



**A**  
**Compendium of  
Research Articles by  
Prospective Researchers  
2019-20**

**Biotechnology**

**Chemistry**

**Microbiology**

**Mathematics**

**Statistics**

**Khandesh College Education Society's  
Post Graduate College of Science, Technology and Research, Jalgaon**

**A**  
**Compendium of Research**  
**Articles by Prospective**  
**Researchers**  
**2019-20**

**Under the**  
**Prospective Researchers' Scheme (PRS)**



**Khandesh College Education Society's**  
**Post Graduate College of Science, Technology and Research,**  
**Jalgaon**

**Recognized by Govt. of Maharashtra vide G. R. No. NGC 2010/247/10 & Affiliated to K. B. C.**  
**North Maharashtra University, Jalgaon**

**Accredited 'B+' Grade by NAAC with CGPA 2.52 in 1<sup>st</sup> cycle**

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Head, Department of Microbiology

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Principal

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### Foreword

I am pleased to go through the 2<sup>nd</sup> edition of compendium containing research articles by Prospective Researcher's. This has resulted due to collaborative efforts from both, students and our teachers. It is indeed an innovative leap towards bridging the gap between formal education and research, with objective of accelerating the participation of students in research activities.

The present volume has fulfilled the need of helping young scientists to publish their 'original scientific thinking' and to provide a common platform to get involved in research projects. This would definitely enhance the research horizons of the students and it would definitely add to their visualization of scientific, social and environmental problems.

I admire this volume, for its efforts towards creating a conducive environment for research in the institution and motivating student community to participate in research. It will also help to boost the creativity and working potency of the students and researchers. I hope this volume would trigger similar innovative activities, encouraging research in all the fields of human concern. I whole heartedly appreciate the efforts of the students, researchers, teachers, and all those involved in bringing out this volume in an excellent manner.

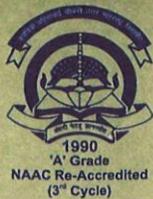
I highly appreciate the dynamic leadership and guidance of principal and supporting faculties of the college to publish research work carried out under the 'Prospective Researchers' Scheme, that will help to inculcate the spirit of research amongst the students. I am sure that this volume will encourage to all teachers and researchers working in the basic as well as allied subject and provide platform for to exchange of knowledge.

I congratulate all the research supervisors & members of the editorial board of this volume and extend my best wishes to all the prospective researchers.

**Shri. N. G. Bendale**

**Hon'ble President, KCE Society, Jalgaon**





॥ अंतरी पेटवू ज्ञानज्योत ॥

## Kavayitri Bahinabai Chaudhari North Maharashtra University, Jalgaon

Umavinagar, Jalgaon - 425 001 (Maharashtra) INDIA  
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**Prof. P. P. Patil**

Ph.D., F.M.A.Sc.

**VICE-CHANCELLOR**



### MESSAGE

I am delighted to know that K. C. E. Society's Post Graduate College of Science, Technology and Research, Jalgaon is publishing volume II as '**A compendium of research articles by prospective researchers**' comprising of research articles prepared by the students. This volume consists of research articles, which are the outcomes of the research projects carried out by the students under the '**Research Promotion Scheme (RPS)**'.

It is appreciable that K. C. E. Society provides financial support to prospective research students to carry out research work in their chosen areas. I am happy to see the wide spectrum of topics under different disciplines; prospective researchers have chosen to work on. Index of the volume indicates that students from Chemical Sciences, Life Sciences and Mathematical Sciences have contributed to this volume by undertaking the seventeen research projects, like green synthesis, chemical recycling, statistical analysis of higher education quality, onion production in India, antibacterial & antifungal activities of microorganisms etc.

I take this opportunity to congratulate the Management, the Principal, Supervisors and Researchers of the project for their contribution to this compendium.

My best wishes to the Vol.II of "**A Compendium of Research Articles by Prospective Researchers.**"

(Prof.P.P.Patil)  
Vice-Chancellor

KBCNMD



॥ अंतरी पेटवू ज्ञानज्योत ॥

## Kavayitri Bahinabai Chaudhari North Maharashtra University, Jalgaon

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### *Preface*

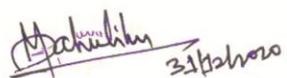
I am very happy to know that Khandesh College Education Society's Post Graduate College of Science, Technology & Research, Jalgaon is publishing "Compendium of Research Articles by Prospective Researchers" in this year (2019-20) under Prospective Researchers Scheme (PRS). Seventeen articles from various subjects i.e. Organic Chemistry, Biotechnology, Microbiology, Statistics and Mathematics are describing the research findings of students and teachers of the college as a team work. The enthusiastic endeavors of the college teachers will definitely help to create a vibrant and congenial environment to foster independent thinking and inculcate creativity among the young generation. It will also provide a platform for post graduate students and teachers from all disciplines to fulfill their wishes and realize their dreams.

I highly appreciate the dynamic leadership of President, KCE Society and guidance of Principal and teachers of college for conducting Prospective Researchers Scheme (PRS) and publishing a Compendium that will help to inculcate the spirit of research amongst the students right from the entry level of post-graduation. It will also help to boost the creativity and working potency of the teachers and newer researchers as well as to encourage multidisciplinary research and team work as projected in National Education Policy-2020.

I take this opportunity to congratulate these future young scientists for their sincere research work as well as Principal and all teachers for their enthusiastic guidance and support.

I wish all the best and bright success to continue this innovative and motivating activity for better achievements. Definitely this activity will help to encourage the young generation towards the research and nation development.

**Place: Jalgaon**  
**Date: 31/12/2020**

  
**Prof. P. P. Mahulikar**  
**Pro-Vice Chancellor**

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**Post Graduate College of Science, Technology and Research, Jalgaon**  
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**Awarded 'B+' Grade by NAAC with CGPA 2.52 in 1<sup>st</sup> cycle**  
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Recognized by Govt. of Maharashtra vide G. R. No. NGC 2010/247/10 & Affiliated to K. B. C. North Maharashtra University, Jalgaon

**Date:** 20<sup>th</sup> December 2020

## FOREWORD

It indeed matter of great pleasure that KCE society's Post Graduate college of Science, Technology and Research, Jalgaon is publishing the second volume of '**A compendium of research articles by Prospective Researchers**' of the projects undertaking by the students. Under the initiative of "Prospective Researchers' Scheme", prospective researcher students have been provided with golden opportunities to undertake multi-disciplinary projects and thereby they get the exposure not only to the scientific and methodological research but also the training about writing of research paper. This year, scheme is continued in which 17 projects (research articles) have been completed by 60 students under the supervision of 13 expert teachers of every department of the college.



I am confident that the articles of the projects presented in this volume will elevate the spirit of scientific attitude amongst the students and teachers as well. Launching new initiative is always easy, but sustaining it for a long period is always difficult. Therefore the efforts taken by the teachers, students and specially co-ordinator of the scheme & the editor of the volume for bringing out this volume is appreciable and commendable.

**Dr. V. S. Zope**  
Principal

## From the Desk of Editor

I am pleased to present to you the Second edition of *A Compendium of Research Articles by a Prospective Researchers* under the activity of 'Prospective Researcher's Scheme' for the year 2019-20. This volume presents the seventeen articles from students and teachers of five Science Departments of college. '**Prospective Researchers' Scheme** is unique research activities of the college in which students undertake research projects under the supervision of teachers.



The outcome of this scheme is reflected in the publication of 'A compendium of research articles of prospective researchers' with ISBN number. At the beginning of an academic year, potential students are identified and small research projects are assigned to them. The innovation and feasibility of research proposal is scrutinized followed by undertaking of projects in the stipulated time. The students are provided with research assistance to complete the research projects within stipulated time period. After completion, projects are evaluated by external experts and best performers are felicitated with cash prizes. This year seventeen research projects were completed from five Departments of Organic Chemistry, Microbiology, Biotechnology, Mathematics and Statistics. The research papers based on their articles is published in a separate volume as "*A Compendium of Research Articles by a Prospective Researchers Volume II*" with ISBN. I thank all the committee members, faculty wise project guides & students for helping me in this endeavor.

I would like to thank the Principal Dr V. S. Zope and our Hon'ble President Shri. N. G. Bendale, KCE Society, Jalgaon, provided help and encouragement to compile the new edition. This volume is result of continuous efforts of teachers & students of this college.

I am very happy to handover this volume to you all.

A handwritten signature in blue ink, which reads "Patil". The signature is written in a cursive style with a horizontal line underneath.

**Mr. Sandip N. Patil**  
**Chief Editor**

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\*Corresponding author and Supervisor

# CHEMISTRY

# Use of High Pressure Autoclave (HPA) for Chemical Recycling of Poly-Ethylene Terephthalate (PET) waste

Varma Nisha, Patil Dhiren, Patil Kaushik, Dhumale Ashwini and Zope Vishvanath S\*  
Department of Chemistry, KCE's Post Graduate College of Science, Technology and Research,  
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## Abstract

HPA is used for high-pressure high-temperature chemical reactions like alkylation, amination, bromination, carboxylation, catalytic reduction, chlorination, dehydrogenation, esterification, ethoxylation, halogenation, hydrogenation, methylation, nitration, oxidation, ozonization, polymerization, sulphonation. Chemical recycling of Polyethylene Terephthalate (PET) waste was studied at different temperatures and autogenious pressures were recorded. Temperature and pressure have been optimised for grater conversion of PET to TPA. The product obtained is characterised by recording FTIR and melting point.

**Key Words:** Chemical Recycling, high pressure autoclave, hydrolysis, PET, TPA

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## Introduction

Nowadays, polyethylene Terephthalate (PET) is commonly used to manufacture water bottles being excellent water and moisture barrier properties. PET is commonly used in making mineral water bottles and soft drinks or carbonated beverage bottles. Huge use of Polyethylene Terephthalate generates considerable amount of waste. Consequently, gigantic amount of synthetic waste is generated; bulk of the waste is disposed in landfills or incinerated. The process of dumping synthetic waste in the land fill is not environmental friendly solution since polyethylene terephthalate (PET) bottles are not biodegradable. Since, the cost of the disposal of waste is increasing without a break, owing to the limited capacity of the landfills is causing soil pollution. As a result, it is of a great interest to chemically recycle and later reuse the materials. Primary recycling of Polyethylene Terephthalate (PET) converts the waste into other products of the virgin polymer. Numbers of researchers<sup>1-6</sup> have dedicated their work to find alternative methods for the recycling of polyethylene Terephthalate (PET), polyurethane (PU) foam, Polyamide (PA). The recovery of the product of depolymerisation of polyethylene terephthalate can be used for conversion of PET.<sup>7-8</sup> We have studied the depolymerisation of PET by alkaline hydrolysis process using pyridine as a catalyst using the high pressure autoclave.

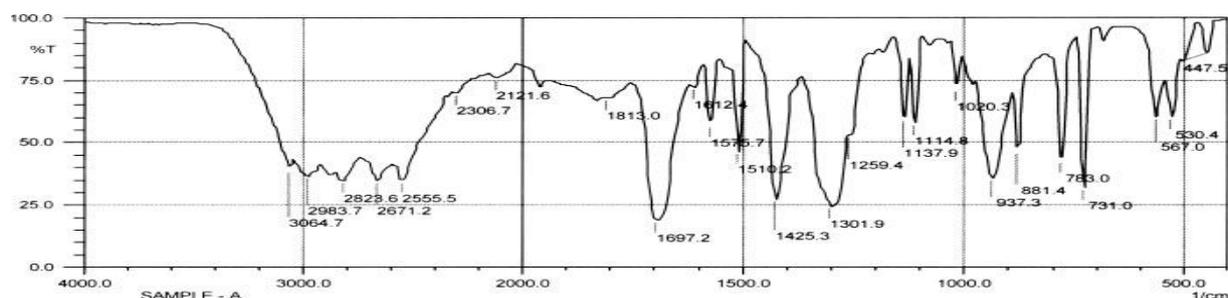
**Materials:** Sodium hydroxide, ethyl alcohol, hydrochloric acid and pyridine were used as such obtained by BDH chemicals. Waste mineral water bottles were used as a source of

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Polyethylene Terephthalate (PET). The bottles were cut into the size of 1 cm<sup>3</sup> (one by one centimeter) into square shape. Amar Equipment Pvt. Ltd manufactured 0.5 L capacity High Pressure Autoclave was used to study.

## Methodology

**Experimental:** 10 g of Polyethylene Terephthalate (PET) waste, which was cut into the 1 cm<sup>3</sup> square shaped pieces, 8 g of Sodium hydroxide and 250 ml of distilled water, were charged into the reaction vessel of HPA. The reaction mixture was homogeneously mixed by string at the rate of 1000 RPM. The reaction was carried out at the temperatures 150, 180, 200, 220 °C the autogenius pressure was recorded at respective temperatures. The reaction was carried for the 120 minutes reaction time. After the completion of reaction time, the vessel was cooled suddenly by circulating cold water in the vessel through inner coil. The reactor vessel was opened by removing the collar when the vessel got completely cooled, Chemical recycling of 10 g PET by alkaline hydrolysis was carried out by refluxing the PET along with water and catalyst for several time intervals. Amount of Sodium Hydroxide was varied for its optimization as 4, 6, 8 gram. The same reaction was carried out using with and without catalyst. 4 ml of pyridine as a catalyst and 8 gram optimized amount of sodium hydroxide were use in the reaction. The reaction was reflux for 120 minutes. The reaction mixture was cooled down on it's own. Once the reaction mixture got cooled, it was collected in the beaker. The TPA is precipitated as a white solid by adding concentrated hydrochloric acid in a reaction mixture. The product obtained was filtered, dried and weighed. The melting point of the product (TPA) was recorded as 249° C. TPA obtained is characterised by recording FTIR as shown in figure 1.



**Fig 1: FTIR spectra of TPA**

Amar Equipment Privet Limited, Kurla, Mumbai was founded in 1974 by Mr. Naresh Shah (Managing Director & Chairman) after completing B.Tech. Mechanical from IIT Mumbai & Masters of Science from University of Michigan, United States. Ever since 1974, Amar has built its foundation on a strong technical team with a blend of vast experience & technological and commercial understanding. Figure 1 shows assembly of high pressure autoclave.

## High Pressure Autoclave:



**Fig 1: High Pressure autoclave assembly**

### Applications:

HPA is used for high-pressure high-temperature chemical reactions like alkylation, amination, bromination, carboxylation, catalytic reduction, chlorination, dehydrogenation, esterification, ethoxylation, halogenation, hydrogenation, methylation, nitration, oxidation, ozonization, polymerization, sulphonation etc. Prominently HPA is used in R&D centers of pharmaceuticals, dyes, chemical, fertilizers, paints, oils, agrochemical, and petrochemicals industries. It has been used by colleges, research institutes, defense organizations. HPA is used, where high pressure reactions and testing is carried out. Some specific uses of HPA are listed below.

- To design new molecules, chemicals & to study the reaction parameters
- To manufacture the chemicals in small quantities in batch or continuous mode
- For synthesizing hydrogenation, acetylation, epoxidation, Grignard reaction, nitration, acylation, photochemical reactions, high throughput catalyst screening and quality control & process improvements
- For supercritical CO<sub>2</sub> solvent extraction, reaction, drying and evaporation system Also for reaction calorimetry to study heat of reaction
- For high pressure storage & transfer of gas / liquid / slurries, acid digestion and Gas hydrate formation.

