedical application (Ramesh P. *et al.*, 2014). The future prospects of this study include testing the efficiency of Fe NPs prepared by green synthesis in bioremediation of heavy metals in soil [Stephens B., 2010] and characterization of Fe NPs by Scanning electron microscopy (Al-Saad K. *et al.*, 2012), uv-visible spectroscopy and FTIR analysis, (Banerjee P. *et al.*, 2014). Further the arena can be widened by green synthesis of various nanoparticles and carrying out a comparative study in which nanoparticle can be used efficiently in reducing the heavy metal concentration in the soil and in water purification (Namratha N. and Monica P., 2013), (Achintya N. *et al.*, 2009). They also play a promising and vital role in the development of rapid and precise environmental sensors which can be used in

the detection of pollutants at molecular levels and also for inactivating harmful bacteria (Stephens B., 2010). The antimicrobial testing of the nanoparticles prepared from green synthesis is an area of wide scope and thus the efficiency of Fe NPs prepared from green synthesis can be checked against the plant pathogen.

### CONCLUSION:

In summary the present work provides a cheap, cost effective, simple method for the preparation of Fe NPs by green synthesis using *Azadirachta indica* which aided in enhancing the growth of moong seeds efficiently and also has a number of promising potential applications in the field of agricultural biotechnology.

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Concentration (ppm)	Sterile bumpertube/ Sterile petriplate	Root length in centimetres (cms)								Average Shoot length (cms)
		Set 1				Set 2				
		Seed 1	Seed 2	Seed 3	Seed 4	Seed 5	Seed 6	Seed 7	Seed 8	
Control	Sterile bumpertube	0.3	-	-	-	-	-	-	-	0.3
Control	Sterile petriplate	1.4	0.1	-	-	-	-	-	-	0.75
FeCl <sub>3</sub>	Sterile bumpertube	1.4	-	-	-	-	-	-	-	1.4
FeCl <sub>3</sub>	Sterile petriplate	1.7	1.1	1.7	1.2	-	-	-	-	1.42
10 ppm	Sterile bumpertube	0.2	-	-	-	-	-	-	-	0.2
10 ppm	Sterile petriplate	1.3	0.8	0.8	1.2	-	1.3	1.2	-	1.08
100 ppm	Sterile bumpertube	-	-	-	-	-	-	-	-	-
100ppm	Sterile petriplate	-	1.6	0.5	-	2.5	-	0.3	0.9	1.16
500 ppm	Sterile bumpertube	1.1	-	-	-	-	-	-	-	1.1
500 ppm	Sterile petriplate	1.0	0.7	1.3	2.1	0.8	0.9	1.4	-	1.17
1000 ppm	Sterile bumpertube	0.4	-	-	-	-	-	-	-	0.4
1000 ppm	Sterile petriplate	0.6	2.0	1.6	1.8	-	1.6	1.3	-	1.48
2000 ppm	Sterile bumpertube	0.6	-	-	-	-	-	-	-	0.6
2000 ppm	Sterile petriplate	1.8	1.6	0.6	1.6	0.6	1.1	1.6	1.9	1.38

**Day 1: Table (1)**

Concentration (ppm)	Sterile bumpertube/ Sterile petriplate	Root length in centimetres (cms)								Average Shoot length (cms)
		Set 1				Set 2				
		Seed 1	Seed 2	Seed 3	Seed 4	Seed 5	Seed 6	Seed 7	Seed 8	
Control	Sterile bumpertube	2.6	-	-	-	-	-	-	-	2.6
Control	Sterile petriplate	7.1	1.5	-	-	-	-	-	-	4.3
FeCl <sub>3</sub>	Sterile bumpertube	1.2	-	-	-	-	-	-	-	1.2
FeCl <sub>3</sub>	Sterile petriplate	5.9	5.2	5.4	5.7	-	-	-	-	5.55
10 ppm	Sterile bumpertube	1.2	-	-	-	-	-	-	-	1.2
10 ppm	Sterile petriplate	5.0	4.2	3.6	4.3	1.8	7.0	4.8	-	3.83
100 ppm	Sterile bumpertube	-	-	-	-	-	-	-	-	-
100ppm	Sterile petriplate	1.2	-	7.5	-	-	6.6	2.5	-	4.45
500 ppm	Sterile bumpertube	2.3	-	-	-	-	-	-	-	2.3
500 ppm	Sterile petriplate	7.5	2.1	9.5	5.3	7.2	4.5	1.3	-	4.67
1000 ppm	Sterile bumpertube	8.9	-	-	-	-	-	-	-	8.9
1000 ppm	Sterile petriplate	4.5	7.0	9.0	6.3	6.4	9.0	-	-	7.03
2000 ppm	Sterile bumpertube	4.5	-	-	-	-	-	-	-	4.5
2000 ppm	Sterile petriplate	7.3	8.3	5.0	11.3	4.1	7.2	8.4	4.0	6.95

Day 2: Table (2)

## USE OF NEEM COATED UREA AS A EFFICIENT FERTILIZER

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### ABSTRACT:

Nitrogen is the most critical nutrient which is required for the growth of crop and this nitrogen is provided by urea as it is a rich source of nitrogen. However 50% of nitrogen provided by urea is lost due to nitrification. Solution to this problem of nitrogen loss is to coat the urea with a substance that can prevent the bacterial activity of nitrification. Neem oil is spread on the urea to produce neem coated urea. Neem has a proven nitrification inhibition property. This way, it slows down the process of nitrogen release from urea. It is best nitrification inhibiting agent. So neem coated urea can be used than that of normal urea.

### INTRODUCTION:

Food production needs to increase to satisfy the demand due to increasing human population worldwide. To minimize these food crises, an increase in the rice production is necessary. The current study was undertaken to evaluate the impact of neem coated urea on rice (*Oryza sativa*). It is a staple food for 2.5 billion people. It is the gain with second highest world wide population. The normal urea has played a key role in increasing rice production in Asia yet it is efficient to only 30-40% or even less because about 30-50% of nitrogen applied is lost by runoff. So the present study was carried out to find out the efficiency of formulation of neem coated urea (an indigenous material with nitrification inhibitor properties).

### MATERIALS AND METHODS:

The field experiment was conducted at a small portion of agricultural land at panvel during rainy season (June to October) of 2016. The soil of the experimental plot was sandy loam with pH=7.3.

Experimental design and treatments: Pusa neem oil micro emulsion coated urea (PNME), Tri neem coated urea (TNU) were prepared by coating with 2% neem cake powder by weight of urea with help of coater and kerosene (ratio=1:2) at a rate of 2ml/100g of urea. The thirty day old seedling was

transplanted at the field on July 24, 2016.

Field technique: The whole quantity of nitrogenous fertilizer was applied at one time just after transplanting the rice seedlings. Before harvesting, the growth parameters & yield attributes of rice were recorded. The rice crop was harvested in second last week of October. The harvested rice was sun dried for 4 days in the fields, after which total biomass yield was recorded. After threshing, cleaning and drying, the grain yield was obtained by:

$$\text{STRAW YIELD} = \text{TOTAL BIOMASS YIELD} - \text{GRAIN YIELD}$$

Laboratory chemical analysis: Laboratory chemical analysis involved the calculation of agronomic N use efficiency and apparent N recovery.

$$\text{a) NUE} = \frac{\text{GRAIN YIELD (F)} - \text{GRAIN YIELD (C)}}{\text{FERTILIZER N APPLIED}}$$

Where F=fertilized plot & C=control plot

$$\text{b) APPARENT N RECOVERY \%} = \frac{\text{N(T)} - \text{N(o)}}{\text{N(a)}}$$

where, N(t)=amount of nitrogen taken up from test plot

N(o)=amount of nitrogen taken up from control plot

N(a)=amount of nitrogen added

### RESULTS AND DISCUSSION:

The factors such as plant height, no. of effective tiller, panicle length, panicle weight was much greater due to use of neem coated urea, which is explained in table no.1.

Grain & straw yields: nitrogen application significantly increased the grain and straw yields and the straw yields were recorded with highest level of nitrogen. Both the neem coated materials produced higher grain & straw yield in rice than prilled urea. The urea coated with neem formulations gave 12-13% higher rice yield than uncoated urea (table no. 2).

### CONCLUSION:

Increasing levels of N increased the nitrogen uptake significantly & each successive

increment of N uptake over the preleading levels of N. As regards the sources of nitrogen, TNU & PNME resulted in significantly more grain, straw & total N uptake than PU.

ORDER: TNU>PNME>PU. The study suggests that to achieve higher rice yields, the rice should be grown at higher level of nitrogen with use of indigenous coating materials such as neem.

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Treatment	Plant height	No. of effective tiller	Panicle length(cm)	Panicle weight(g)
Prilled urea	89.8	6.7	24.7	2.07
PNME	89.9	6.9	26.0	2.10
TNU	90.2	7.0	26.3	2.16

**Table1: Growth and yield attributes influenced by modified urea material**

Treatment	Grain yield	Straw yield	Grain uptake	Straw uptake	total	Agronomic "n" use efficiency
Prilled urea	32.9	614.6	33.8	36.4	70.2	3.47
PNME	34.6	64.8	36.4	40.6	76.0	4.70
TNU	35.0	65.8	37.1	42.0	76.0	4.69

**Table2: Yields, N recovery, agronomic N use efficiency & apparent N recovery % as influenced by modified urea materials.**

**POLLUTION IMPACT STUDY USING ASPARTATE AMINOTRANSFERASE (AspAT) AND ALANINE AMINOTRANSFERASE (AlaAT) AS A MOLECULAR BIOMARKER IN MUSCLE AND LIVER TISSUES OF *Harpodon nehereus* COLLECTED FROM WELL KNOWN FISH LANDING CENTER SASSOON DOCK, MUMBAI.**

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**ABSTRACT:**

Fish is a most valuable bio-monitor of environmental pollution. Continuous contamination by heavy metals and organic pollutants in the marine environment is a severe problem in present world. *Harpodon nehereus* commonly called as Bombay duck is one of the famous mostly consumed estuarine fish of west coast of India. The study on disturbance in enzyme AspAT and AlaAT with respect to a change in environment makes the attractive molecular marker of stress. The present study aims to analyse the levels of enzymes Aspartate aminotransferase (AspAT) and Alanine aminotransferase (AlaAT) in the muscle and liver tissues of *Harpodon nehereus* collected from Sassoon dock, Mumbai coast of Maharashtra. The level of AspAT in the muscle and liver tissues was found to be 7.15 KA (IU/ L) and 2.14 KA (IU/ L) respectively. AlaAT levels in the muscle and liver tissues was found to be around 4.8 KA (IU/ L) and 7.43 KA (IU/ L) respectively. When compared to normal reference values of various studies on enzyme activity, it was found that there is a decrease in AspAT and AlaAT values which indicates that the functions of liver and muscle tissues are affected due to man-made pollution stress.

**Keywords:** *Harpodon nehereus*, AspAT, AlaAT, Sassoon dock, Pollution stress.

**INTRODUCTION:**

Fishes are the most affected organisms due to marine pollution and this has become a severe problem in the present world. *Harpodon nehereus* (Bombay duck) is one of the famous and most common marine fish of west coast of Maharashtra constituting about 10% of the total marine fish landings (Fig. 1). It is widely accepted as sea food and provides a good source of nutrition to common people of west coast of Maharashtra. It is the only species of the Family: Scopelidae forming a major fishery along the West Coast of India including states of Gujarat and Maharashtra. Sassoon dock is the oldest and most popular fish

landing centre of Mumbai from where most of the sea foods are supplied throughout the Mumbai region. Pollution in the marine environment affects the existence and metabolic activity of aquatic organisms. Heavy metals are known for their strong effect on biological tissues. Metal ions once absorbed in to the body are capable of reacting with variety of active binding sites and disturbing the normal physiology of an organism which may lead to the death of the organisms (M. Babu *et al.*, 2016). The most important factor of metabolic activities of an aquatic organism is its enzymes. Enzymes determine the major

metabolic activities of a cell and thus of an organism. The study on enzyme with respect to a change in environment makes the attractive index of stress, hence, could be due to this reason enzyme analysis are becoming increasingly important for the determination of toxic effects of chemical pollutants in the field of environmental toxicology (Leena Muralidharan, 2014). Transaminases are important enzymes known to play a key role in mobilizing L-amino acids for gluconeogenesis and function as links between carbohydrate and protein metabolism under altered physiological, pathological conditions (K AnusiyaDevi, et al. 2016; Prashanth, 2012; Shwetha *et al.*, 2012). The quantitative estimation of enzyme helps to evaluate the health status of the organism and thus the extent of pollution in their surroundings. The enzyme Aspartate aminotransferases (AspAT) and Alanine aminotransferase (AlaAT) are enzymes involved in protein metabolism. AspAT are the most active and widely distributed transaminase. AlaAT are active and more abundant in liver compared to other tissue (Leena Muralidharan, 2014). The variation in the activity of these marker enzymes affects the growth and development of an organism (Osman *et al.*, 2010; Prashanth, 2012). These enzymes are also sensitive to metal pollution thus indicating the toxic effects of metal pollution in the marine environment. In the present study the two enzymes were assessed to evaluate the toxic effects of various kinds of man-made pollutants and heavy metals in the marine ecosystem.