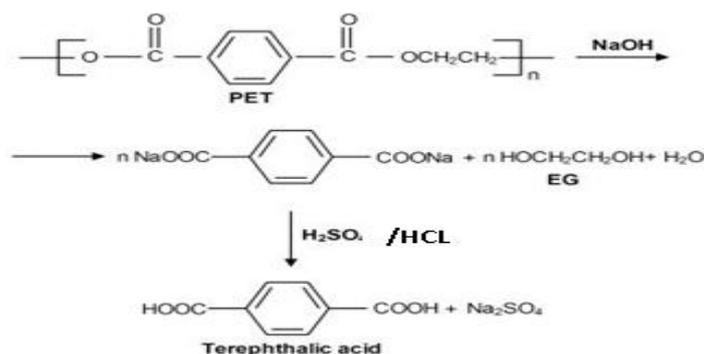
### Salient Features:

HPA is available as Stirred & non-stirred reactors, pressure vessels with the volume ranging from 5 ml to 500 ml for research laboratories and for industrial and pilot plant it is up to 2000 liter. The vessel is made up of SS-316/316L, Hastelloy B/C, Monel, Inconel, Nickel, Titanium, Tantalum lined, Zirconium etc. The maximum design pressure is up to 700 & temperatures up to 650 °C. It is provided with high torque maintenance free zero leakage magnetic drive coupling. It is meant for to design complete pilot plant with automatic

temperature, pressure, RPM, motor torque/ current, liquid and gas. It is also provided with condenser for distillation or reflux, thermicfluid heating and cooling system etc. It is fully automated PC controlled high pressure systems and completely flame, explosion proof, ATEX certified systems suitable

## Results and Discussions

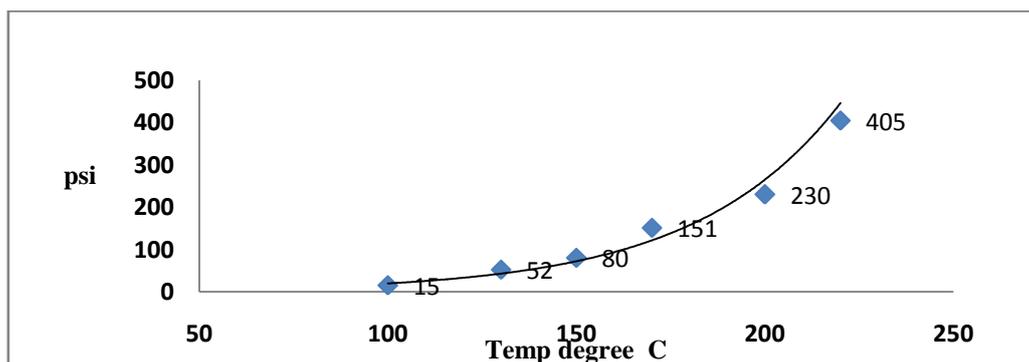
**i. Reaction mechanism:** The reaction mechanism for depolymerization of polyethylene Terephthalate to Terphthalic acid is represented as given below.



**ii. Measurements of Autogeniuos pressure:** The pressure exerted by the steam of water in the closed vessel is referred as autogeniuos pressure. The autogeniuos pressure was recorded at different temperature when three fourth of the vassal of 500 ml capacity is filled by water. Figure 2 shows variation of pressure with temperature. It is evident from the figure the trend is same as theoretical.

Temp <sup>0</sup> C	100	130	150	170	200	220
Pressure (psi)	15	52	80	151	230	405
Pressure (bar)	2	3.8	7.0	10.2	16.0	28.0

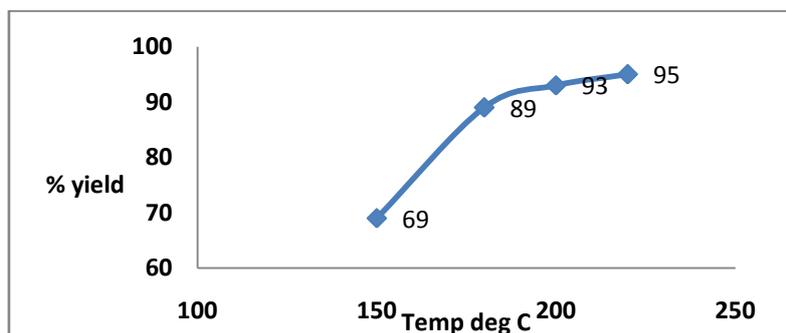
**Table 1: Variation of Temperature versus Pressure**



**Fig 2: Variation of Temperature versus Pressure**

**ii. Conversion of PET to TPA using HPA:** The variation of percenatge yield for the conversion of PET into TPA with temperature of autoclave at respective autogenous pressure is as shown in figure 3. It is evident from the graph that, % yield is 69 at 150<sup>0</sup>C and at autogenous pressure 80 psi and then it suddenly increases up to 89% at 180<sup>0</sup>C.

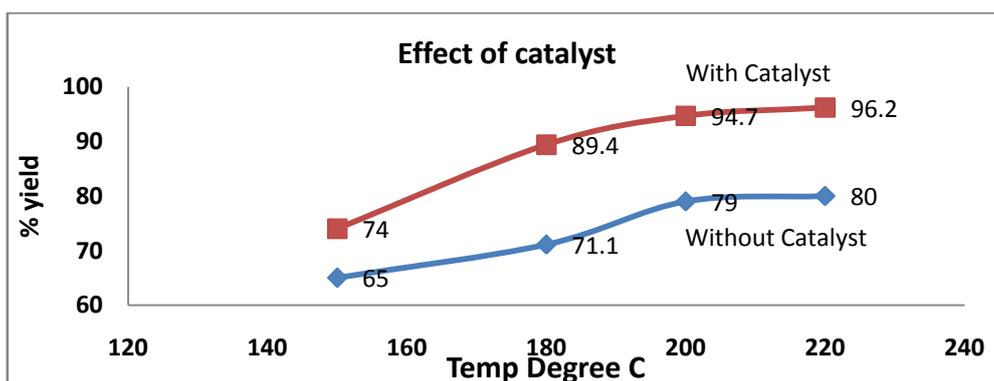
Thereafter there is no significant increase in % yield. It reaches to maximum to 95% at 220°C and at Autogenous pressure 405 psi.



**Fig 3: Variation of % yield versus Temperature**

**iii. Conversion of PET to TPA by using reflux method (with and without catalyst):**

Figure 4 indicates same trend of increase of percentage yield with and without catalyst. But at every temperature increase in percentage yield is more when catalyst was used than without catalyst. The maximum percentage yield at 220°C was found to be 96.2 and 80% with and without catalyst respectively.



**Fig 4: Variation of % yield versus Temperature (with and without catalyst)**

**Conclusions**

1. High pressure autoclave is used for study of depolymerisation of plastics waste
2. Calibration curve is set up by measuring autogenous pressure at different temperature.
3. Maximum percentage yield for obtaining monomer TPA by depolymerization reaction is 95% at 220°C using high pressure autoclave.
4. Maximum percentage yield for obtaining monomer TPA by depolymerization reaction is 96.2 and 80% with and without catalyst respectively at 220°C using reflux method.
5. 8 gram of sodium hydroxide required for depolymerization of 110 gram of PET has been optimized.

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# One-pot Multicomponent Synthesis of 2,4,5 triaryl-imidazole Derivatives by using CMC-Ce<sup>IV</sup> as reusable Catalyst

Chaudhari Kamlesh, Patil Nilesh, Patil Ganesh, Khatik Nisar and Patil Ravindra M.\*  
Department of Chemistry, KCES's Post Graduate College of Science Technology &  
Research, Jalgaon

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## Abstract

CMC-Ce<sup>IV</sup> was prepared by metathesis strategy and characterized by FT-IR techniques. The ensuring catalyst has been successfully applied in the one-pot four-component reaction of various aromatic aldehyde and benzil to the synthesis of 2,4,5 triaryl-imidazole derivatives. The catalyst was recovered and reused for five cycles without considerable loss of activity. The advantages of the protocol include rapid reactions with good yields and simple workup. The synthesized compounds were characterized by FT- IR technique.

**Keywords:** CMC-Cu<sup>II</sup>, Imidazole derivatives, Multi-component reaction, Metathesis Reaction.

\*Corresponding author: rpatil1734@gmail.com

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## Introduction

Imidazole is an important core organic molecule. It is found in many naturally occurring compounds like vitamin B<sub>12</sub>, histidine, histamine, pilocarpine alkaloids, and biotin.<sup>1-3</sup> It is also showing good activity as herbicide, plant growth regulator, anti-epileptic, anticonvulsant, anti-inflammatory, analgesic, anticancer, etc.<sup>4-8</sup> Also, imidazoles are found as the main core molecule in drugs like Omeprazole, Pimobendan, Losarton, Olmesartan, Eprosartan, and Trifenagrel.<sup>9</sup>

Owing to their wide range of biological advancement, synthesis of title compounds are still of intrigue. The available reported method for the synthesis of substituted imidazoles suffers from drawbacks such as the catalysts used for synthesis are either toxic or expensive and requires harsh reaction condition. Therefore, a need still exists for further development of an efficient, reusable, inexpensive and eco-friendly catalyst for the synthesis of substituted imidazoles. In organic synthesis, the product yield and reaction time are extremely important. The increase in reaction steps results in a decrease in final product yield and increase in total reaction time. Multicomponent reactions help to solve this problem. By novel developing multicomponent reaction strategies, synthesis of the desired product in the one-pot method is possible thereby increases the product yield and reducing reaction time required for the reaction.

The interest in metal NPs, attributable to their high surface area, incredible availability, high

biocompatibility and low toxicity. In addition, the high catalytic activity of metallic NPs can be accounted due to its Lewis acid site.<sup>10</sup> Considering these facts, we have decided to synthesize 2,4,5 triaryl-imidazole derivatives of various substituted benzaldehydes and benzil efficiently using CMC-Ce<sup>IV</sup> as a recoverable and reusable catalyst in ethanol as a solvent via. Multi-component reactions.

## Materials and Methods

All reagents used were of laboratory grade. Melting points were determined in open capillaries. The purity of compound was checked by TLC. IR spectra were recorded on Shimadzu FT-IR (Affinity Model) using KBr.

### Preparation of Cu(II)carboxymethylcellulose (CMC- Ce<sup>IV</sup>) Catalyst

The Ce(IV) carboxymethylcellulose (CMC- Ce<sup>IV</sup>) catalyst was prepared by metathesis reaction of ceric ammonium nitrate and Na-CMC. The yellow solid was precipitated which was left to equilibrate in a solution for overnight. The resulting yellow solid was separated from the solution and washed thoroughly with distilled water. The wet CMC- Ce<sup>IV</sup> was dried at 60<sup>0</sup>C in the oven till constant weight.

### General procedure for synthesis of 2,4,5 triaryl-imidazole derivatives by using CMC- Ce<sup>IV</sup> as catalyst:

In 150 ml round bottom flask, a mixture of benzaldehyde (10 mmol), benzil (10 mmol) and ammonium acetate (10mmol), as ammonia source, and CMC-Ce<sup>IV</sup>(20mg) were stirred and refluxed in ethanol for appropriate time (Table 1). The progress of the reaction was monitored by TLC. After completion of the reaction, the precipitate thus obtained was wash with ethanol and then purified by recrystallization from ethanol to get corresponding pure product ((Scheme 1).

## Results and Discussions

### Optimized Reaction Conditions:

To optimize the reaction condition, we performed the model reaction with different amount of CMC- Ce<sup>IV</sup> catalyst loaded as shown in **Table 1**.

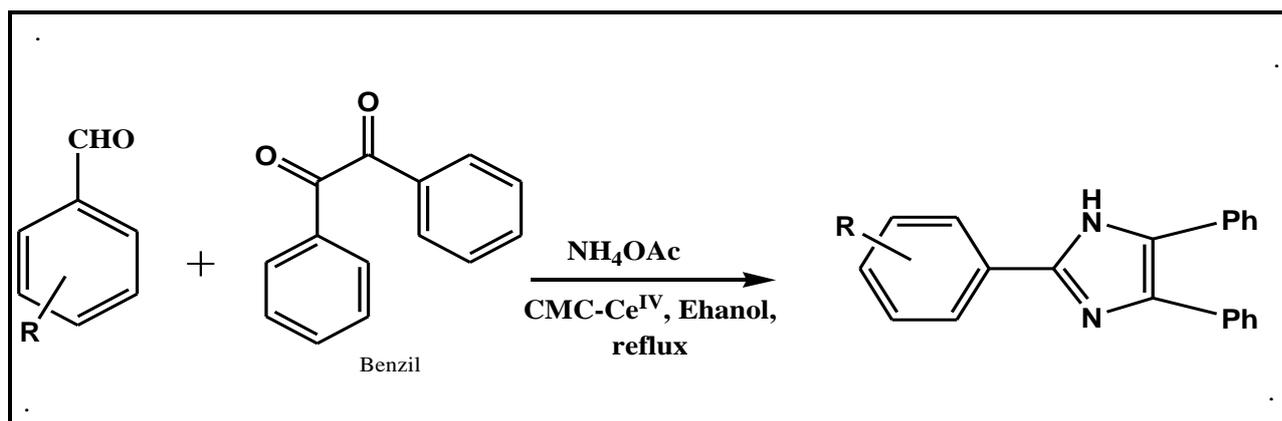
It was found that, the 20 mg catalyst is sufficient to push the reaction forward. To investigate the role of solvent in model reaction was performed in different solvent like ethanol, water, 50% ethanol or another organic solvent.

**Table 1: Optimized amount of catalyst loaded**

Entry	Catalyst (mg)	Time (min)	Yield (%)
1	5	80	60
2	10	50	82
3	15	35	90
4	<b>20</b>	<b>15</b>	<b>94</b>
5	25	15	93

It was observed that, when we used pure ethanol as a solvent the yield of product increases up to 97% and time also reduced about 10 min. Imidazole formation was increases in ethanol, while the same reaction occurred slowly in water and another organic solvent.

After the study of above optimized reaction conditions were explored for the synthesis of series of 2,4,5 triaryl-imidazole derivatives from various substituted benzaldehydes and benzil efficiently using CMC-Ce<sup>IV</sup> catalyst as shown in **Scheme 1** and the results are summarized in Table 2.



**Scheme 1: Synthesis of 2,4,5 triaryl-imidazole derivatives by using CMC-Ce<sup>IV</sup> as catalyst**

### Spectral data of compounds (1-4)

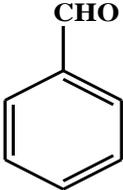
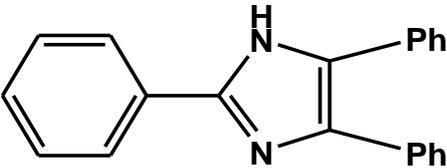
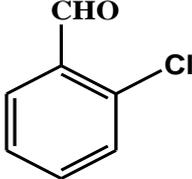
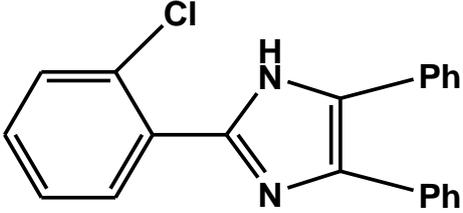
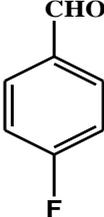
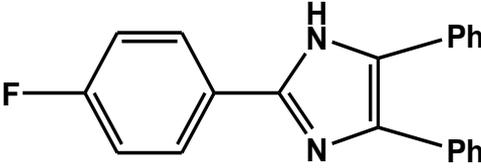
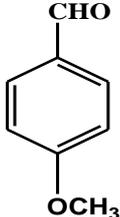
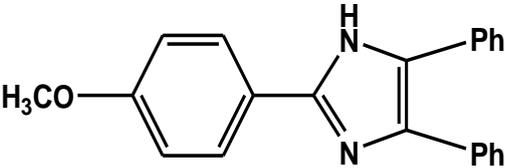
1) Cream White solid, IR (cm<sup>-1</sup>): 3340(N $\tilde{H}$ ); 1668(C=N), 1587(C $\tilde{N}$ ), 1320 (C $\tilde{N}$ ), 1510 (C=C aromatic), 3061(C= $\tilde{C}H$ ).

2) Yellow solid, IR (cm<sup>-1</sup>): 3319(N $\tilde{H}$ ); 1670(C=N), 1583(C $\tilde{N}$ ), 1311 (C $\tilde{N}$ ), 1520 (C=C aromatic), 3064(C= $\tilde{C}H$ ).

3) Lemon Yellow solid, IR (cm<sup>-1</sup>): 3322(N $\tilde{H}$ ); 1648(C=N), 1590(C $\tilde{N}$ ), 1313 (C $\tilde{N}$ ), 1534 (C=C aromatic), 3066(C= $\tilde{C}H$ ).

4) Brown solid, IR (cm<sup>-1</sup>): 3340(N $\tilde{H}$ ); 1668(C=N), 1579(C $\tilde{N}$ ), 1315 (C $\tilde{N}$ ), 1519 (C=C aromatic), 3067(C= $\tilde{C}H$ ).