**Fig. 1: Fresh *Harpodon* species collected from Sassoan dock, Mumbai**

## **MATERIALS AND METHODS:**

The fresh fishes measuring 26-30cm and 160-180gm were collected from Sassoan dock during the post monsoon season (Oct- Nov, 2016), kept in ice bags and brought to the laboratory. Fishes were dissected to remove the muscle and liver tissues. The tissues were washed with chilled distilled water and 10% homogenate was prepared in 0.9% saline. The analysis of enzyme AspAT and AlaAT was done by Reitman and Frankels Method (1957). The optical density was measured at 510 nm on a colorimeter (green filter). Entire experiment including additions of chemicals was performed in a sterile condition and the chemicals used for the analysis were of AR grade.

## **RESULTS AND DISCUSSION:**

AspAT and AlaAT are enzymes frequently used in diagnosis of damage caused by pollutants in various tissues (De La Torre *et al.*, 2000; M. Babu. *et al.*, 2016). Pollution of the aquatic ecosystem stresses the animals and disturbs their metabolism by altering the enzyme activity, damage and dysfunction the tissues and hindering growth all that associated with biochemical changes (Osman *et al.*, 2010). Analysis of biochemical parameters could help to identify the target organs of toxicity as well as the general health status of animals. It may also provide an early warning signal in stressed organism (David *et al.*, 2010; Prashanth, 2012). In the present study, activity of AspAT and AlaAT decreased significantly in both, the muscle and liver tissues of *Harpodon nehereus* (Table 1). Similar inhibitory effects of the above mentioned enzymes exposed to toxicants were observed by, Verma. *et al.* (1976; 1981); Joshi (1978) and Gabriel *et al.* (2012). They were due to cellular disturbance and damage to these organs causing leakage of these enzymes from the tissue into circulatory system. Isreal (2012) reported biochemical changes in certain tissues of *Cirrhinamrigala*

(Hamilton) (Cyprinidae: Cypriniformes) exposed to fenthion. Baby Joseph (2011) analysed the effect toxicity of pesticide on selected biomarkers in fishes. Abilash (2005) studied toxic, physico-morphological and behavioural responses of *Oreochromis mossambicus* exposed to commercial grade endosulfan and reported alterations in the cellular morphology of pesticide treated fish. Satyanarayan (2004) studied impact of some chlorinated pesticides on the haematology of the fish *Cyprinus carpio* and *Puntius ticto*. Dhasharathan (2000) studied about the effect of endosulfan and butachlor on the digestive enzyme and proximate composition of the fish, *cyprinus carpio*. Thus with agreement to the above research work done by various authors and the decreased levels of enzyme AspAT and AlaAT from present investigation it may be clear that the liver and muscle tissues of fish *Harpodon nehereus* are affected due to pollution stress.

### CONCLUSION:

Decreased values of AspAT and AlaAT are due to tissue damage probably caused by various toxicants present in the marine environment. Thus from the above result and discussion it can be concluded that there is damaged caused to the tissues of fish *Harpodon nehereus* collected from Sassoon dock, Mumbai due to various kinds of pollutants in the marine ecosystem. The present study was carried out so that the result from the above investigation will help to keep a continuous check on pollution stress by reducing pollution so that consumable organisms are not affected and disturbance in the aquatic ecosystem caused by various anthropogenic activities could be prevented.

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Month (post-monsoon)	Enzymes			
	Aspartate aminotransferase		Alanine aminotransferase	
	Liver	Muscle	Liver	Muscle
October- November 2016	2.14 IU/ L ±0.02	7.14 IU/ L ±0.01	7.43 IU/ L ±0.01	4.8 IU/ L ±0.02

Values are expressed as Mean± Standard deviation

**Table 1: Changes in the activity of enzymeAspAT and AlaAT in the muscle and liver tissues of *Harpodon nehereus* collected from Sassoon dock Mumbai**





# SECTION III

## SHORT COMMUNICATIONS



## **SYNTHESIS OF SILVER NANOPARTICLES CAPPED IN STARCH AND THEIR EFFECT ON SOIL MICROFLORA AND SOIL EXOENZYME ACTIVITY**

*Aryan Patel, Onkar Kadam and Dr. Shubhada Walvekar*

*Biotechnology Department, Mithibai College.*

### **ABSTRACT:**

The study involves rapid formation of silver nanoparticles through chemical reduction capped in Ragi starch. Starch stabilized the particles in aqueous medium. The particles were monitored using UV-visible spectrophotometer. The effect of these particles on soil microflora was determined by serial dilution method for a period of 3 days. The effect of these nanoparticles on soil exoenzyme activity was studied for a period of 30 days and 45 days.

**Keywords** - silver nanoparticles, starch, soil exoenzyme action

### **INTRODUCTION:**

Silver is well known for its health benefits as well as for its medicinal properties. The use of silver in the form of its nanoparticles is an accepted method in medicine and pharmaceutical industry (Ihegwuagu 2014). The antimicrobial effects of these nanoparticles are well known. Silver nanoparticles have applications in the fields of sewage treatment, improvement in quality of the environment, removal of dyes etc. With increasing focus on green synthesis of nanoparticles the natural compounds like starch, sugar have gained importance as safe reducing and capping agents for silver nanoparticles (Amin, 2012). Silver is safe for animal cells but it is highly toxic to bacteria and fungi (Golda, 2012). Biogenic synthesis of these particles is on soil health and its exoenzyme activity is studied in the present study. Silver nanoparticles capped with starch from ragi were prepared and their effects on soil phosphatase activity was studied in the present work.

(Bryaskova, 2011) with little modifications. The reagents used were of analytical grade. Ragi powder was purchased from local market. Starch was obtained after removal of proteins using standard methods. The solution of 1% silver nitrate and reducing agent were mixed in the proportion of 1:4. The mixture was microwaved at 100% power for 1 min. The complex was cooled and centrifuged at 10,000rpm for 10 min. The UV absorption spectrum was noted for the particles to obtain the absorption maxima.

The effect of these particles was studied on soil bacterial flora using serial dilution method up to  $10^{-6}$  dilution. 5ml of nanoparticle solution was added in each pot of soil. The colony count and enzyme activity was estimated after a period of 30 days and 45 days. The effect of the nanoparticles on soil phosphatase activity was estimated qualitatively for the period of 30 days and 45 days, comparing it with the positive and negative control solutions (Tatabhai, 1969).

### **MATERIALS AND METHODS:**

The synthesis of silver nanoparticles was carried according to the method suggested by

### **OBSERVATIONS:**

Synthesis of nanoparticles was indicated by the solution turning brown due to reduction of

silver from silver nitrate. The UV absorption spectrum showed absorption maxima at 470 nm.

The effect of silver nanoparticles on soil bacterial flora showed gradual reduction in number of colonies in the period of 30 days and 45 days. This reflects the bactericidal effect of silver on bacterial cells.

Soil exoenzymes represent bacterial activities of soil. The reduction of bacterial colonies was reflected in the analysis of phosphatase activity. The activity was observed to be reduced in both the observations as compared to the control. The intensity of yellow colour developed was reduced in the treated samples than the control sample. The activity was estimated qualitatively comparing the positive and negative control tubes.

## CONCLUSION:

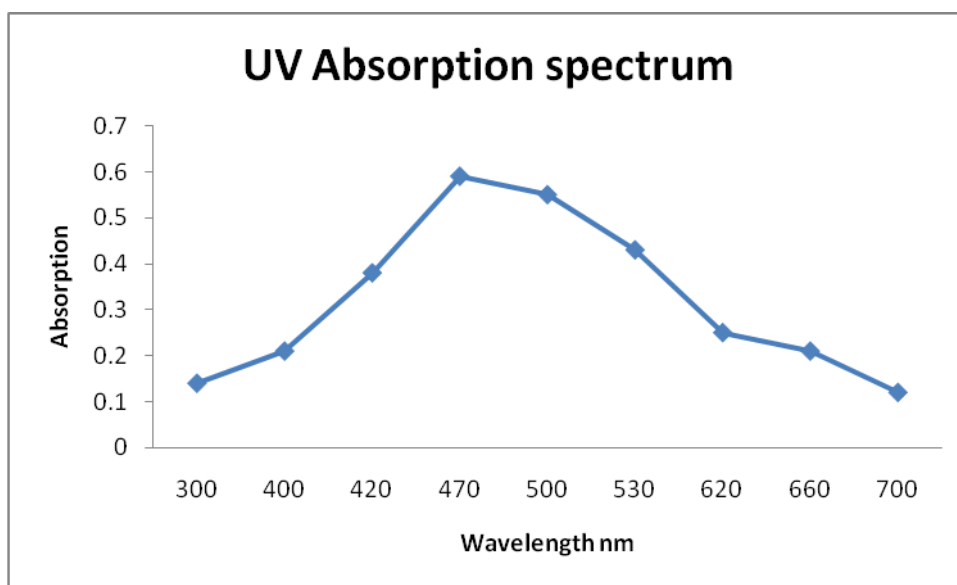
The starch capped silver nanoparticles were found to be effective against bacterial growth of oil. The activity of phosphatase enzyme activity was also reduced after the addition of the nanoparticle solution. It indicated that the bactericidal effect of silver remains unaltered after capping it with starch. This method could have applications in getting rid of soil pathogens as well as in pharmaceutical industry where the bactericidal action gains significant importance in development of new pharmaceuticals.

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No. of colonies	10 <sup>-4</sup>	10 <sup>-5</sup>	10 <sup>-6</sup>
Control	54	39	23
30 days	52	28	14
45 days	49	18	09

**Table 1. Number of bacterial colonies in control and nanoparticle treated soil:**



**Figure 1. -Absorption maxima at 440 nm:**

## MICROFLORA ASSOCIATED WITH HYDROPONIC SYSTEMS

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### ABSTRACT:

Hydroponics is a viable method of producing vegetables, crops and foliage plants. The demand for hydroponically grown produce has risen dramatically in recent years. Various environmental concerns, reduction in arable land, and scarcity of water which are the primary concerns of traditional cultivation can be easily conquered with the use of hydroponics. One of the major reasons for rapid development of hydroponics was to control soil-borne diseases affecting crop plants leading to economic losses. However, since hydroponic systems employ a nutrient medium for the growth of plants, various kinds of microorganisms are also known to thrive in it. There are many factors that influence the behavior and health of beneficial micro-organisms, such as total dissolved solids (salts), oxygen content, the type of growth medium used, pH and various nutrient levels. The present work is an early attempt to determine the different types of microflora that exists in a hydroponic setup and their probable effect on plant growth.

**Key words:** Hydroponics, Microflora, Nutrient medium.

### INTRODUCTION:

Field soil has two major functions in plant growth. Soil acts as a reservoir to retain nutrients and water and it also provides support to the plant through its root system. Well- drained, pathogen free soil of uniform texture is the ideal medium for plant growth but soil does not always occur in this form. Pathogenic microorganisms are a common occurrence in field soils affecting crop production. Hence the need for the development of *soilless culture*/hydroponics. Hydroponics is a term that was coined in the 1930s to describe all methods of growing plants in liquid media for commercial purposes (Gericke, 1937). Initially, it was expected that soilless cultivation would be the solution to prohibit pathogenic microorganisms. However, since hydroponic systems employ nutrient rich media for the growth of plants, various microorganisms also

thrive in it. The diversity of root-infecting microorganisms is less in hydroponic systems compared to soil. However, root diseases do occur and are sometimes more serious in soilless systems (Stanghellini & Rasmussen, 1994). This contamination of microorganisms in soilless systems can come from plant material, growing media, insects, workers in the greenhouse and irrigation water (Postma *et al.*, 2008). The colonization is often triggered by environmental conditions, such as steady temperature and moisture regimes, and by the fact that root colonizers are easily spread by the recirculating water. The availability of water in hydroponic systems favors all zoosporic pathogens with the temperature of the nutrient solution being the most important environmental factor controlling the proliferation of these root diseases (Stanghellini & Rasmussen, 1994).