**Table 2: Synthesis of 2,4,5 triaryl-imidazole derivatives (1-4)**

Sr. No.	Substituted benzaldehydes	Product	Time (min)	Yield (%)	Melting Point (°C)
1.			20	88	210
2.			25	92	250
3.			20	90	252
4.			15	94	212

### Conclusion:

The CMC-Ce<sup>IV</sup> NPs were prepared by the ion exchange reaction. The inclusion phenomenon of sodium carboxymethyl cellulose with ceric ammonium nitrate was successfully characterized by FT-IR techniques. We have developed a simple and efficient protocol for one-pot synthesis of 2,4,5 triaryl-imidazole derivatives from various substituted benzaldehydes and benzil efficiently using CMC-Ce<sup>IV</sup> as a catalyst. The high catalytic activity of CMC-Ce<sup>IV</sup> was accounted due its Lewis acid sites. The advantages of procedure includes simplicity of operation, good yields, wide substrate scope, no chromatographic separation technique, an easy recovery of the catalyst and recyclability of catalyst.

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# Efficient One Pot Synthesis of Schiff's Bases By Using CMC-Cu<sup>II</sup> Catalyst

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## Abstract

CMC-Cu<sup>II</sup> was prepared by metathesis strategy and characterized by FT-IR techniques. The ensuring catalyst has been successfully applied in the one-pot four-component reaction of various aromatic aldehyde and substituted aniline to the synthesis of Schiff's Bases. The catalyst was recovered and reused for five cycles without considerable loss of activity. The advantages of the protocol include rapid reactions with good yields and simple workup. The synthesized compounds were characterized by FT- IR technique.

**Keywords:** CMC-Cu<sup>II</sup>; Schiff's Bases; Metathesis Reaction; Multi-component reaction.

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## Introduction

Green chemistry techniques continue to grow in importance. The aim of green chemistry involved the use of raw material obtained from renewable sources, environmentally benign catalysts and reagents. Also involved the developments of more efficient protocols.<sup>1</sup>

A Schiff base is a compound with a functional group that contains a carbon-nitrogen double bond with the nitrogen atom connected to an aryl or alkyl group<sup>2</sup>. Many biologically important Schiff base have been reported which possess antibacterial,<sup>3</sup> antifungal,<sup>4</sup> antimicrobial<sup>5</sup> and anti-HIV<sup>6</sup> activities. Owing to their wide range of biological advancement, synthesis of title compounds are still of intrigue.

The interest in metal nano particles ((NPs), attributable to their high surface area, incredible availability, high biocompatibility and low harmfulness, has developed significantly. In addition, the high catalytic activity of metallic NPs can be accounted due to its Lewis acid site.<sup>7</sup> The stabilization of NPs on suitable stabilizing agents presents some advantages for example increases in NPs reactivity, stability, selectivity, reusability, easy separation and recovery from reaction mixture and decreased agglomeration. Multi-component reactions (MCRs) are eco-friendly process as they obey green chemistry principles.<sup>8</sup>

Considering these facts, we have decided to synthesize Schiff bases of various substituted benzaldehydes and aromatic amines efficiently using CMC-Cu<sup>II</sup> as a recoverable and reusable catalyst in ethanol as a solvent via. Multi-component reactions (**Scheme 1**).

## Materials and Methods

All reagents used were of laboratory grade. Melting points were determined in open capillaries. The purity of compound was checked by TLC. IR spectra were recorded on Shimadzu FT-IR (Affinity Model) using KBr.

### Preparation of Cu(II)carboxymethylcellulose (CMC- Cu<sup>II</sup>) Catalyst

The Cu(II) carboxymethylcellulose (CMC- Cu<sup>II</sup>) catalyst was prepared by metathesis reaction of copper sulphate and Na-CMC. The sky blue solid was precipitated which was left to equilibrate in a solution for overnight. The resulting sky blue solid was separated from the solution and washed thoroughly with distilled water. The wet CMC-Cu<sup>II</sup> was dried at 70<sup>0</sup>C in the oven till constant weight.

### General procedure for synthesis of Schiff's base by using CMC-Cu<sup>II</sup> as catalyst:

In 100 ml beaker, a mixture of substituted benzaldehyde (10 mmol), substituted aniline (10 mmol) and CMC-Cu<sup>II</sup>, (10 mg) was taken in 2 ml ethanol and stirred vigorously at room temperature for appropriate time (Table 3). The precipitate thus obtained was filtered off and wash with ethanol and then purified by recrystallization from ethanol to get corresponding Schiff's base in pure and crystalline form as shown in **Scheme 1**.

## Results and Discussion

### Optimized Reaction Conditions:

To optimize the reaction condition, we performed the model reaction with different amount of Cu<sup>II</sup>-CMC catalyst loaded as shown in **Table 1**.

**Table 1: Optimized amount of catalyst loaded**

Entry	Catalyst (mg)	Time (min)	Yield (%)
1	5	5	71
2	10	5	92
3	15	5	84
4	20	17	69

Here we found that, the 10 mg catalyst is sufficient to push the reaction forward.

The yield of product without catalyst is only about 50% and the time required was also more i.e. about 30 min to complete the reaction.

To investigate the role of solvent in model reaction was performed in different solvent like ethanol, water, 50% ethanol or another organic solvent as shown in **Table 2**.

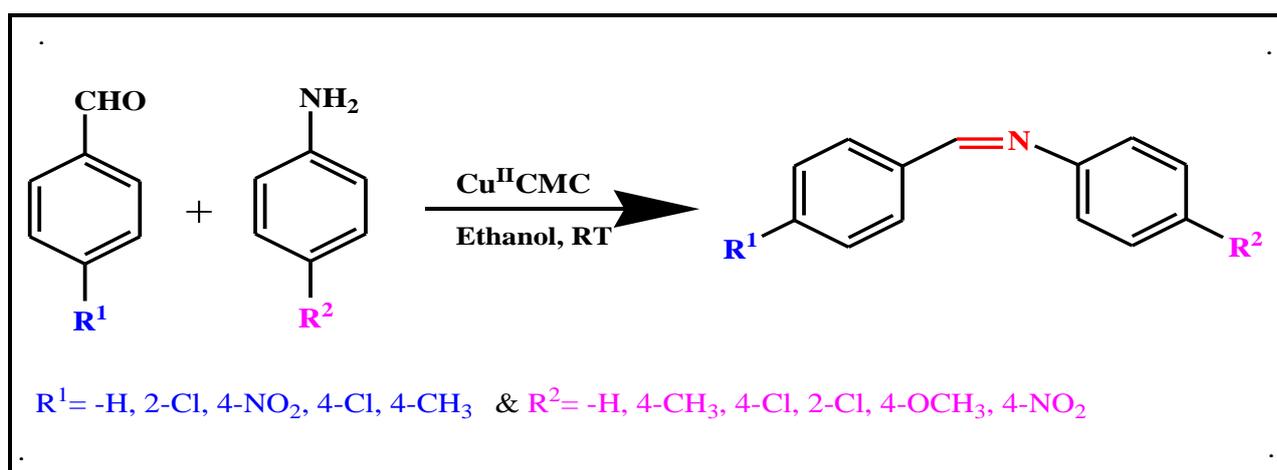
It was observed that, when we used ethanol as a solvent the yield of product increases up to

90% and time also reduced about 10 min. Schiff base formation was increases in ethanol, while the same reaction occurred slowly in water and another organic solvent.

**Table 2: Effect of solvent on synthesis of Schiff's base**

Entry	Solvent	Catalyst(mg)	Time(min)	Yield (%)
1.	Solvent free	10	30	50
2.	Water	10	17	46
3.	30% aq. Ethanol	10	10	69
4.	50% aq. Ethanol	10	5	82
5.	<b>Pure Ethanol</b>	10	<b>5</b>	<b>92</b>
6	DMSO	10	30	49
7	Toluene	10	25	52

After the study of above optimized reaction conditions were explored for the synthesis of series of Schiff's base from substituted benzaldehyde and substituted aniline catalyzed by CMC- $\text{Cu}^{\text{II}}$  as shown in **Scheme 1** and the results are summarized in **Table 3**.



**Scheme 1: synthesis of Schiff's base by using CMC- $\text{Cu}^{\text{II}}$  as catalyst**

### Spectral data of compounds (1-4)

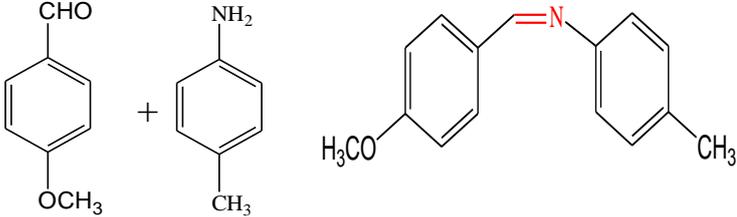
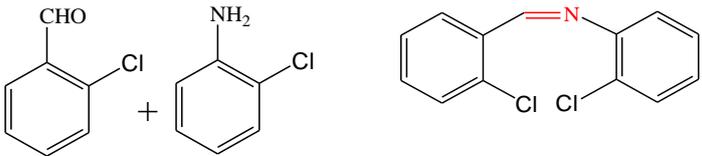
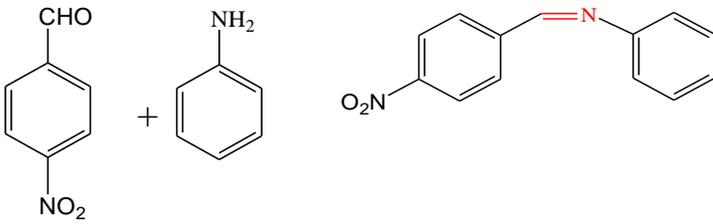
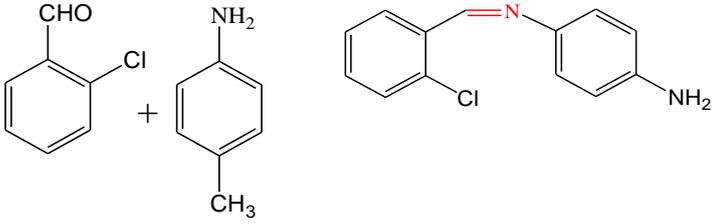
1) Olive green solid, **IR** ( $\text{cm}^{-1}$ ): 1678(C=N), 1292(C-N), 1242 (C-O), 1504 (C=C aromatic), 3007(C=C-H), 2941(C-C-H).

2) Lemon Yellow solid, **IR** ( $\text{cm}^{-1}$ ): 1693(C=N), 1274(C-N), 1460 (C=C aromatic), 3064(C=C-H), 2924(C-C-H).

3) Lemon Yellow solid, **IR** ( $\text{cm}^{-1}$ ): 1622(C=N), 1352(C-N), 1352 (N-O), 1517 (C=C aromatic), 3080(C=C-H), 2922(C-C-H).

4) Yellow solid, **IR** ( $\text{cm}^{-1}$ ): 1691(C=N), 1296(C-N), 1624 (C=C aromatic), 3064(C=C-H), 2870(C-C-H).

**Table 3: Synthesis of Schiff's base derivatives (1-4)**

Sr. No.	Starting Compounds	Product	Time (min)	Yield (%)	Melting Point (°C)
1.		5	92	92	
2.		10	90	102	
3.		5	89	44	
4.		34	87	110	

### Conclusion:

The CMC-Cu<sup>II</sup> NPs were prepared by the ion exchange reaction. The inclusion phenomenon of sodium carboxymethyl cellulose with copper sulphate was successfully characterized by FT-IR techniques. We have developed a simple and efficient protocol for one-pot synthesis of Schiff's bases from substituted benzaldehyde, and substituted aniline using Cu<sup>II</sup>CMC as a catalyst. The high catalytic activity of CMC-Cu<sup>II</sup> was accounted due its Lewis acid sites. The advantages of procedure includes simplicity of operation, good yields, wide substrate scope, no chromatographic separation technique, lack of by-products, avoiding use of hazardous solvents, an easy recovery of the catalyst and recyclability of catalyst.

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## Synthesis of Azo Dyes by Using Grinding Method

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### Abstract

The efficient and green approach for the synthesis of azo dyes has been developed by diazo coupling reactions of aromatic amines with  $\beta$ -Naphthol catalyzed by Lewis acid by grinding method at room temperature. This green methodology aims to overcome the limitations and drawbacks of the previously reported methods. Moreover the attractive advantages of the process include mild condition with excellent conversions, simple product isolation process and inexpensive procedure.

**Keywords:** Azo dyes, Coupling reaction, Lewis acid, Green reaction.

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### Introduction

Green chemistry techniques are an alternative process to help to conserve resources and can reduce costs. The replacement of conventional solvents with green solvent ethanol which is harmless to health and is available in large quantities is an interesting basic approach along this line.<sup>1-3</sup>

The diazo coupling reactions involving aromatic diazonium salt can be regarded the most versatile reaction in organic chemistry.<sup>4-6</sup> Synthesis of azo dyes by grinding under solvent free condition afforded azo dyes in 25-70% yield. In grinding three components mixture electron donor (coupling agent), sodium nitrate and amines formed colour reaction mixture.<sup>7</sup>

We have used the  $\text{AlCl}_3$  as a Lewis acid for the synthesis of Azo dyes from aromatic amines, sodium nitrate and coupling agent at ice cold condition in water as solvent with good to excellent yield within 5-10 min as shown in **scheme 1**.

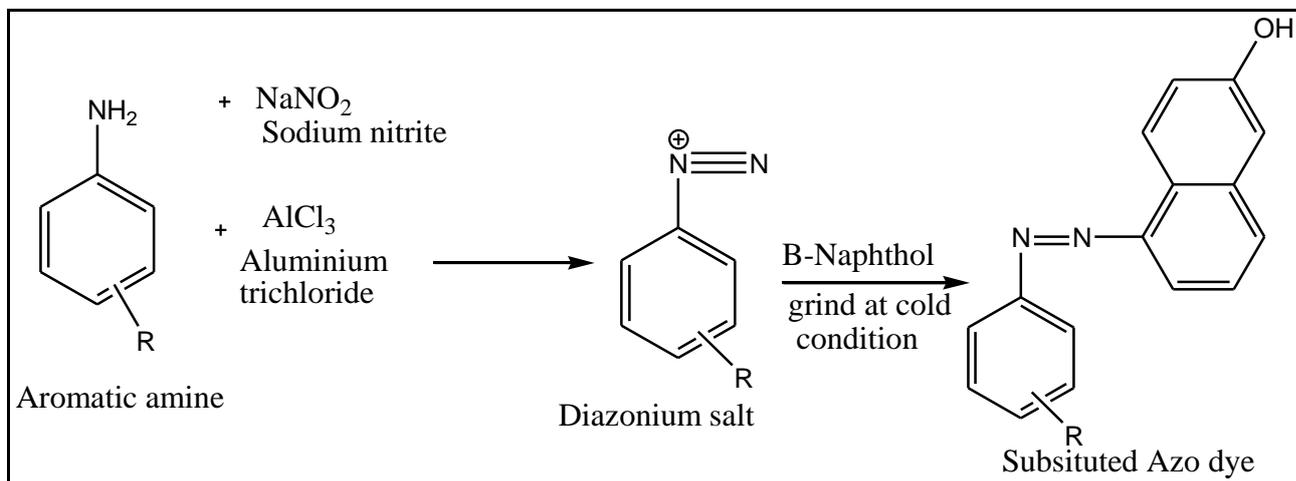
### Materials and Methods

All reagents used were of laboratory grade. Melting points were determined in open capillaries. The purity of compound was checked by TLC. IR spectra were recorded on Shimadzu FT-IR (Affinity Model) using KBr.

### General Procedure for Synthesis of Substituted Azo dyes

Aromatic amine (10 mmol), sodium nitrite (10 mmol) and  $\text{AlCl}_3$  (20% of 10 mmol) as a catalyst were taken in a beaker with ice cold condition. This cold mixture was transferred to

mortal and add coupling agent i.e. B-Naphthol (10 mmole) and then grinded for 10 min with 2 drops of water. The reaction was monitored by TLC. The solid product was obtained from an intermediate melt and then was laid up at room temperature for 5 to 10 min. The mixture was transferred to cold water and then was filtered off. The crude product was recrystallized by ethanol as per **scheme 1**.



**Scheme 1: Synthesis of substituted Azo dyes using Grinding Method**

## Results and Discussions

### Optimized Reaction Conditions

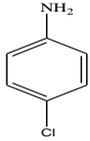
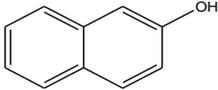
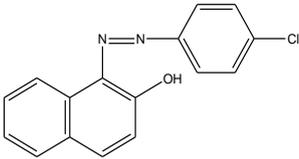
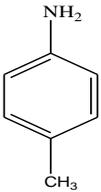
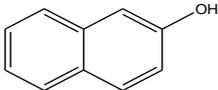
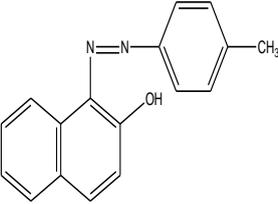
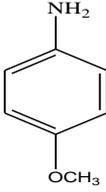
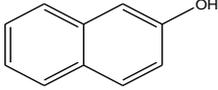
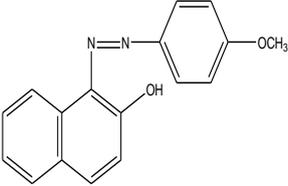
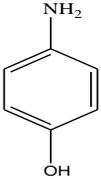
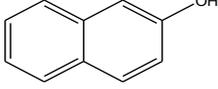
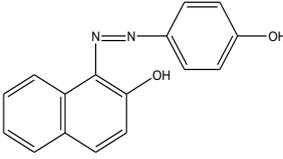
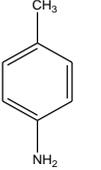
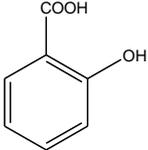
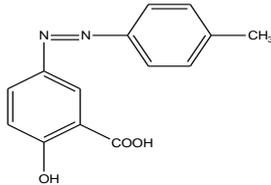
The reaction condition for amount of catalyst was optimized by carrying out the model reaction of 4-Chloroaniline with sodium nitrite, aluminium trichloride, and B-Naphthol, grind at cold condition with and without catalyst. It was observed that only 60% of the solid product was obtained for one hour reaction time. When the reaction was performed with Glycine as a catalyst, increase in the yield was noticed significantly as summarized in Table 1. Use of 20 mol% Glycine was sufficient to precede the reaction forward.

Higher amounts of the catalyst of the catalyst did not improve the results to a greater extent. So, aluminium trichloride was an efficient catalyst and 20 mol% aluminium trichloride was chosen as a quantitative catalyst for reaction, the result is summarized in Table 2.

**Table 1: Optimized amount of catalyst loaded**

Entry	Catalyst (mole %)	Time (min.)	Yield (%)
1	0	40-30	60
2	5	25	80
3	10	20	85
4	15	15	88
5	20	10	93
6	25	10	93

**Table 2: Synthesis of substituted azo dyes**

Sr.No.	Starting Compound	Coupling Compound	Product	Time (min.)	Yield (gm)	Melting Point(°C)
1.				10	93	168-170
2.				15	89	130-132
3.				10	92	136-138
4.				10	90	190-194
5.				15	90	132-134

### Spectral data of compounds (1-5)

#### IR Stretching frequency of the compounds 1 to 5

- 1) IR  $\text{cm}^{-1}$ : 3434 (Ar-OH), 1618 (C=C), 1496 (N=N), 1142  $\text{cm}^{-1}$  (C-O)
- 2) IR  $\text{cm}^{-1}$ : 3420 (Ar-OH), 1615 (C=C), 1500 (N=N), 1153  $\text{cm}^{-1}$  (C-O)
- 3) IR  $\text{cm}^{-1}$ : 3435 (Ar-OH), 1619 (C=C), 1457 (N=N), 1205  $\text{cm}^{-1}$  (C-O)
- 4) IR  $\text{cm}^{-1}$ : 3445 (Ar-OH), 1620 (C=C), 1486 (N=N), 1253  $\text{cm}^{-1}$  (C-O)
- 5) IR  $\text{cm}^{-1}$ : 3424 (Ar-OH), 1646 (C=C), 1500 (N=N), 1121  $\text{cm}^{-1}$  (C-O)

#### Conclusion

We have developed a green and simple method for the synthesis of substituted azo dyes from aromatic amine, sodium nitrite, and 20 mol% aluminium trichloride. These moieties having

broad application scope in pharmaceuticals. All the products were simply purified by recrystallization from water and hence the method is free from column chromatographic purification. Mild reaction conditions, use of green solvent, high atom economy and the lack of by-products are among the other advantages of this method.

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# **MICROBIOLOGY**

# **Studies on Effect of Mono-Sodium Glutamate (MSG) On Growth of Microorganisms**

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## **Abstract**

Monosodium glutamate (MSG), the sodium salt of the non-essential amino acid-glutamic acid, commonly known as Ajinomoto, is the most widely used flavour enhancer. Though there are certain claims regarding the safety in the usage of Monosodium Glutamate in food, conflicts do exist among the public and the Governing bodies. The present study, focus on the evaluation of effect of MSG against two species of Gram-negative bacteria. The growth kinetics of the organism under MSG was studied and was compared with non-monosodium glutamate growth curves. The Bacterial species shows high degree of tolerance to MSG also effect of different pH and temperature condition on digestion of MSG was evaluated.

**Keywords-** Ajinomoto, monosodium glutamate (MSG)

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## **Introduction**

Food industries employ the addition of certain agents, to preserve the food or enhance the texture, color or flavor, without deteriorating the food constituents and its quality. Traditionally, ingredients high in free amino acid are used to improve the palatability. The IUAPC name of compound is Sodium 2-Aminopentanedioate also known as Ajinomoto or Monosodium glutamate (MSG) is used in food for sour flavor. (Tushar, 2017) The U.S. Food and Drug Administration (FDA) have declared MSG to be a "Generally Recognized as Safe" (GRAS) ingredient. Under normal conditions, humans can metabolize relatively large quantities of glutamate, which is naturally produced in the gut by exopeptidase enzymes in the course of protein hydrolysis. The use of MSG as a food additive and the natural level of glutamic acid in foods are not toxicological concerns in humans. A popular belief is that large doses of MSG can cause headaches and other feelings of discomfort, known as 'Chinese Restaurant Syndrome' (CRS), but double-blind tests fail to find evidence of such a reaction. The European Union classifies it as a food additive permitted in certain foods and subject to quantitative limits. Although we are still remain oblivious to effect of Ajinomoto (Monosodium glutamate (MSG) on bacteria. The present study was focused to evaluate the effect of Ajinomoto on isolated organism from human gut flora to give better understanding of its effect on growth of microbes.

## **Materials and methods**

### **Collection of sample**

The Monosodium glutamate was procured from local market of Jalgaon, Maharashtra, India.

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Properties of MSG Monosodium glutamate (MSG, also known as sodium glutamate; IUPAC name- Sodium 2- aminopentanedioate is the sodium salt of glutamic acid, one of the most abundant naturally occurring non-essential amino acids (Tushar, 2017)

#### **Isolation of microbes from human gut flora**

Stool sample of three month old baby was obtained aseptically and enriched in 100 ml nutrient broth at 37<sup>0</sup>C for 24 hrs in incubator. To isolate the gut flora the loopful aliquot from enriched broth was streak on the sterile Nutrient agar plate and incubated the plates at 37<sup>0</sup>C for 24 hrs in incubator.

#### **Characterization of isolates**

Isolate were characterized according to the procedures given in Aneja KR. All strains were subjected to the biochemical test and carbohydrate fermentation (Glucose, mannitol, sucrose)

#### **Effect of Monosodium Glutamate on Growth of Isolated organism -**

Four flasks containing 100 ml of nutrient broth and 1% of Monosodium Glutamate were inoculated with 1ml broth culture of Isolates A, B, C and D respectively. These flask were incubated for 24 hrs at 37<sup>0</sup>C and growth was measured and 600nm.

#### **Tolerance of isolates to different concentrations of Monosodium Glutamate**

To find out the tolerance to Monosodium Glutamate concentrations the isolates subjected to different concentration prepared in Nutrient broth were incubated for 24 hrs at 37<sup>0</sup> C and growth was measured and 600nm .

#### **Effect of temperature on organisms in presence of Monosodium Glutamate**

The isolates were subjected to different temperature such as 37°C, 45 °C, 50°C and incubated for 24 hrs then subjected to spectrophotometer analysis.

#### **Effect of pH on organisms in presence of Monosodium Glutamate**

The pH of medium was adjusted 5, 6,7,8 and 9 using 0.1N hydrochloric acid (HCl) and 0.1N Sodium hydroxide (NaOH) . The medium was incubated at 37°C for 24hr then subjected to spectrophotometer analysis.

#### **Results and Discussions**

The present study was aimed to isolate microbes from human gut and to evaluate the effect of various environmental factors such as pH, temperature and different concentrations of Monosodium Glutamate on growth kinetics of these microbes.

Two microbial isolates were isolated from human gut sample and identified biochemically as *Escherichia coli* *Pseudomonas aeruginosa* respectively.

#### **Effect of Monosodium Glutamate Concentration on growth of isolates**

It was found that Monosodium Glutamate does not inhibit growth of the isolates at any concentration. Increasing concentration of Monosodium Glutamate lead to a decrease in the number of bacteria recovered but not restricts the growth completely. Higher growth rate was observed up to 500 mg/L of concentration and moderate growth was observed at 500-750 mg/L. The growth was completely restricted at 1000 mg /L of monosodium glutamate concentration.

No significant Effect of Monosodium Glutamate was found on growth kinetics of isolates when grown in presence and absence of monosodium Glutamate for 48 hrs. Also the growth at different temperature and pH in presence of monosodium Glutamate affected the growth of isolates.

**Table1.0 Effect of Monosodium Glutamate on growth kinetics of isolates**

Growth Response				
	Presence of MSG		Absence of MSG	
Time Hours	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>
0	0.05	0.05	0.05	0.05
6	0.19	0.19	0.17	0.35
12	0.24	0.09	0.22	0.15
18	1.27	1.21	1.55	1.31
24	1.55	1.53	1.71	1.69
30	1.72	1.71	1.72	1.72
36	1.76	1.73	1.78	1.81

All value recorded as OD at 600nm

**Table 2.0 Growth of Isolates in different pH in presence of MSG**

pH	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>
5	1.351	1.388
6	0.852	0.815
8	1.313	1.630
9	0.198	0.299

**Table 3.0 Optical density (600nm) of Isolatesat different Temperature in presence of MSG**

Temperature	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>
RT	1.449	2.005
37 <sup>0</sup> C	0.108	0.114
45 <sup>0</sup> C	0.424	0.455
50 <sup>0</sup> C	--	--

### Conclusion

- Current study focuses on effect of MSG on human gut flora.
- The organisms were isolated from human gut in presence of MSG showed accelerated growth as compared to growth without monosodium glutamate.
- Isolates may have taken MSG in metabolic pathway accelerating its growth
- The isolates showed the high degree of tolerance to MSG at elevated temperature, pH.

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# **Larvicidal activity of proteins extracted from *Bacillus Subtilis* against vector mosquitoes**

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## **Abstract**

Mosquito larvicidal activity was rendered by *Bacillus* species was found to be effective in control of the mosquito larvae. The extracellular proteins from the isolates were extracted and tested for its mosquito larvicidal activity against larvae of *Aedes* mosquito. The proteins were evaluated for its toxicity and the percentage mortality against the larvae was determined. The result revealed that 80 % mortality against *Aedes* at 200 mg, This study concludes that non spore formers of common microbial isolates from the natural environment were also able to kill the mosquito larvae through their protein which are non-toxic to human population.

**Keywords** *Bacillus*, *Aedes*, larvicidal activity etc.

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## **Introduction**

Mosquitoes transmit disease agents which are responsible for more than 500 million clinical cases estimated by the World Health Organization. The increase in number of resistant varieties of mosquitoes, ineffectiveness of chemical insecticides, necessitates the development of vector control strategies. Thus microbial insecticides can be considered as alternatives to chemical insecticides. Mosquitoes are the disease causing vectors within almost all tropical and subtropical countries are responsible for the transmission of pathogens causing some of the most life threatening and debilitating diseases of man, like malaria, yellow fever, dengue fever, chikungunya, filariasis, encephalitis, etc. (Chandra et al 2008) There is no specific treatment for these vector borne diseases. There is provocative interest in research for larvicidal compound from natural sources. (Kishore et al 2006) The indiscriminate use of neurotoxic insecticides problems to non target organisms and insecticides resistance. The first insecticidal component of *Bacillus Subtilis* used in mosquito control is acting by the production of toxin during sporulation and vegetative stages of *Bacillus subtilis*. Control of such diseases is becoming increasingly difficult because of increasing resistance of mosquitoes to pesticides (Plearnpis, 2001). Control of such diseases is becoming increasingly difficult because of increasing resistance of mosquitoes to pesticides. Therefore the need for alternative, more effective and environmental friendly

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control against mosquitos become obligatory. In Present study soil samples from garden were collected and used for screening of bacteria. In this paper attempt has been made to find out alternative, more effective and environmental friendly control against mosquito's by using microbial derived larvicidal compound.

## **Material and Methods**

### **Collection of mosquitos' larvae**

Larvae were collected from various breeding habitats such as around households, waste water, store waters, ponds, ditches, plastic containers .Larvae density was calculated by the following

$$\text{Larvae density} = \frac{\text{Number of Larvae collected}}{\text{Number of dips}}$$

Collected larvae were maintained in plastic jars. Larvae collected from fields were morphologically identified according to the classification keys provided in photographic manual of mosquito identification

### **Soil and water sample collection**

Soil and water samples were collected from P. G. College campus, Maharashtra. Total 20 samples comprising soil and water samples were collected.

### **Screening and Isolation of microorganisms**

The soil and water samples were enriched in Nutrient broth & incubated at 37<sup>0</sup>C for 48h after enrichment period; one loopful broth was inoculated on to Nutrient agar medium and incubated at respective temperatures. Colonies thus obtained were isolated, identified and were utilized to study their mosquito larvicidal activity.

### **Maintenance of culture**

Isolate was maintained in triplicates on nutrient agar medium and incubated at 37<sup>0</sup>C for 24 h. Then slants were stored for further investigation.

### **Larvicidal assay**

Isolated bacteria were inoculated in sterile nutrient broth and incubated for 2 weeks. The enriched broth was centrifuged at 5000 rpm for 20 minutes. The cell free supernatant obtained was used for testing its toxicity towards mosquito larvae. For Preliminary testing ten larvae of mosquito vector *Aedes* were introduced in each of the test solution as well as the control. For each of the dose three replicates were maintained at a time. All the isolates were incubated at room temperature up to 48hrs. Percentage mortality of effective isolates was determined after 24hrs and toxicity assay was carried out for all the bacterial isolates and water was kept as a control

### Extraction of Metabolite from the Bacterial Isolates

The larvicidal compounds were extracted from bacterial isolates by filtering Broth and then centrifuged at 15000 rpm, for 20 min to obtain cell free supernatant. Equal volume of ethyl acetate was added to the cell free supernatant and kept under shaker conditions for 1 hour. Metabolite was extracted as middle layer after allowing the mixture to settle in separating funnel. The layer of secondary metabolite was collected. Ammonium sulphate precipitation of extracted metabolite was done. Purification was performed by dialysis method.

### Results and Discussion

Five bacterial isolates were isolated and used for larvicidal activity. The effective isolate were identified based on their morphological and Biochemical characteristics.

### Determination of larvicidal activity

For the screening 13 microbial isolates were used .The isolate was considered most toxic strain ensuring mortality rate was 97% at 12 hrs exposure.

### Evaluation of larvicidal Activity of crude extract of *Bacillus Subtilis*

**Table 1. Determination of larvicidal Activity**

Sr. No.	Concentration (mg)	Mortality			
		2 Hrs.	4 Hrs.	8 Hrs.	12 Hrs.
1	50	0	0	0	35
2	100	0	0	0	50
3	150	0	0	40	60
4	300	0	0	55	80
5	500	0	0	65	97

It is evident from the Table 1 that the mortality rate is dependent on concentration of protein more than 50 %mortality is considered as effective concentration. Lowest activity was recorded at 50 mg which on increasing ten times concentration is reached to maximum.

### Conclusions

- An efficient larvicidal culture was isolated from soil sample.
- The effective isolate was partially characterized.
- The microbes were used for their larvicidal activity to control of mosquito larvae.
- This study concludes that non spore formers of common microbial isolates from natural environment were also able to kill mosquito larvae through their metabolites which are non toxic to human population.

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## **Effect of Environmental Stresses on Food Borne Microbes**

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### **Abstract**

Some microorganisms can induce adaptive responses to environmental stresses, which can enhance their tolerance to these stresses and may promote persistence under adverse conditions. Stress responses of food borne pathogens can have profound effects on their survival in foods. The exposure to sub lethal stress may produce a spectrum of adaptive responses for various stresses like chemical and physical like pH, temperature salt and UV light effect shows microbial tolerance indicate that variation in the magnitude for each parameter studied. Understanding the mechanisms underlying the microbial responses to different stresses will improve the effective use of intervention strategies to inhibit the survival of pathogens in foods.