## METHOD AND METHODOLOGY:

- **Isolation of microflora (actinomycetes, bacteria and fungi) from hydroponic systems by serial dilution method.**

1ml Nutrient solution from Deep water Culture was diluted with 9ml sterile distilled water. Serial dilutions till  $10^{-6}$  were prepared. Similarly, 1 gm cocopeat from Trough System was diluted with 9ml sterile distilled water and dilutions were prepared.

0.1 ml of  $10^{-4}$ ,  $10^{-5}$  and  $10^{-6}$  dilutions were plated on Nutrient Agar and Potato Dextrose Agar. The Nutrient Agar plates were incubated at 37°C for 24 hrs whereas PDA plates were incubated at 25°C for 3-5 days.

## RESULTS:

- **Following microflora were isolated (Figure 1)**

A variety of bacterial and fungal colonies were obtained

## DISCUSSION:

In the present study, in general, more bacterial and fungal colonies were isolated from the Trough System as compared to the Deep water Culture System. Fungal colonies obtained showed aseptate mycelium suggesting the possibility of oomycetous fungi. Previous studies have reported the presence of aquatic fungi like *Pythium* in hydroponic systems. *Pythium*, which in soil-grown cucumbers and tomatoes is not considered to be a serious threat, has become severe in hydroponic conditions. It has also been suggested that the relatively low microbial diversity in hydroponic systems compared to soil (Menzies *et al.*, 2005) leads to an increased severity of opportunistic pathogens, such as *Pythium* (Paulitz, 1997). Both Craft and Nelson (1996) and Chen *et al.* (1988) have demonstrated the

association of high microbial activity with suppression of *Pythium* diseases. Once a zoosporic pathogen comes into the system, it will multiply and spread by self-dispersal through the recirculating solution and by root-to-root contact (Stanghellini & Rasmussen, 1994). In periods of stress, such as during fruiting when the temperature is high and there is a low concentration of dissolved oxygen in the nutrient solution, disease epidemics can take place (Cherif *et al.*, 1997; Stanghellini & Rasmussen, 1994; Gold & Stanghellini, 1985).

## CONCLUSION:

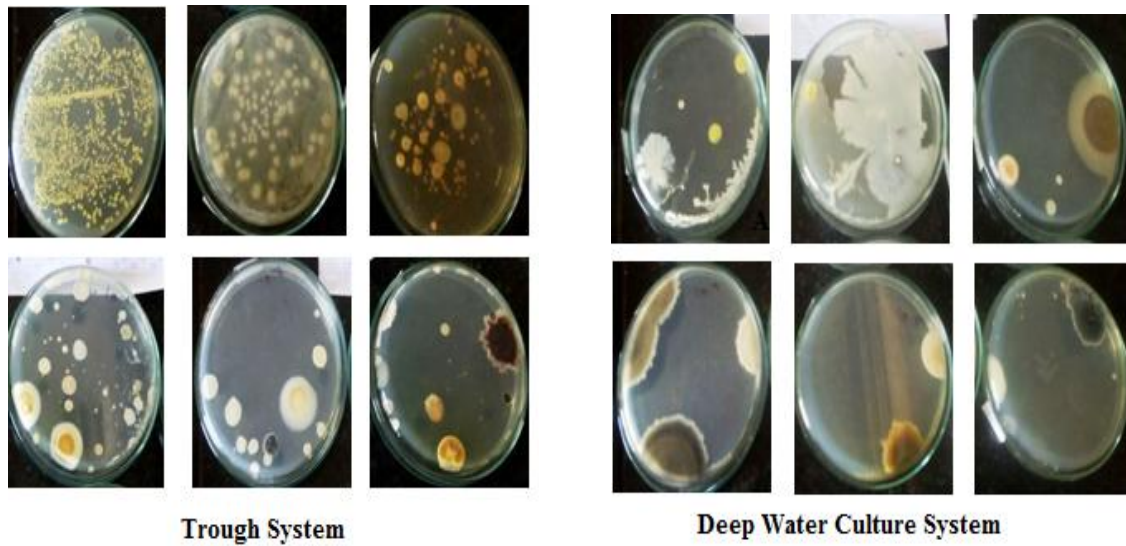
- A variety of bacterial and fungal colonies were obtained
- More number of bacterial and fungal organisms was isolated from the Trough system as compared to the Deep Water Culture Syst;em.

## REFERENCES

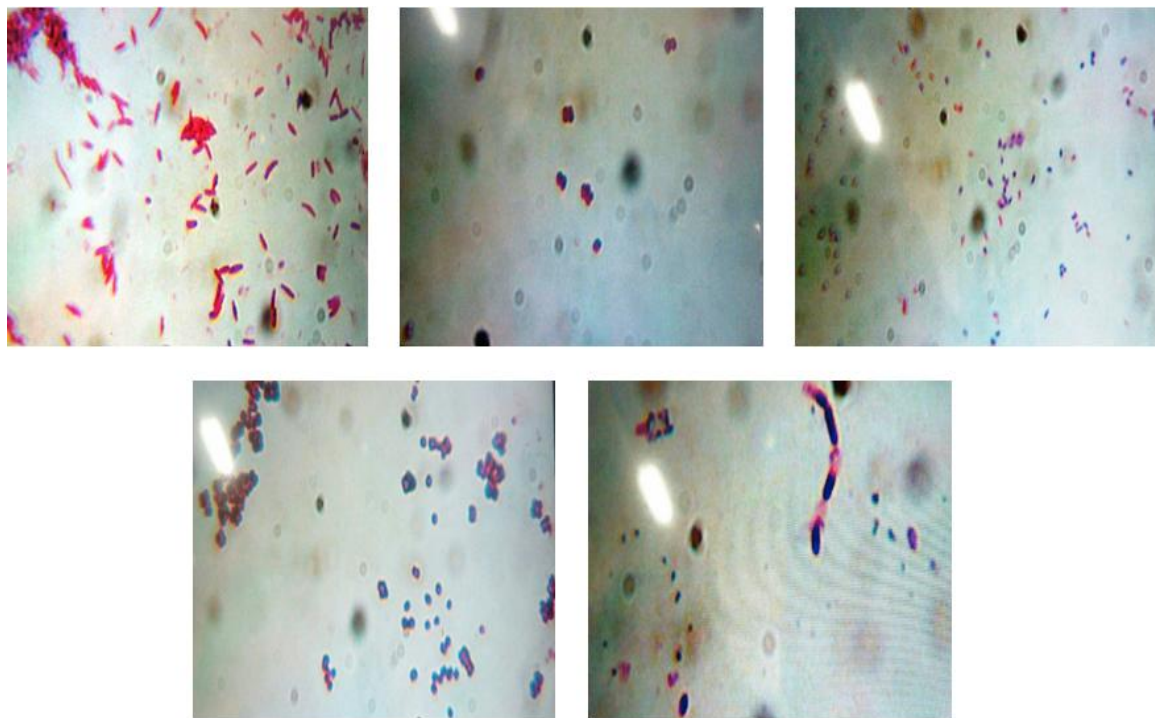
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**Fig.1 Different bacterial and fungal colonies obtained in the Trough System and Deep water Culture System**