**Keywords:** Food borne, Stress, Stress Response,

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### **Introduction**

Microorganisms play an important role in production, storage and consumption of foods. They are found in water, air, soil and in foods. Micro-organisms also perform useful functions in some branches of the food industry. One of the major limitations is microorganisms cause contamination of food that may cause spoilage. (Hurst,1977) Similarly, when food is exposed to room temperature and not refrigerated, it may get spoiled, due to the occurrence of micro-organisms. Foodborne pathogens face a broad spectrum of stresses in all links of the food chain. (World Health Organization, 2015) During traditional food processing microbial cells are more likely to be killed than injured or stressed. Microorganisms can tolerate small changes in environmental parameters through inducing adaptive responses. Among the known foodborne outbreaks, there is an increasing involvement of stress-adapted strains, which are difficult to control with traditional intervention strategies. (Bouwknegt, et al. 2017) Adaptive responses of foodborne pathogens to stresses are thus of paramount significance in food safety. Stresses to these microorganisms in foods during processing include physical stresses, such as heat, high pressure, desiccation, and irradiation, chemical stresses, such as acids, salts, and oxidants, and biological stresses, such as microbial antagonism.

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## Methods and Materials

**Sample Collection-** the Mix fruits pulp, cheese, Bread was purchased from local market.

Isolation and Screening of bacteria enrichment

For the isolation Sterile Nutrient Agar plate are prepared. The sample was serially diluted (10-10) in sterile saline solution. 1ml of appropriate dilution streaked on Nutrient agar plated and Incubated the plate at 37<sup>0</sup> C for 24-48hrs.



**Fig 1. Sample Collection-Mix Fruit Pulp, Cheese, Bread**

### Acid Tolerance pH

Acid Tolerance was studied by sterile nutrient agar medium adjusted 1.0 pH 3 to 10. With 0.1 N HCl or 1% NaOH. 0.1 ml culture suspension of each isolate was spread on the respective Plates. All the plates are incubated at 37<sup>0</sup>C for 24 hrs. Isolates which were growing on agar were considered to be acid tolerant strains.

### Temperature Tolerance

The temperature tolerances was determined using sterile nutrient agar medium plates inoculated with 0.1ml culture suspension of each isolate and were incubated at respective temperature.

### Salt tolerance

Sterile nutrient agar medium adjusted to NaCl concentrations from 0.5to 3.0% 0.1ml culture suspension of each isolate was spread on the respective plate.

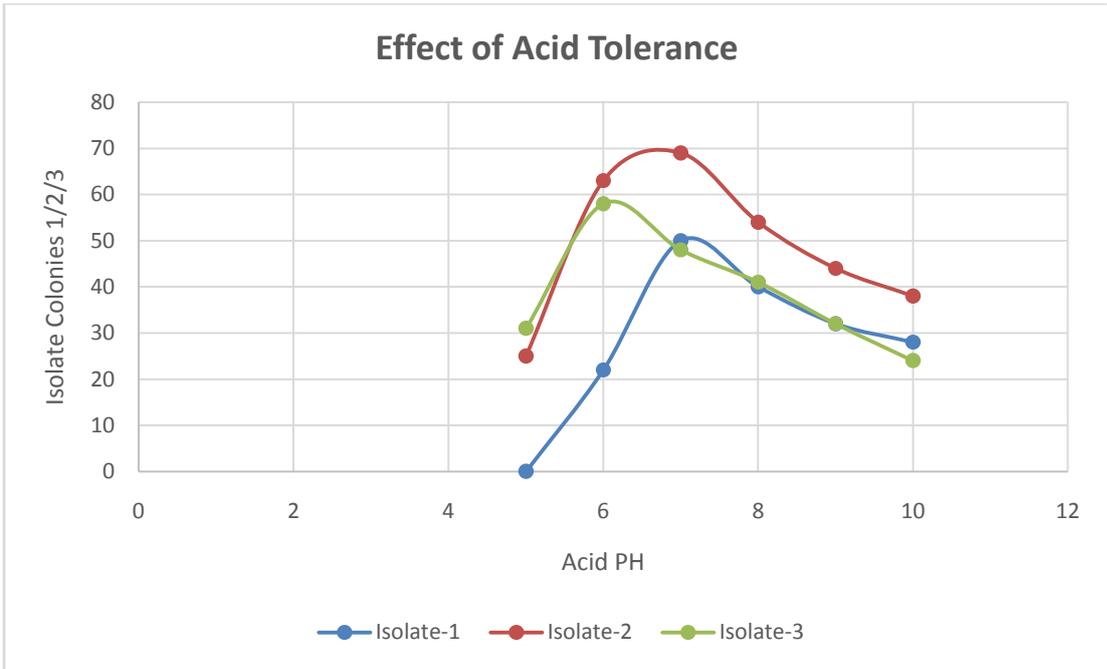
### UV Radiation

Sterile nutrient agar plates were exposed to UV light in chamber for varying time interval (30 to 180 sec.) a set for control. Then above plates were incubated 30<sup>0</sup> C for 24 hrs.

## Results and Discussions

### Primary screening of isolation Bacteria

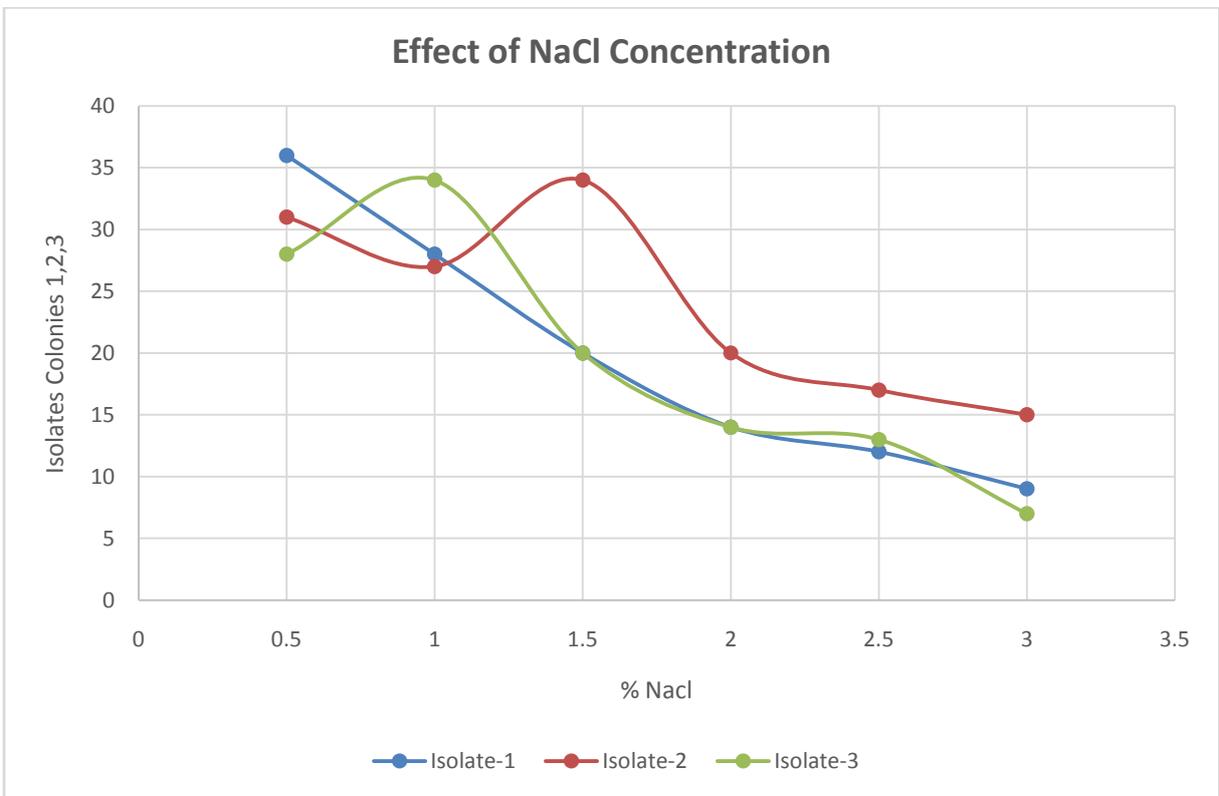
From different samples, a total of 3 isolates were obtained. The isolate was identified by morphological characteristics and gram staining as per procedure given by Aneja. The colonies were randomly selected and identified as *Escherichia coli*, *Salmonella typhimurium*, *Listeria monocytogenes*



**Fig.1 Acid Tolerance**

Optimum pH for isolate FB1 and FB2 was 7 and 6 with growth inhibition at pH 5.5 and 6, respectively. In contrast, FB3 exhibited optimum growth at pH 7.

The Isolates did not show grow under temperature stress at 10° C, and 60° C.



**Fig. 2 Effect of NaCl**

The isolates did not shown growth above 2.5% NaCl concentration and experience stress

**Table .1 Effect of UV Radiation**

Time [Sec.]	Number of organisms survive after UV treatment		
	FB1	FB 2	FB3
30	32	48	36
60	24	43	28
90	22	19	20
20	3	14	13
150	8	9	11
180	1	7	6

Isolate exhibited maximum resistance to UV light with no inhibition till 3 mins of UV exposure, respectively

**Conclusion**

Food borne pathogenic bacteria have adapted to face the challenges of changing environment in the form of the stress.

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## Studies on Bioethanol Production Using Orange Peel

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### Abstract

This paper deals with the development of a citrus peel waste biorefinery that employs low environmental impact technologies for production of ethanol. The present study focuses on production of second generation bioethanol, which is distinguish from the first generation and subsequent generations of biofuels by its use of lignocellulosic biomass as raw material. Orange (*Citrus sinensis*) peel waste was used as substrate for bio-fuels production. The fermenting microorganisms obtained through screening of microorganisms to optimize its use of xylose accumulated in the hydrolysate. The isolation and characterization of stress tolerant, high potential ethanol producing yeast strains from various fruit peel was done. Yeast from pineapple, orange, grape, wine have been isolated, characterized on the basis of morphological and physic-chemical characters. Optimization for various physical and chemical factors for production of bioethanol by yeast was done. The isolation and cultivation of yeast in appropriate medium has been carried out using the orange peel as substrate the results reveals that temperature 40<sup>0</sup> C and pH 4.0 give maximum yield.

**Keyword:** bioethanol, yeast, orange peel, alcohol etc

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### Introduction

Pollution and fuel crisis are two major problem of developing India. Bioethanol may be used as alternative of fossil fuel. Bioethanol is free of sulphur and its carbon is of vegetable origin, it does not contribute the global warming. A biofuel alternative to the fossil fuels used to power industries would aid in the reduction of greenhouse gases. The production of cellulosic ethanol made from citrus fruit peels makes a more efficient alternative to gasoline. The pectin, sugars, seeds, and cellulose of the orange peel is used to create the ethanol fuel. Citrus fruits are renewable and constantly demanded, making them more accessible than gasoline. Companies in agriculture production can use citrus peel ethanol as fuel for trunks and machinery. 'Their own products' waste can then serve two purposes to businesses, fuel and food. Companies who produce the citrus fruits should convert the peels into usable ethanol fuel to power their machinery used in production. Also, the ethanol fuel can be produced for other companies to use as a substitution to gasoline. Businesses then can sell citrus ethanol

along with the fruits.

## **Materials and Methods**

### **Isolation of microorganism (Yeast)**

Different samples (Grapes, Orange, Wine Bread and Soil) were collected from local market and surrounding. One gram of each sample was inoculated in 250 ml YEPD broth at 30° C for 3 days. After 3 days incubation, 100µl of suspension was spread on a plate containing YEPD agar. The plates were incubated at 30° C for 3 days. After incubation, the single colony formed was picked, and cells were observed under a microscope.

### **Preparation of Orange peels for ethanol production**

Fruit samples of oranges were collected from local market and their peels were extracted.

Orange peels were washed and, cut in small pieces and kept it in the sunlight for few days.

### **Substrate Pretreatment**

The orange peel was subjected to acid pretreatment which involves the use of sulfuric, nitric or hydrochloric acids to remove hemicelluloses components and expose cellulose for enzymatic digestion while alkali pretreatment refers to the application of alkaline solutions to remove lignin and various uronic acid substitutions on hemicelluloses that lower the accessibility of enzymes to the hemicelluloses and cellulose. The acid hydrolysis is used for physic-chemical pretreatment for substrate. 1N HCL was prepared and the substrate (dried orange peels) was soaked in it for overnight. After overnight incubation substrate were separated from HCL and wash with distill water to remove the remaining HCL content. After washing with water, the substrate were autoclaved at 121° C for 20 minutes. After autoclaving the Substrate were used for ethanol production.

### **Preparation of Growth Medium**

The growth medium (Inoculum Medium) prepared for ethanol production consists of glucose (20 g/l), Ammonium sulphate (0.8 g/l), KH<sub>2</sub>PO<sub>4</sub> (0.8 g/l), Magnesium sulphate (4 g/l), Yeast extract (3.2 g/l), in the production medium Glucose as carbon resource is replaced with orange peels in 250 ml of conical flask containing 100 ml of distilled water (pH-5.5). The flasks were autoclaved at 121°C for 20 minutes. The cells of *Saccharomyces cerevisiae* were aseptically cultured in inoculum Medium and incubated at 30°C for 24hrs.

### **Ethanol production**

Using the above prepared growth medium the isolates were process for ethanol production. The substrates concentration was chosen from 5 to 50%. After incubation every flask were centrifuge to remove cell debris and kept for distillation process.

250 ml of production medium was prepare using Ammonium Sulphate (0.8 gm/lit), KH<sub>2</sub>PO<sub>4</sub>

(0.8 gm/lit), Magnesium Sulphate (4gm/lit), yeast extract (3.2 gm/lit) and Substrate (orange peels) as carbon resource in 250 ml distilled water. Media were autoclaved at 121° C for 20 minutes. After autoclaving, media were cooled and inoculated with 5% of isolated test culture. The flasks were incubated anaerobically for 8 to 10 days. The anaerobic condition was maintained by using Teflon tape for coating cotton plugged flasks. After incubation of 8 to 10 days the fermented mixture were centrifuge at 5000 rpm for 10 minute. The supernatant were collected and debris were discarded. The supernatant were subjected to the distillation at 78.5° C (boiling point of ethanol). Distillate is collected and further checked for qualitative estimation. Qualitative estimation of ethanol was done by Potassium dichromate method

### **Results and Discussions**

Orange peels are an efficient material for production of ethanol rather than burning it with other agricultural wastes. Ethanol production from orange peels can satisfy the growing need of ethanol; present study can be economical and also reduce the agricultural waste.

### **Primary Product Isolation**

The raw ethanol yield was measured by ethanol assay using potassium dichromate method. Also the odour and colorless appearance was checked and also showed flammable property.

### **Potassium Dichromate Test**

The samples were withdrawn in every 24hrs and the changes in ethanol concentration were qualitatively estimated and at the end of fermentation at seventh day changes in ethanol concentration were quantitatively estimated using specific gravity method and it was seen that, *Sacchromyces cerevisiae* used orange peels efficiently for ethanol production in anaerobic condition than in aerobic condition and as substrate orange peels showed highest ethanol production.

### **Conclusion**

The orange peels are an efficient material for production of ethanol rather than burning it with other agricultural wastes. Ethanol production from orange peels can satisfy the growing needs of ethanol. Present study can be economical and also reduce the agricultural waste.

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## **Studies on Bio-plastic Producing Microorganisms**

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### **Abstract**

Bioplastic is a biodegradable material that come from renewable sources and can be used to reduce the problem of plastic waste that is suffocating the planet and polluting the environment. Polyhydroxyalkanoates (PHA) are storage substances produced and stored by many cells, plants and bacteria only in times when they lack important nutrients. A particular property of these plastics-related biopolymers is that their composition and hence their material properties can be regulated by the fermentation process. A PHB producing bacterial isolate have been isolated from soil samples collected from industrial effluent sewage. Initial characterization was done on basis of Sudan black dye. Positive test isolates partially characterized by procedure laid down by Aneja (2013) further test performed as per procedures. Results show 28 mg PHB produced per 100 ml. The production of PHB also depends on parameters like pH, carbon source, incubation time and NaCl concentration. Some organisms are able to combine hydroxy carbonic acids to polyesters due to their specific chemical structures.

**Keywords:** PHA, Bioplastic, Biodegradation , Polyhydroxyalkanoates

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### **Introduction**

The amount of plastic produced using petrochemical materials increases every year and the exact time needed for its biodegradation is unknown. Therefore, they contribute to environmental pollution. Biodegradable plastic (i.e. PHB) offers a good alternative to the conventional plastic but its production cost is much higher than conventional plastic. PHBs can be produced using microbial isolates and cost effective raw materials like potato waste which do not find any valuable application. Use of potato waste for PHB production may significantly reduce the production cost. Over 150 different PHAs are known, and there are good reasons for such a large variety. Cells produce hydroxy carbonic acids from standard intermediary products of the energy metabolism and fatty acid synthesis. When key components in the food supply are lacking, organisms and cells turn to alternative metabolic pathways and form PHAs. Specific metabolic pathways are chosen on the basis of the carbon source provided to the cells, which leads to the creation of PHAs with different chemical structures. This principle is almost ideal for controlling the chemical composition and the characteristics of PHAs through biotechnological processes. When long-chain carbon sources

such as palm oil are used, microorganisms will produce a larger number of PHAs consisting of short-chain 3-hydroxybutyrate and long-chain 3-hydroxyhexanoate molecules.

## **Materials and methods**

### **Collection of samples**

The samples were collected from waste water samples including industrial effluent, domestic sewage and transfer it into sterile container (Mikkili I, Karlapudi AP, Venkateswarulu TC, et al. (2014).

### **Enrichment and isolation of sample**

The soil samples (1g each) were directly inoculated in LB Medium (100 ml) and incubated at 37<sup>0</sup> C temperature for 3 days.

### **Isolation of Microorganisms**

The isolation was done on LB agar plates and then the plates were incubated at room temperature for 24hrs. The representative isolated bacterial colonies were picked up, purified and preserved on nutrient agar slants till further use. First selection of isolates in case of PHB production is depends on its colony appearances. White colored or mucoid colonies can be assumed as PHB positive. (Mikkili I, Karlapudi AP, Venkateswarulu TC, et al. 2014)

### **Rapid screening of isolates for PHB positive bacterial isolates**

All isolates to be screened for PHB production were taken and these isolates were subjected qualitatively tested for PHB production following viable colony staining method of screening using Sudan black B dye (Jaunet *al.*, 1998).

Ethanol solution of (0.05%) Sudan Black B was spread over the colonies and the plates kept undisturbed for 30 minutes. They are washed with ethanol (96%) to remove the excess stain from the colonies. The dark blue coloured colonies were taken as positive for PHA production. Of all the isolates strongly positive for the viable colony staining and were selected for further experimentation.

### **Biochemical Characterization of Isolates**

Isolate was characterized according to the experiment discussed in Aneja KR. All strains were subjected to the biochemical test and carbohydrate fermentation (Glucose, mannitol, sucrose)

### **Production and Quantification of crude Bioplastic (PHB)**

The strongly Sudan Black B positive isolates were subjected to production of Bioplastic. It was performed according to Singh and Parmar by sodium hypochlorite-chloroform method; the five selected isolates were cultured in Minimal Davis Medium at 37 °C for 3 days. After incubation, 10 ml of culture was centrifuged at 6000-rpm for 10 minutes and supernatant was

discarded. The pellet was suspended in 5 ml of 4 % sodium hypochlorite and 5 ml of hot chloroform and incubated at 37 °C for 1 hour. After incubation, the suspension was centrifuged at 3000 rpm for 10 minutes. Upper and middle phases were discarded, 5 ml of hot chloroform was added to the bottom phase, and then 5 ml of ethanol and acetone mixture (1:1) was added to precipitate the granules. The precipitate was allowed to evaporate for dryness at 30 °C, and then the weight of PHB was measure (Sathianachiyar and Devaraj 2013).

### **Optimization of Carbon and Nitrogen Sources for PHB Production**

Effect of media ingredients like carbon and nitrogen sources on PHB production was determined by simply replacing the carbon source with other carbon sources (glucose, sucrose, mannitol, raffinose, xylose galactose, lactose) and nitrogen source with other nitrogen sources (peptone, tryptone, ammonium sulphate, ammonium acetate, diammonium hydrogen phosphate ammonium dihydrogen phosphate, ammonium persulphate and Ammonium chloride).

### **Results and Discussion**

#### **Isolation of different PHB producing microorganisms**

Different soil samples were collected from areas varying in environmental conditions and subjected to enrichment followed by isolation on nutrient agar plate.

#### **Screening of PHB Producers**

The colony which showed whitish colored appearance with round shape was chosen. The isolates were subjected for visual screening for PHB production using Sudan black B. They were tested for PHB production following the viable colony screening method based on the intensity of staining. The isolates obtained from industrial soil proved higher PHB producing organism.



**Figure 3: Sudan Black staining of colonies**

#### **Characterization of bacterial isolate**

#### **Biochemical Characteristics of the isolates**

The most efficient PHB producing bacterial isolates was subjected to a set of morphological and biochemical tests for the purpose of identification. Morphological and biochemical tests were done by the standard procedures with 24 hrs old culture.

The strongly Sudan Black B positive isolates were subjected to production of Bioplastic. It was performed according to Singh and Parmar 2011 by sodium hypochlorite-chloroform method; the five selected isolates were cultured in Minimal Davis Medium at 37 °C for 3 days. The precipitate was allowed to evaporate for dryness at 30 °C, and then the weight of PHB was measure It was able to produce 289 mg of PHB per 100ml.

### **Conclusion**

The present study was designed for the isolation of effective poly-hydroxybutyrate producing strains from soil to yield maximum PHB under optimized conditions. The isolates were able to produce PHB in considerably good quantity compared to other isolated species. Consequently, the effect of various parameters like carbon source, incubation time, pH and NaCl concentration on PHB production were seen to be species specific. Similarly, the production from broth and fermentation methods gave much better results.

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# **BIOTECHNOLOGY**

# Polyphenol oxidase potentials of two wild mushroom species isolated from *Microporus xanthopus* and *Polyporus arcularius*

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## Abstract

Crude enzyme extracts were prepared from *Polyporus arcularius* and *Microporus xanthopus*. The partially purified extract of crude polyphenol oxidase (PPO) extracts from each mushroom was prepared by three phase portioning. Catechol and pyrogallol were the most preferred substrate with maximum enzymatic activity. The results reveal that the enzymes were most active lower substrates concentration that is 5 mM and 10mM of pyrogallol and catechol for both the mushrooms. In citrus fruits the sweet lime and orange and in vegetables the black eyes were the potent natural inhibitor for PPO. Also, sodium metabisulphite and ascorbic acid were strong inhibitors of the enzyme activities for both mushrooms.

**Keywords:** *Microporus xanthopus*, *Polyporus arcularius*, Polyphenol oxidases, TPP.

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## Introduction

The polyphenol oxidase (PPO) enzyme involved in this reaction is tyrosinase, which is of monophenol, O-diphenol: oxygen oxidoreductase; (EC 1. 14. 18. 1) a copper protein widely distributed in nature (Casanola, 2006). Enzymatic browning in fruits and vegetables is often an undesirable reaction and responsible for unpleasant sensory qualities (Unal, 2007). When cell membrane integrity is disrupted, phenolic substrates encounter the enzyme and are converted to O-quinones (monophenolase activity), followed by the oxidation of diphenols to O-quinones (diphenolase activity) which are then polymerized to undesirable brown, red, or black pigments (Chisari, 2007). PPO activity is undesirable in food production processes, there are many industrial processes in areas such as cosmetics, clinical methods, pharmaceuticals and textile that require the enzyme activity (Seetharam, 2002).

Mushrooms contain high nutritional value and are of great help to human body health. (Gurgu, 2017). *Microporus xanthopus*, also referred to as *Polystictus xanthopus*. The mature fruiting bodies have thin, funnel-shaped caps that are concentrically zoned in various shades of brown and which are supported by a yellow-footed stem. The *Polyporus arcularius*, however, is its delicately fringed, finely hairy ("ciliate," in Mycologese) cap margin. The cap color ranges from vey dark brown to pale tan.

The aim of this work was to isolate, partially purify and characterize the PPO from mushroom and determine some of its enzymatic properties. The enzyme was characterized at different substrates (substrate preference), natural and chemical inhibitors. Till date now upto our knowledge no work has been done on this mushrooms. This is the first kind of report.

## **Materials and Methods**

### **Collection of Mushrooms**

The *Microporus xanthopus* and *Polyporus arcularius* were collected in the month of september from a pine forest of Akkalkuwa, Dist-Nandurbar, Maharashtra with GPS reading and it was identified by Dr. Tanveer Khan, Taxonomist, Department of Botany, H.J. Thim College of Arts and Science, Mehrun, Jalgaon and herbarium preserved in laboratory also.

The natural fruit bodies of mushrooms were stored at – 4°C until studied.

### **Preparation of crude extract**

The mushrooms collected during the season and shed dried. After drying washed with tap water and purified water and crude extract was prepared by using the method described by ( Saki, 2018).

### **Three phase partitioning**

With slight variations, the three phase partitioning was carried out as mentioned in the (Saki 2018).

### **Polyphenol oxidase enzyme Assay**

Polyphenol-oxidase activity was determined by the customized method of Fujita et al. (1995). One unit of polyphenol-oxidase (U) was defined as the amount of enzyme necessary to cause an increase of 0.001 OD in the absorption for each minute of reaction time.

### **Substrate preference Assay**

Enzyme activity was measured using seven substrates (catechol, pyrogallol, tyrosine, quercetin, gallic acid, , phloroglucinol) with two different concentrations ( for catechol, pyrogallol and tyrosin 50-100 mM) while for gallic acid,quercetin, phloroglucinol and retinal hydrate: 50 mM) and the enzyme activity was calculated by using above equation.

### **Selection of substrate concentration for optimum**

Maximun amount of enzyme activity was shown in the substrates (Catechol and Pyrogallol) so for optimum concentration for these substrates at varying concentration for these two different substrates and the enzyme activity was calculated by using above equation.

### **Effect of natural inhibitors on PPO of *Polyporous arcularis***

The natural inhibitory effect of the vegetables Black eyes, Butter beans, Cluster beans and

citrus fruits like orange, pineapple and sweet lime was studied by using catechol as a substrates against for both the mushrooms.

### Effect of synthetic inhibitors on PPO

The synthetic inhibitory action of various chemicals like, Sodium Azide, Sodium Metabisulfite and Ascorbic Acid, Citric Acid at 5 mM constant conc on mushroom and PPO was studied by using Catechol as a substrates for both the mushrooms.

### Results and Discussions

#### Three phase partitioning

**Table 1 Partial purification of PPO**

TPP phases	Organisms	Enzyme Activity ( $\mu\text{g/ml/min}$ )	Specific Activity ( $\mu\text{g/ml/mg/min}$ )
1:1 (Lower layer)	<i>Polyporous arcularis</i>	48.16	53.93
	<i>Microporous xanthopus</i>	63.78	70.78
1:1.5 (Lower layer)	<i>Polyporous arcularis</i>	52.27	55.81
	<i>Microporous xanthopus</i>	69.26	77.52

Table, it is clear that in partial purification from TPP the maximum amount of enzyme is present in 1.1.5 concentration in lower layer with 77.52 and 55.81 specific activity in *Microporous xanthopus* and *Polyporous arcularis*.

#### Polyphenol oxidase enzyme Assay

Table 2, Shows that the *Microporous xanthopus* and *Polyporous arcularis* produces the 75.40 and 53.52 specific activity.

Table 2: Comparative activity of Polyphenol oxidase enzyme

Sample	Enzyme Activity ( $\mu\text{g/ml/min}$ )	Specific Activity ( $\mu\text{g/ml/mg/min}$ )
Tomato	77.20	36.04
Banana	67.53	29.96
Potato	68.19	31.83
Apple	78.82	37.06
<i>Polyporous arcularis</i>	48.80	53.52
<i>Microporous xanthopus</i>	63.76	75.40

#### Substrate preference Assay

Table 3 Studies on Substrate preference assay for PPO

Substrate (mM)	Catechol		Pyrogallol		Tyrosine		Quercetin	Retin hydrate	Galic Acid	phloroglucinol
	50	100	50	100	50	100	50	50	50	50
<i>Polyporous arcularis</i>	207.20	65.19	375.89	29.25	16.28	04.05	153.75	19.18	08.16	06.11
<i>Microporous xanthopus</i>	192.68	205.75	347.88	336.97	10.69	60.28	112.15	67.29	06.17	04.09

From above observations it is clear that the Catechol and pyrogallol were the most preferred substrate at a conc of 5 and 100 mM. *Polyporous arcularis* shows the maximum activity 375 at conc of 50 mM of pyrogallol, while 207 at catechol and lowest activity at 100 mM. Whereas in *Microporous xanthopus* maximum activity at 50 mM pyrogallol 347 while 205 activity was seen in 100 mM catechol conc. And the most detested substrate for both the mushrooms was phloroglucinol, galic acid, and tyrosin, and intermediate substrate was quercetin and retin hydrate.

#### Selection of substrate concentration for optimum

**Table 4. Determination of optimum substrate concentration for PPO**

Mushrooms	Enzme activity µg/ml/min					
	Catechol			Pyrogallol		
	5 mM	15 mM	20 mM	5 mM	15 mM	20 mM
<i>Polyporous arcularis</i>	65.79	13.66	10.75	27.87	28.66	17.5
<i>Microporous xanthopus</i>	92.58	8.33	7.25	117.15	36.46	30.25

Table 4 it is clearly seen that as the concentration of substrate increases the enzyme activity was decreased. *Polyporous arcularis* shows maximum activity 65 at 5 mM conc of catechol by comparing both the substrate while *Microporous xanthopus* shows 117 activity at 5 mM of pyrogallol in both substrates.

#### Effect of natural inhibitors on PPO of *Polyporous arcularis*

Table 5 it is clear that the most potent natural inhibitor was sweet lime, orange, and pineapple in citrus fruits while in vegetables the most effective inhibitor was black eyes.

Table 5 Effect of natural inhibitors on PPO activity

Inhibitor	Enzyme Activity (µg/ml/min)	Specific Activity (µg/ml/mg/min)
Black eyes	27.12	12.66
Butter beans	70.75	32.83
Cluster beans	45.49	21.10
Orange	23.73	10.78
Pineapple	23.19	11.18
Sweet lime	4.05	1.8
Control	52.11	24.39

#### Effect of synthetic inhibitors on PPO

Table 6 Effect of natural inhibitors on PPO activity

Inhibitor	<i>Polyporous arcularis</i>	<i>Microporous xanthopus</i>
	Specific Activity (µg/ml/mg/min)	
Sodium Azide	10.78	6.59
Ascorbic acid	3.56	1.38
Sodium metabisulphide	12.28	5.39

Citric acid	18.24	3.89
Control	21.69	8.41

By observing the above results it is concluded that ascorbic acid was the most effective synthetic inhibitor for both *Polyporous arcularis* and *Microporous xanthopus* while citric acid shows promising inhibitory action against *Microporous xanthopus* PPO and is less effective in *Polyporous arcularis*. Sodium azide has also showed promising results for both the mushrooms.

### Discussion

Phenolics are crucial substrates of PPO; diphenol and triphenol compounds were selected to measure PPO substrate specificity. PPO showed substrate preference for diphenols, with most activity detected with catechol, followed by pyrogallol. In contrast, the PPO had low activity towards monophenols and triphenols such as tyrosin, phloroglucinol, and gallic acid. Similar results were reported by (Zaini et al., 2013), which showed extreme preference towards catechol. Our results of inhibitory action of black eyes enzyme activity was 12 which is having very much similar to the (Gomes et al., 2001) it indicates the PPO value of beans is in between 10 to 14 U/ml/min. Our results of natural inhibitors with matched with the (Antoniolli et al. 2012) who studied that pineapple contains ascorbic acid, reviewing the antioxidant properties of pineapple which controls PPO activity. Cysteine and other sulfhydryl compounds present in pineapple extract also contribute to PPO inhibition.

### Conclusion

- Specific activity of *Polyporous arcularis* and *Microporous xanthopus* 53.52 and 75.40 respectively.
- Out of seven substrate, Pyrogallol and Catechol were the most preferred substrate at 50 and 100 mM conc for *Polyporous arcularis* and *Microporous xanthopus* .
- At 5 mM conc of catechol *Polyporous arcularis* shows maximum activity, while *Microporous xanthopus* shows maximum activity at 5 mM of pyrogallol.
- Sweet lime is the more effective inhibitor against *Polyporous arcularis*, intermediate inhibitor was orange in citrus fruits while in vegetables black eyes were most valuable.
- Out of synthetic inhibitors, ascorbic acid was more potent inhibitors for both the mushrooms.
- Further chemical characterization of PPO is warranted along with purified fraction or secondary metabolites of each plant.