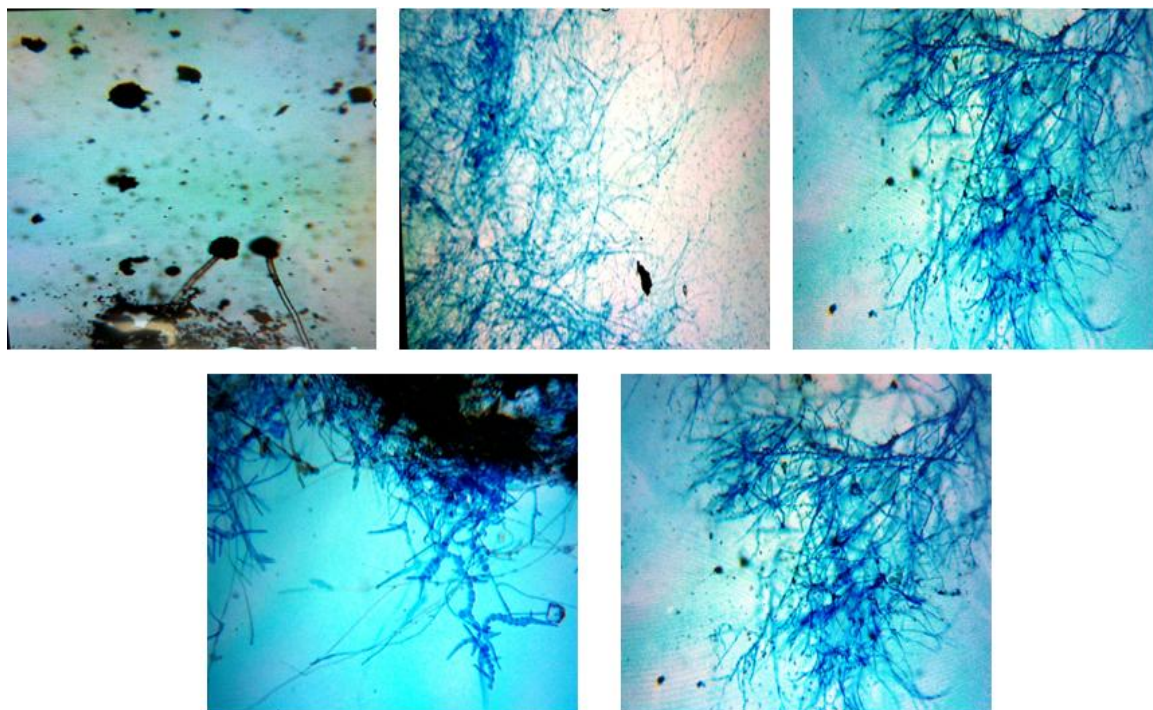


**Fig. 2 Different bacterial colonies obtained**

**Table 1: Colony characteristics and gram nature of bacteria isolated from the Trough system and Deep water Culture system**

Sr. No	System	Colony Characteristics						Gram Nature
		Shape	Margin	Diameter	Colour	Elevation	Transparency	
1	Trough System	Circular	Entire	4 mm	White	Convex	Opaque	Gram -ve rods
2		Irregular	Undulate	2 mm	Orange	Raised	Translucent	Gram -ve rods
3		Circular	Entire	1.5 mm	Orange	Raised	Opaque	Gram +ve cocci
4		Circular	Entire	3 mm	Light orange	Flat	Translucent	Gram +ve oval
5		Circular	Entire	5 mm	Yellow	Flat	Translucent	Gram +ve cocci
6		Circular	Entire	1 mm	Off-white	Convex	Opaque	Gram +ve cocci in pairs
1	Deep Water Culture	Irregular	Undulate	14 mm	Off-white	Flat	Opaque	Gram -ve rods
2		Circular	Entire	0.1 mm	White	Convex	Opaque	Gram +ve cocci

<b>Table 2: Colony characteristics of fungi isolated from the Trough system and Deep water Culture system</b>					
<b>Sr. No</b>	<b>System</b>	<b>Shape</b>	<b>Diameter</b>	<b>Colour</b>	<b>Elevation</b>
1	Trough System	Circular	30 mm	White	Convex
3		Irregular	25 mm	Dark Brown	Raised
4		Oval	30 mm	White	Raised
5		Irregular	25 mm	Grey	Convex
1	Deep Water Culture	Circular	20 mm	Black	Flat
2		Irregular	20 mm	Black	Flat
3		Circular	12 mm	Bottle Green	Raised



**Fig. 3 Different fungal colonies obtained**



# SECTION IV

# REPORTS



**First pre-conference workshop, held on 20<sup>th</sup> August 2016**

<b>Time</b>	<b>Details</b>
1:30 pm – 1:45 pm	Inaugural Function
1:45 pm – 2:45 pm	Speaker 1: Culturing of algae as a support practice -Dr. M. A. Deodhar Associate Professor, V. G. Vaze College of Arts, Science and Commerce, Mumbai, Maharashtra, India.
2:45 pm – 3:45 pm	Speaker 2: ‘Agri Mall: a novel concept’. -Ms. Kavita Jadhav Entrepreneur, Agro service centre, Nagar
3:45 pm – 4:00 pm	Tea Break
4:00 pm – 5:00 pm	Speaker 3: Marker Assisted Selection (MAS) in Plants -Dr. Shivani Patel Head, Department of Biotechnology, N. M. Virani college, Rajkot, Guj Rath