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## **Studies on antibacterial and antifungal activity of spices (Cinnamon, Black pepper and Cumin)**

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### **Abstract**

Antimicrobial properties of three spices namely Cinnamon, Black pepper and Cumin were tested against different microbes including couple of bacteria and a fungus. Agar well diffusion method was implemented for this purpose in presence of standard antimicrobials. Microbial growth area over culture media plates, encompassing the antimicrobials provide data for analysis. Results obtained confirmed the antimicrobial nature of spices with their characteristic action against specific micro-organism.

**Keywords:** Antibacterial activity, Antifungal activity, Spices, Agar-well diffusion

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### **Introduction**

Spices are aromatic and pungent food ingredients, like herbs. They can have significant antioxidative effects. Total equivalent antioxidant capacities and phenolic contents (Folin-Ciocalteu) of 32 spices was reported till now. Spices can also have antimicrobial effects. Out of 46 spice extracts evaluated, many exhibited antibacterial activity against food borne pathogens. Gram-positive bacteria (e.g. *Bacillus subtilis*) are generally more sensitive than Gram-negative bacteria (*Escherichia coli*).

Cinnamon is a popular spice which is found all over India, belongs to the family *Leuraceae*. It is obtained from the inner bark of several tree species from the plant genus *Cinnamomum*. Cinnamon is used mainly as an aromatic condiment or flavouring additive in a wide variety of cuisine, sweets, snacks, tea and traditional foods. The aroma and flavour of cinnamon is attributed to its essential oil and cinnamaldehyde, the principal component, along with some other constituents like eugenol.

Cinnamon consists of a variety of resinous compounds, including cinnamaldehyde, cinnamate, cinnamic acid, and numerous essential oils including trans-cinnamaldehyde, cinnamyl acetate, eugenol, L-borneol, caryophyllene oxide,  $\beta$ -caryophyllene, L-borneyl acetate, E-nerolidol,  $\alpha$ -cubebene,  $\alpha$ -terpineol etc. Spicy taste and fragrance are due to absorption of oxygen by cinnamaldehyde. As cinnamon ages, it darkens

in color, improving the resinous compounds.



**Fig. 1. *Cinnamomum Verum***

**Fig.2. *Cuminum Cymium***

**Fig.3. *Piper nigrum***

Cinnamon contains large amounts of highly potent antioxidants that neutralise the harmful free radicals, formed every day in the body. Since cinnamon contains high levels of flavonoid acting as plant proteins, with powerful antioxidant property, the spice shows anti-inflammatory effect. In addition, cinnamon shows good resistance against large number bacteria and certain fungi that renders their applications in the food industry, for food preservation. *Cuminum cyminum*, commonly known as Cumin, is a flowering plant belonging to family *Apiaceae*. Its native territory belongs to the middle east of India. Cumin seed are nutritionally rich, providing high amount of fats, proteins, and dietary fibre. Moreover, vitamin B and E along with several dietary minerals, especially iron, are also present in considerable amount within cumin seed. The most common traditional medicinal use of cumin is for indigestion. Similarly, it also helpful for treating diabetes. Black pepper is native to south India and has been known to Indian cooking, since at least 2000 BC. It is a flowering vine in the family *piperaceae*, cultivated for its fruit known as a peppercorn, which is usually dried and used as spice and seasoning. Black pepper composed of carbohydrates, protein, fibre, moisture and fat as well as minerals, including potassium, calcium and phosphorous and magnesium. The main volatile flavour compound in black pepper are terpenes. Among medicinal importance's, black pepper helps in losing weight, cleanses intestine and stomach and useful in prevent cancer. It consists potassium that helps in regulating heart rate and high blood pressure. Moreover, black pepper is reported to for producing red blood cells treating constipation.

## Materials and methods

### • Pre-treatment

Samples of three spices namely *Cinnamon*, *Black pepper* and *Cumin* were procured from local market of Jalgaon (Maharashtra). 10 gm of dry powder of all spices was packed in Soxhlet apparatus. This was for extraction of respective soluble bioactive molecules from the rhizome, using methanol as solvent system. Based on polarity of volatile solvent, they were concentrated on water bath (50° - 70°c) under reduced pressure. The concentrated extract was kept in desiccator till used. Standard samples of spices were obtained from HI-MEDIA.

### • Test Microorganisms

Two different strains were used for testing antibacterial activity includes *E. coli* (gram-negative), *Bacillus subtilis* (gram-positive). For testing antifungal activity, a strain of *Aspergillus niger* was used (Naz et al. 2010). The bacterial cultures were maintained on Nutrient Agar (NA), whereas the fungal cultures were maintained on potato dextrose agar (PDA) and preserved at 4°C. All cultures were sub-cultured periodically under stationary condition, on the same medium (Borate et al. 2014).

#### ➤ Antibacterial Assay

Effect of all spices extracts on Gram-bacterial species was assayed by agar well diffusion method. Antimicrobials present in the extracts (100 mg / 2 ml) are allowed to diffuse out into the medium to interact with the test organisms, seeded in freshly prepared plates. Resulting zones of inhibition (diameter in mm) is expected to be uniformly circular, due to confluent lawn of growth.

### Reagents:

1. Muller Hinton Agar Medium (1L): The medium was prepared by dissolving 33.9g of the commercially available Muller Hinton Agar Medium (Hi-Media) in 1000ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121° for 15 minutes. The autoclaved medium was mixed well and poured in molten condition, into 100mm petriplates (25-30ml/plate).
2. Nutrient broth (1L): Nutrient broth was prepared by dissolving 13 g of commercially available nutrient broth into 1000ml distilled water, followed by shaking till complete dissolution.
3. Ciprofloxacin (Standard antibacterial agent)

**Protocol:**

1. Petri plates containing 20ml Muller Hinton medium were seeded with 24h incubated culture of (0.1ml) bacterial strains (*E. coli* and *Bacillus subtilis*).
2. Wells of approximately 2mm was bored using well-borer and exactly 100µl of all extracts (each with hexane, methanol, acetone and water) were added.
3. All plates were incubated at 37°C for 24h., followed by measurement of inhibition zone.
4. Ciprofloxacin, a standard antibacterial agent at a concentration of 200 µl in a well was used as the positive control (Praveen et al. 2014).

**Antifungal Assay**

The fungicidal effect of the spices can be assessed by the agar well plate method using fungus *Aspergillus niger*. It involves inhibition of mycelial growth of the fungus, observed as a zone of clearance near wells.

**Reagents**

1. Potato Dextrose Agar medium (PDA): The medium was prepared by dissolving 39g of the commercially available potato dextrose agar in 1000ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121° for 15 minutes. The autoclaved medium was mixed well and poured in molten condition, into 100 mm petri plates (25-30 ml/plate).
2. Potato Dextrose Broth (PDB): Potato dextrose broth was prepared by dissolving 13 g of commercially available PDB in 1000ml distilled water and dissolved the medium completely.
3. Flucanazole (standard antifungal agent)

**Protocol:**

1. PDA medium was prepared and poured into the petri plates.
2. Wells of about 2 mm diameter were aseptically punched on each agar plate using sterile borer.
3. Fungal strain culture (0.1 ml) were aseptically inoculated and evenly spread using sterile glass spreader on the surface of PDA plate.
4. 100 µl of the spices extracts are then inoculated into the wells and plates are incubated at 37 °C for 24 h.
5. Flucanazole was used as standard.
6. The diameter of zones of inhibition was recorded in mm (Borate et al.2014).

**Results and Discussions**  
**Antibacterial Activity**

As mentioned in table 1, largest zone of clearance was noticed as 14 mm, in case of Cumin among all three spices when tested against Gram negative bacteria (*E. coli*) at least concentration. On the contrary, largest zone of clearance was observed as 16 mm, in case Cinnamon when all the three spices were tested against Gram positive bacteria (*B. subtilis*) at same concentration. This indicates the superior antibacterial activity of Cumin as well as Cinnamon against respective specific bacteria. Nevertheless, black pepper also exhibited tentative antibacterial activity against both types of bacteria.

**Table 1. Antimicrobial activity of spices**

Spices / Microbes	<i>Cinnamon</i>		<i>Cumin</i>		<i>Black pepper</i>	
	Concentration ( $\mu$ l)	Zone of inhibition (mm)	Concentration ( $\mu$ l)	Zone of inhibition (mm)	Concentration ( $\mu$ l)	Zone of inhibition (mm)
<i>E. coli</i>	0.1	12	0.1	14	0.1	12
	0.2	16	0.2	18	0.2	16
	0.2 (Std.)	32	0.2 (Std.)	32	0.2 (Std.)	32
<i>Bacillus subtilis</i>	0.1	16	0.1	12	0.1	12
	0.2	15	0.2	17	0.2	14
	0.2 (Std.)	36	0.2 (Std.)	28	0.2 (Std.)	34
<i>Aspergillus niger</i>	0.1	14	0.1	15	0.1	16
	0.2	16	0.2	17	0.2	19
	0.2 (Std.)	32	0.2 (Std.)	30	0.2 (Std.)	32

**Antifungal activity**

Table 3.1 tabulated the values of zone of inhibition corresponding to antifungal activity of three spices carried out using fungus *Aspergillus niger*. Results shown that maximum zone of inhibition that is 16 mm at the least concentration of 0.1 $\mu$ l, was obtained for the spice black pepper. It highlighted the role of this particular spice to be used as fungus-resistant agent. It should be noted that both other spices too, exhibited same nature up to a large extent.

**Conclusion**

From the present work, it is concluded that all the three types of spices clearly exhibited antibacterial as well as antifungal activities. In case of antibacterial activity for Gram positive bacteria, cumin can be used as most effective spice, whereas that of for Gram negative

bacteria, Cinnamon proved to be comparatively significant, when used at their least concentration. Similarly, black pepper claimed its usefulness to resist fungi among all spices. Collectively, all spices confirmed their traditional medicinal importance and also provide scope for their economic production, in terms of drugs and supplementary products.

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## **Phytochemical analysis of *Eriophorum comosum* (cotton grass) and *Ficus exasperata* (*brahmas banyan*) along with their antibacterial Activity**

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### **Abstract**

Phytochemical properties of couple of plants namely ‘cotton grass’ and ‘brahmas banyan’ were analyzed, confirming the presence of secondary metabolites in both plants. Similarly, their antibacterial properties were also tested against a Gram-positive and a Gram-negative bacterium. Agar well diffusion method was implemented for this purpose in presence of standard antimicrobials. Microbial growth area over culture media plates, encompassing the antimicrobials, provide data for analysis. Results obtained confirmed the antibacterial nature of both plants with their characteristic action against specific bacteria, in particular solvent systems.

**Keywords:** Phytochemical analysis, Antibacterial activity, Agar-well diffusion

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### **Introduction**

The genus *Eriophorum* commonly known as cotton-grass or cotton-sedge, is characterized by the presence of smooth silky white or brownish red elongated perianth bristles. There are about 25 species in the genus and distributed in the cool temperate regions of Himalaya and south-east Asia. In India, three species namely *E. comosum*, *E. microstachyum* and *E. scheuchzeri* are recorded so far and most of them are confined in the northern regions. It has the flowering and fruiting season from November to March. Habitat point of view, it is naturally growing in the rock crevices at an altitude of over 2000 m from sea level and the environmental conditions at Nilgiri Plateaus, comparable to that of its original habitats in Himalayan region. The leaves and roots of this plant are astringent and have been used in the post treatment of diarrhea.

On the other hand, *Ficus exasperata*, commonly known as Brahma's Banyan, is a deciduous tree, up to 70 feet tall, native to parts of Africa, Arabian Peninsula and India. The tree has smooth grey bark. Alternately arranged, ovate-elliptic leaves have very rough surface,

making them look like sandpaper. Young leaves are often lobed. Sap is sticky, not milky. Figs arise in leaf axils, 1-2 in number. They are rough, spherical or ovoid, 1-1.5 cm in diameter and yellow, orange or red in colour. Its forage (leaves plus stems less than 6 mm in diameter) contained 14.6 g and 19.9 g crude protein per 100 g dry matter in the wet and dry season, respectively. The associated species in the community are *Ficus asperifolia*, *Ficus sur*, *Ficustsjahela*, *Ficushispida* etc. *Ficus exasperata* is used as analgesic, anti-arthritic, diuretic, wound healing, anti-parasitic, vermi-fugue etc. The plant parts are also used as animal fodder. Rough leaves are widely used as sandpaper for polishing wooden, metal or ivory articles, like kitchen utensils, chairs etc.



**Fig. 1** *Eriophorum comosum*



**Fig.2.** *Ficus exasperate*

## Materials and Methods

- **Pre-treatment of plants**

*Eriophorum Comosum* and *Ficus exasperata* was collected from Akkal-kuva village, Nandurbar, Maharashtra and further processed for cleaning to prevent the deterioration of phytochemicals in it. All plants were shade dried, to remove the water content from entire plant, for longer storage. After complete drying, whole plants are grinded in blender to obtain fine powdered form which finally applied for Soxhlet extraction using different solvents (Hexane, Methanol, Acetone and water).

- **Test Microorganisms**

Two different strains were used for testing antibacterial activity includes *E. coli* (gram-negative) and *Bacillus subtilis* (gram-positive) (Naz et al. 2010). The bacterial cultures were maintained on Nutrient Agar (NA) and preserved at 4°C. All cultures were sub-cultured periodically under stationary condition, on the same medium (Borate et al. 2014).

### ➤ **Antibacterial Assay**

Effect of both plant extracts on bacterial species was assayed by agar well diffusion method. Antimicrobials present in the plant extracts are allowed to diffuse out into the medium to interact with the test organisms, seeded in freshly prepared plates. Resulting zones of inhibition (diameter in mm) is expected to be uniformly circular, due to confluent lawn of growth.

- **Reagents:**

4. Muller Hinton Agar Medium (1L): The medium was prepared by dissolving 33.9g of the commercially available Muller Hinton Agar Medium (Hi-Media) in 1000ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121° for 15 minutes. The autoclaved medium was mixed well and poured in molten condition, into 100mm petri plates (25-30ml/plate).
5. Nutrient broth (1L): Nutrient broth was prepared by dissolving 13 g of commercially available nutrient broth into 1000ml distilled water, followed by shaking till complete dissolution.
6. Ciprofloxacin (Standard antibacterial agent)

- **Protocol:**

5. Petriplates containing 20ml Muller Hinton medium were seeded with 24h incubated culture of (1ml) bacterial strains (*E. coli* and *Bacillus subtilis*).
6. Wells of approximately 10mm was bored using well-borer and exactly 200µl of both plant extracts (each with hexane, methanol, acetone and water) were added.
7. All plates were incubated at 37°C for 24h., followed by measurement of inhibition zone.
8. Ciprofoxin, a standard antibacterial agent at a concentration of 200 µl in a well was used as the positive control (Praveen et al. 2014).

## **Results and Discussion**

- **Phytochemical screening (secondary metabolites)**

Chemical tests were carried out on the aqueous extract of given plant using standard procedures. Results obtained are shown with details in table 1 and table 2 for both plants extracts. From the data, it is cleared that both plants exhibit presence of various secondary metabolites (biomolecules) in different solvent extractions of hexane, methanol, acetone and water.