**Second pre-conference workshop, held on 19<sup>th</sup> November 2016**

<b>Time</b>	<b>Details</b>
1:30 pm – 1:45 pm	Inaugural Function
1:45 pm – 2:30 pm	Speaker 1: Status of GM technology in India. - Dr. S. V. Sawardekar, Associate Professor, Plant Biotechnology Centre, DBSKKV, Dapoli, Maharashtra, India
2:30 pm – 3:15 pm	Speaker 2: Biodiversity Protection: The ‘Gene Banks’ - Dr. A. D. Rane, Associate Professor, DBSKKV, Dapoli, Maharashtra, India
3:15 pm – 3:30 pm	Tea Break
3:30 pm – 4:15 pm	Speaker 3: ‘Sustainable agriculture: Bhagirath model’ -Dr. Prasad Devdhar, President, Bhagirath Gramvikas Pratishthan, Kudal.
4:15 pm – 5:00 pm	Speaker 4: Plant Breeders’ rights –Mrs. Anushree Lokur Associate Professor, Ramnarain Ruia College, Matunga, Mumbai.



### **Report of First Pre-conference Workshop**

Department of Biotechnology and Microbiology, V.P.M.'s B.N. Bandodkar College of Science, Thane, had organized the first pre-conference workshop for International Conference on “Emerging technologies for Sustainable Agriculture” on 20<sup>th</sup> August 2016 in Patanjali Auditorium, between 1:30-5:15 pm.

Total 330 students and fifteen faculty members of B. N. Bandodkar College of Science participated in the workshop. Eight students from other colleges also registered for the International Conference and attended the workshop. Ms. Sayli Daptardar, Assistant Professor, Department of Biotechnology and Microbiology, compered the event.

The event began by lightening the lamp. Dr. Ashwini Tilak, Assistant Professor, Department of Biotechnology and Microbiology, recited Sarasvati Vandana to worship Goddess Sarasvati.

Convener of the conference, Honorable Principal Dr. (Mrs) M. K. Pejaver, while addressing the audience, emphasized on the significance of pre-conference workshops for undergraduate students in terms of understanding research work at International level. She urged the students to be interactive and inquisitive throughout the sessions.

Dr. Kalpita Mulye, Organizing Secretary of the conference, shared with the gathering, the whole idea of organizing a conference on ‘sustainable agriculture’. She highlighted the significance of modern technology for improving the agricultural yield, and the urgent need for collaborative efforts between academic and industrial sectors to accomplish this task. Dr. Kalpita also talked about various curricular and co-curricular activities planned during current academic year based on the theme of the conference.

Dr. Kalpita’s speech was followed by talks by the invited speakers. Ms. Rutuja Gaikwad, Assistant Professor, Department of Biotechnology and Microbiology, introduced the first guest speaker of the workshop, Dr. M.A. Deodhar, Associate Professor, V. G. Vaze College, Mulund. Dr. Deodhar enlightened the listeners on ‘Culturing of algae as a support practice’. She discussed various aspects of algal culture, the temperature and light conditions, as well as the different type of reactors used for the same. She also talked over various applications of algal culture, such as the production of algal polysaccharide as a gelling agent, thickening agent and emulsifier. Importance of microalgae in pharmaceuticals and food, feed and pigment production was shared. Dr. Deodhar, after her speech, answered queries by many students regarding cultivation of algae in laboratory.

The second speaker for the workshop was Ms. Kavita Jadhav, a young entrepreneur, who delivered a talk on ‘Agri Mall: a novel concept’. Ms. Rucha Kulkarni, Assistant Professor, Department of

Biotechnology and Microbiology, briefly introduced Ms. Kavita. The audience congratulated Ms. Kavita for receiving an award by 'ZeeMarathi', the 'Uncha Maza Zoka' Puraskar for her outstanding contribution in the field of agriculture. Ms. Kavita elaborated on her idea of Agri Mall as a 'one stop solution' for farmers, where quality seeds and other farming equipments are made available at affordable prices. She shared that the mall not only organizes training programs and provides consultancy for farmers, but also promotes the concept of organic farming amongst them. She also mentioned that Agri Mall provides soil and water testing services to farmers and also has a library where farmers have an easy access to various books related to agriculture. The audience was thrilled, realizing the enormous efforts put in and dedication with which Ms. Kavita is working. The session was followed by a brainstorming question-answer session in which students as well as teachers actively participated.

After the tea break, Ms. Zahera Momin, Assistant Professor, Department of Biotechnology and Microbiology, briefly introduced the last speaker of the workshop, Dr. Shivani Patel, Head, Department of Biotechnology, M and N Virani College of Science, Rajkot, Gujarat. Dr. Shivani delivered a lecture on the topic 'Marker Assisted Selection (MAS) in Plants'. By using simple terms and giving examples from day to day life, Dr. Shivani discussed the advantages of marker assisted selection of plants over conventional plant breeding and also over transgenic technology. She shed light on different types of molecular markers used in MAS, essential requirements of MAS, procedure and molecular mechanism of MAS. She also discussed a case study in which MAS technique was used to obtain an improved variety of Basmati rice. She concluded the session with discussion on potential benefits of MAS and the challenges faced in their use.

Mrs. Jayashree Pawar, organizing Secretary of the conference, gave the vote of thanks. The workshop was concluded with the National Anthem. Feedback of the participants for the workshop was also collected. The event has been captured by recording the same.

### **Report of Second Pre-conference Workshop**

Department of Biotechnology and Microbiology, V.P.M.'s B.N. Bandodkar College of Science, Thane, had organized the second pre-conference workshop for International Conference on “Emerging technologies for Sustainable Agriculture” on 19<sup>th</sup> November 2016 in Patanjali Auditorium, between 1:00-5:30 pm.

Total 194 students and thirteen faculty members of B. N. Bandodkar College of Science participated in the workshop. Two participants from other institutions registered for the International Conference and attended the workshop. Ms. Sayali Daptardar, Assistant Professor, Department of Biotechnology and Microbiology, compered the event.

The event began with the recitation of Sarasvati Vandana by Dr. Ashwini Tilak, Assistant Professor, Department of Biotechnology and Microbiology.

Convener of the conference, Honorable Principal Dr. (Mrs) M. K. Pejaver, in her welcome address appreciated and congratulated the first year students for their presence in spite of the ongoing examination and also said that the pre-conference workshop would enable the student to prepare for the International conference. She also mentioned about future plans of collaborations with DBSKKV for carrying out various research activities.

Dr. Kalpita Mulye, Organizing Secretary of the conference, thanked Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth (DBSKKV), Dapoli for the collaboration and also emphasized on the fact that learning about the technologies for sustainable agriculture from the experts would help us in understanding the current scenario and the challenges encountered in the same. She gave a brief overview of the first pre-conference workshop and also briefed the audience about the topics that were going to be discussed in this workshop. She highlighted the two contrast concept namely genetic modification and conservation through gene bank that would be discussed in first session.

This was followed by speech from Dr. N. B Gokhale, Incharge Biotechnology Unit, College of Agriculture, DBSKKV. He said that this collaboration would help in inculcating a practical approach in the students. He also informed that the graduates from other biological sciences can also now apply for Masters in agriculture science, DBSKKV with changed norms that are going to be implemented.

Dr. Gohkale's speech was followed by talks by the invited speakers. Ms. Rutuja Gaikwad, Assistant Professor, Department of Biotechnology and Microbiology, introduced the first guest speaker of the workshop, Dr. S.V. Sawardekar, Associate Professor, Plant Biotechnology Centre, DBSKKV, Dapoli, Maharashtra. Dr. Sawardekar delivered a lecture on the topic “Status of GM technology in India.” He stated that agricultural sector is a major contributor to India's GDP and this contribution can be

further increased through biotechnological interventions. He discussed the global as well as Indian scenario regarding the commercial availability of GM crops. He informed the audience about different agencies that regulate the release of a new GM crop variety. He also spoke about two genetically modified plant varieties released by his team namely Panvel-3 in rice and Dapoli-2 in Finger millet. Dr. Sawardekar, after his speech, answered the queries by audience regarding the criteria for genetically modification of a crop and its economics when released in public sector.

The second speaker for the day was Dr. A. D. Rane, Associate Professor, DBSKKV, Dapoli, Maharashtra, India who delivered a lecture on 'Biodiversity Protection: The Gene Banks'. Dr Rane was introduced to the audience by Ms. Rucha Kulkarni, Assistant professor, Department of Biotechnology and Microbiology. His lecture focused on the need for conservation of biodiversity and challenges faced for the same. He elaborated on several *in situ* and *ex situ* methods of conservation of biodiversity. He discussed how anthropogenic activities pose a threat to genetic diversity. He introduced the concept of *ex situ* gene bank and informed the audience about National Gene Bank which includes facilities like seed bank, *in vitro* gene bank, cryo bank and field gene bank.

The third speaker for the workshop was Dr. Prasad Devdhar, President, Bhagirath Gramvikas Pratishthan, Kudal, who delivered a talk on 'Sustainable agriculture: Bhagirath model'. Dr. Mrs. Jayashree Pawar, Co-ordinator-Biotechnology, briefly introduced Dr. Devdhar. The audience congratulated Dr. Devdhar as his organization received the award by 'Zee Marathi', the 'Uncha Maza Zoka Puraskar' for their contribution in the field of agriculture. The objective of Bhagirath Gramvikas Pratishthan is making rural India self-reliant. Dr. Devdhar started the session with a documentary depicting positive impact of his organization on the farmers in Kudal. He said that their theme is development through society's co-operation. Bhagirath Pratishthan has construed over 5,000 biogas plants of Deenbandhu model, in Kudal, Sindhudurg district. They have also tried to make farmers self reliant by training and providing them assistance in starting agriculture based business like poultry farming, animal husbandry etc. He encouraged the students to explore rural India and obtain first hand information about the challenges faced by them which will help the students in developing a need based approach. In summary his talk conveyed that maximum utilization of available resources and their conservation by a proper scientific method helps to attain a systematic development.

Dr. Mrs. Kalpita Mulye, Co-ordinator-Microbiology, briefly introduced the last speaker of the workshop, Mrs. Anushree Lokur Associate Professor, Ramnarain Ruia College, Matunga, Mumbai. Mrs. Lokur delivered a lecture on the topic "Plant Breeders' rights". She began her session with the explanation of basic concepts and significance of intellectual property rights. She introduced the audience to 'The protection of plant variety and farmer's right Act 2001' that not only protects the interest of breeders but also farmers. She explained the difficulty of applying conventional patenting

criteria for plant varieties, and described the modified criteria for patenting the same. She discussed the rights and duties of the breeder of a protected variety. She also described how the act protects farmer's rights and has a provision for protection against unintentional infringement by farmers. She briefed on the lacunae concerned with the Act that need to be considered in order to make it more effective.

All the speakers were given a token of appreciation in the form of a box that contained turmeric, seeds of marigold, basmati rice, tulsi, beetle nut and red chilies that represented Indian agriculture.

Dr. Mrs. Jayashree Pawar, organizing Secretary of the conference, gave the vote of thanks. The workshop was concluded with the National Anthem. Feedback of the participants for the workshop was also collected. The event has been captured by recording the same.

### Pre-conference Lecture Series

Date	Speaker	Topic
7 <sup>th</sup> December 2016	Ms. Jilpa Nijasure	Sustainable Agriculture
2 <sup>nd</sup> January 2017	Dr. Jayashree Pawar	Introduction to Agriculture related Molecular Biology Tools and Techniques
3 <sup>rd</sup> January 2017	Dr. Jayashree Pawar	GPCRs – Basics to Advances
5 <sup>th</sup> January 2017	Dr. Malali Gowda	Reforestation Technology to combat local climate change

## Conferences/ Seminars/ Workshops Conducted In Last Five Years

Department	Topics
<b>2015 – 16</b> Zoology and Environmental Science	International conference on Ecosystem Services of Wetlands ‘Ardrabhumi 2016’
<b>2014 – 15</b> Botany National conference on ‘Emerging	Trends in Bioinformatics and Taxonomy’
<b>2013 – 14</b> Zoology and Environmental Science	National Conference on Biodiversity Status and Challenges in Conservation – ‘Faveo 2013’
<b>2012 – 13</b> Information Technology Chemistry	National Conference – FUTECH National Conference on Phytochemistry : Recent Trends and Challenges Mathematics National Seminar on Recent Research Trends in Mathematics
<b>2011 – 12</b> Department of Chemistry and Research Committee Botany	National Seminar on Avenues on Scientific Research Proposal Grants National Seminar on Evolving of Scientific terminology in Environmental Science in Regional Language
Information Technology Biotechnology	National Conference on Cloud Technology National Conference on Biotechnology in Diagnostics



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