**Table 1. Phytochemical analysis of *Eriophorum comosum***

Sr.No.	Solvent	Test	Secondary metabolite	Procedure	Observation	Inference
1 (a)	Hexane	Benedict's test	Carbohydrates	0.5ml filtrate+0.5ml Benedict's reagent+heat=coloured precipitate	No coloured precipitate	absent
(b)	Methanol				Coloured compound	present
(c)	Acetone				Coloured compound	present
(d)	Aqueous				Coloured compound	present
2 (a)	Hexane	Ferric chloride	Phenolic compounds	0.5ml + 5% neutral ferric chloride= dark green colour	Colored compound	present
(b)	Methanol				Coloured compound	present
(c)	Acetone				Colored compound	present
(d)	Aqueous				No coloured compound	absent
3 (a)	Hexane	Biuret test	Proteins	2ml filtrate+1drop (2%) CuSO <sub>4</sub> + 1ml ethanol(95%) +KOH= pink ethanolic layer	Pink colour	present
(b)	Methanol				Pink colour	present
(c)	Acetone				No change	absent
(d)	aqueous				Pink colour	present
4 (a)	Hexane	Wagner's test	Alkaloids	A drop of wagner's reagent + 1ml filtrate= reddish brown precipitate	Noppt	absent
(b)	Methanol				No ppt	absent
(c)	Acetone				No ppt	absent
(d)	Aqueous				No ppt	absent
5 (a)	Hexane	Borntrager's test	Glycosides	2ml filtrate+ 3ml chloroform+ 10% ammonia= pink colour	No colour	absent
(b)	Methanol				Pink colour	present
(c)	Acetone				No colour	absent
(d)	Aqueous				No colour	absent
6(a)	Hexane	Alkaline reagent test	Flavonoids	0.5ml filtrate+10% ammonium hydroxide=yellow colour	no colour	absent
(b)	methanol				Yellow colour	present
(c)	Acetone				no colour	absent
(d)	Aqueous				Coloured compound	present
7(a)	hexane	Saponification test	Fixed oil and fats	5 ml filtrate+15mlDWW+shake =layer of foam	No layer of foam	absent
(b)	methanol				No layer of foam	absent
(c)	acetone				No layer of foam	absent

(d)	aqueous				No layer of foam	absent
8(a)	Hexane	Salkowski test	Steroids	2ml filtrate+2ml chloroform +2ml Sulphuric acid=red colour	No change	absent
(b)	Methanol				No change	absent
(c)	Acetone				No change	absent
(d)	Aqueous				Red colour	present
9(a)	Hexane	Libermann-brchard's test	Phytosterols	1ml filtrate+2ml acetic anhydride + 2 drops conc. H <sub>2</sub> SO <sub>4</sub> = colour change	No colour change	absent
(b)	Methanol				Pink colour	Present
(c)	Acetone				Pink colour	Present
(d)	Aqueous				No color change	Absent

**Table 2. Phytochemical analysis of *Ficus exasperata***

Sr. No.	Solvent	Test	Secondary metabolite	Procedure	Observation	Inference
1 (a)	Hexane	Benedict's test	Carbo-hydrates	0.5ml Filtrate+0.5ml Benedict's reagent+heat =coloured precipitate	No coloured precipitate	absent
(b)	Methanol				No Coloured compound	absent
(c)	Aqueous				Coloured compound	present
2 (a)	Hexane	Ferric chloride	Phenolic compounds	0.5ml + 5% neutral ferric chloride= dark green colour	No colour	absent
(b)	Methanol				No colour	absent
(c)	Aqueous				No coloured compound	absent
3 (a)	Hexane	Biuret test	Proteins	2ml filtrate+1drop(2%) CuSO <sub>4</sub> + 1ml ethanol(95%) +KOH= pink color ethanolic layer	No pink colour	absent
(b)	Methanol				No pink colour	absent
(c)	Aqueous				No pink colour	absent
4 (a)	Hexane	Wagner's test	Alkaloids	A drop of wagner's reagent + 1ml filtrate= reddish brown precipitate	Reddish brown ppt.	present
(b)	Methanol				No ppt.	absent
(c)	Aqueous				No ppt.	absent
5 (a)	Hexane	Borntrager's test	Glycosides	2ml filtrate+ 3ml chloroform+ 10% ammonia= pink colour	No colour	absent
(b)	Methanol				Nocolour	absent
(c)	Aqueous				No colour	absent
6(a)	Hexane	Alkaline reagent test	Flavonoids	0.5ml filtrate+10% ammonium hydroxide=yellow colour	No colour	absent
(b)	Methanol				Nocolour	absent
(c)	Aqueous				No colour	absent

7(a)	Hexane	Saponification test	Fixed oil and fats	5 ml filtrate+15mlDWW+shak	No layer of foam	absent
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(b)	Methanol			e =layer of foam	No layer of foam	absent
(c)	Aqueous				No layer of foam	absent
8(a)	Hexane	Salkowski test	Steroids	2ml filtrate+2ml chloroform +2ml Sulphuric acid=red color	No change	absent
(b)	Methanol				No change	absent
(c)	Aqueous				No change	absent
9 (a)	Hexane	Libermann-brchard's test	Phytosterols	1ml filtrate+2ml acetic anhydride + 2 drops conc. Sulphuric acid= colour change	No colour change	absent
(b)	Methanol				Redcolour	present
(c)	Aqueous				No color change	absent

- **Antibacterial Activity**

The antibacterial activity of the *Eriophorum comosum* and *Ficus exasperate* extracts against Gram-negative bacteria (*E.coli*) and Gram-positive bacteria (*B. subtilis*) is given below in table 3. According to data obtained, it was noticed that *Eriophorum comosum* extracts in methanol as well as acetone exhibited the zones of inhibition of 19 mm and 4 mm respectively, against Gram negative bacteria namely *E.coli*. On contrary, there was no such clear zone noticed against Gram positive bacteria namely *B. subtilis*. This confirmed the antibacterial nature of concerned plant, particularly again Gram negative bacteria.

In case of *Ficus exasperata*, there was no zone of clearance observed in any solvent extracts for *E.coli* while for *B. subtilis*, only such zone of 3 mm was noticed in hexane extracts. This confirmed the antibacterial nature of respective plant against Gram positive bacteria, specifically using hexane as solvent.

**Table 3. Antibacterial activity of *Eriophorum comosum* and *Ficus exasperata***

<i>Eriophorumcomosum</i>			<i>Ficusexasperata</i>		
Extracts	Zone of inhibition (mm)		Extracts	Zone of inhibition (mm)	
	<i>E. coli</i>	<i>B. subtilis</i>		<i>E.coli</i>	<i>B. subtilis</i>
Standard(Ciprofoxacin)	38	32	Standard(Ciprofoxacin)	30	30
Acetone	19	-	Hexane	-	3
Methanol	04	-	Methanol	-	-
Aqueous	-	-	Aqueous	-	-

## Conclusion

From the present work, it is concluded that both plant species exhibited variable antibacterial

activities. *Eriophorum comosum* plant species proved to be more effective against Gram-negative bacteria in aceto-methanol extracts, while *Ficus exasperate* in hexane extract confirmed its antibacterial action against Gram-positive bacteria. Similarly, both plants exhibited presence of secondary metabolites of varying range. Phytochemical studies concluded that *Eriophorum comosum* contains carbohydrates, phenolic compounds, proteins, glycosides, flavonoids, steroids and phytosterols. On the other hand, *Ficus exasperate* contains carbohydrates, alkaloids, steroids and phytosterols, in particular solvent extracts.

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# STATISTICS

## **Statistical Process Control Tools For Some Quality Parameter Of Brake System**

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### **Abstract**

The aim of the study is the safe operation of motor vehicle required for continuous adjusting of its speed which changes in traffic conditions. The breaks and the tires along with the steering system are the most important safety and critical accident avoidance component of a motor vehicle. In this case we study the critical parameters of those components of break system and then capability has been verified.

**Keywords:** Caliper, Wheel cylinder, industrial manufacturing process, I and MR Chart

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### **Introduction**

The wheel cylinder is one of the important break system components. The wheel cylinder is four wheeler motor vehicle parts and it is used in break system of four wheeler vehicle. Some are critical parameters of break system are main bore diameter and depth of port hole. 30 units of wheel cylinder were inspected for measurement of main bore diameter. Digital air gauge was used for this purpose. Here our objective is to check whether the bore size is uniform over the entire length of cylinder or not.

### **Objective and Scope**

- To observe industrial manufacturing process.
- To observe the environment and culture.
- To identify sources and causes of variation in industrial process.
- Use/Scope of statistical techniques on industrial process.
- To carry out the process stability and capability analysis on collected
- To carry out the gauge capability analysis on collected data sets.
- To suggest the some possible action for improvement.

### **Data collection and description**

#### **Data collection on caliper of break system:**

The caliper bracket is four wheeler components and it is used in break system. The bracket is one of the important parts of break system; produced in Foundation Brake Manufacturing Pvt. Ltd. Some of the parameters of brackets are critical and we want to see those parameters

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are capable or not for break system. So we have decided to collect data on the basis of following quality characteristics

**Specification limits:** Only Upper specification is given in the table 1.

**Table1: Upper specification Limit**

Parameters	Targets (mm)	USL (mm)	LSL (mm)	Fig. 1:Break Caliper 
CD Parameters:	<b>134</b>	<b>134.1</b>	<b>133.9</b>	
Counter Diameter	<b>61.5</b>	<b>61.55</b>	<b>61.45</b>	
Seal Gr. Diameter	<b>56.43</b>	<b>56.56</b>	<b>56.3</b>	

### Material and Methods

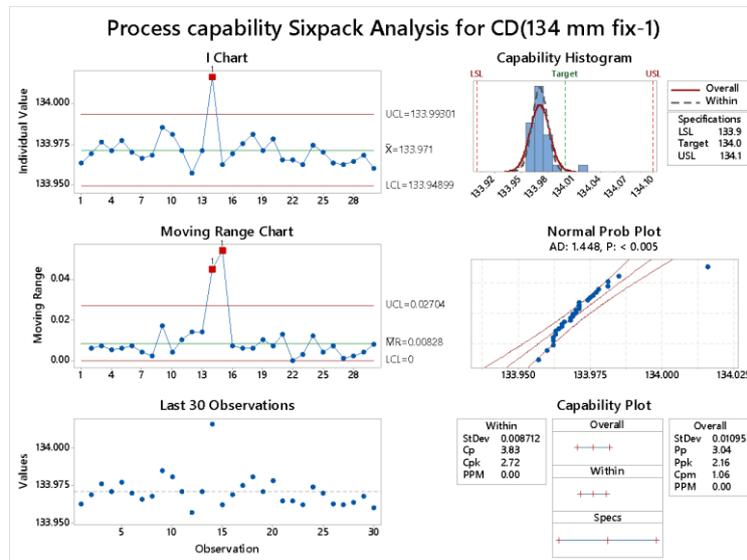
An MR chart plots the moving range over time to monitor process variation for individual observations. Use the MR chart to monitor process variation when it is difficult or impossible to group measurements into subgroups. This occurs when measurements are Expensive, production volume is low, or products have a long cycle time. Statistical techniques can be useful for throughout the product cycle, including development activities. Prior to manufacturing; in quantifying process variability, in analyzing this variability relative to product requirements or specifications and in assisting development and manufacturing in eliminating or greatly reduces this variability. This general activity is called process capability analysis it is frequently convenient to have a simple, quantitative way to express Process capability. One way to do so is through the process capability ratio PCR. It is denoted by Cp Determining the capability of the measurement system is an important aspect of many quality and process improvement activities.

The purpose of most measurement systems capability study is to

- 1) Determine how much of the total observed variability is due to the gauge or instrument.
- 2) Isolate the components of variability in the measurement system. Assess whether the instrument or gauge is capable (i.e., is it suitable for the intended application).

## Data Analysis for Caliper

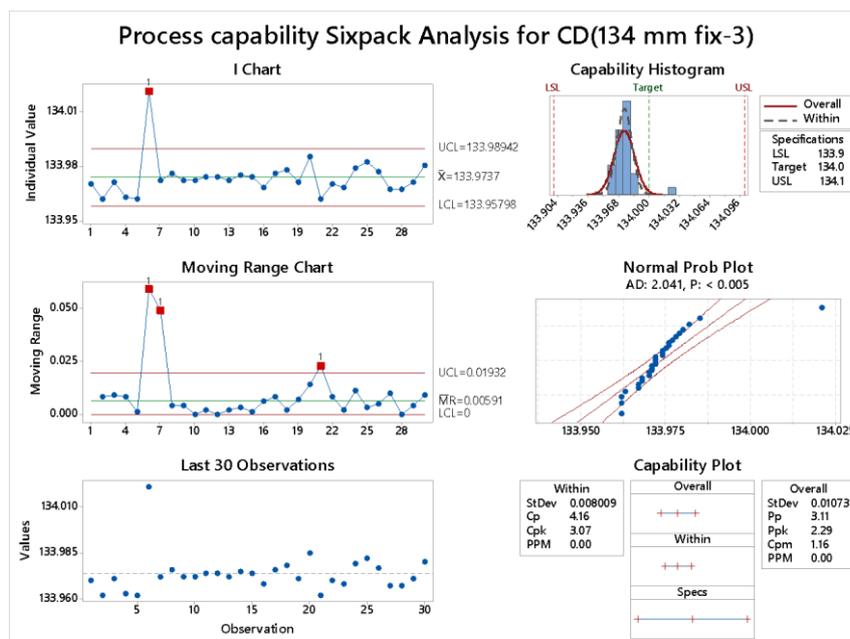
➤ Parameter : CD 134



**Fig. 2: Process Capability Analysis of CD (134mm Fix-1)**

### Interpretation:

1. Data distribution shows approximately normal.
2. From I chart we observe that one point is out of control and MR chart
3. Shows two points are out of control. The process is not under control.
4. From the capability analysis it is concluded that the process is well capable. In this parameter ppm=0 i.e. highly capable process.



**Fig 3.Process Capability Analysis of CD (134mm Fix-3)**

### Interpretation:

- 1) Data distribution shows approximately normal.
- 2) From I chart we observe that one point is out of control and MR chart shows three points are out of control. The process is not under control.
- 3) From the capability analysis it is concluded that the process is well capable. In this parameter ppm is zero. i.e. highly capable process

In a same manner we plotted control chart for remaining 2 parameter of Caliper and 6 parameters for wheel cylinder and interpreted the result in final conclusion

### Gauge Capability Analysis:

#### 1. Gauge Capability study for caliper:

Parameter: CD (134±0.10)

**Table2:** specification Limit for parameter CD

USL	LSL	$\sigma_{gauge}^2$	$\sigma_{total}^2$	$\sigma_{Product}^2$	P/T ratio
134.1	133.9	0.0007754	0.00036	0	0.835357

### Interpretation:

- 1) Here, precision to tolerance ratio (or P/T) ratio is greater than 0.1. Hence gauge is not capable.
- 2) We conclude that gauge capability is poor as precision to tolerance ratio (P/T) ratio is 0.835357.

Similar result obtains from rest 3 parameter and interpreted in final conclusion

#### Gauge Capability study for Wheel Cylinder:

Parameter: Depth Port-A (7.5±0.5)

**Table 3:** specification Limit for parameter Depth Port

USL	LSL	$\sigma_{gauge}^2$	$\sigma_{total}^2$	$\sigma_{Product}^2$	P/T ratio
8	7	0.0080484	0.0086333	0.0005849	0.535122

### Interpretation:

- 1) Here, P/T ratio is greater than 0.1. Hence gauge is not capable.
- 2) We conclude that gauge capability is poor as P/T ratio because this ratio is not less than 0.1.

### Overall conclusion and suggestion

Table showing overall conclusion analysis of Cp, Cpk, Pp and Ppk:

**Table 4: Process capability statistics for caliper**

Compound	parameter	Observation	CP	CPK	Pp	Ppk
Caliper	CD	Fix-1	3.83	2.72	3.04	2.16
		Fix-2	4.29	3.37	4.24	3.33
		Fix-3	4.16	3.07	3.11	2.29
		Fix-4	2.1	1.78	1.98	1.68
	Counter Diameter	Fix-1	19.47	1.87	12.35	1.19
		Fix-2	3.48	0.22	1.63	0.1
		Fix-3	8.52	0.52	8	0.49
		Fix-4	8.79	0.21	7.56	0.18
	Seal Gr. Diameter	Fix-1	16.16	1.53	5.16	1.28
		Fix-2	4.87	1.49	4.28	1.31
		Fix-3	6.36	1.92	5.99	1.82
		Fix-4	7.83	2.53	7.42	2.4

**Table 5: Process capability statistics for wheel cylinder**

Compound	parameter	Observation	CP	CPK	Pp	Ppk
Wheel Cylinder	Depth Port-A	Fix-1	1.87	1.35	2.07	1.49
		Fix-2	1.73	1.08	1.6	1
		Fix-3	1.71	1.18	1.83	1.26
		Fix-4	1.82	1.27	1.7	1.19
		Fix-5	1.91	1.31	1.77	1.22
		Fix-6	1.77	1.22	1.88	1.29
	Depth Port-B	Fix-1	1.54	1.05	1.69	1.22
		Fix-2	2.25	1.33	1.9	1.29
		Fix-3	1.6	0.99	1.87	1.15
		Fix-4	1.74	1.07	1.78	1.12
		Fix-5	2.27	1.49	1.9	1.15
		Fix-6	2.13	1.36	2.01	1.1
	Bore Diameter	Fix-1	2.94	1.5	2.98	1.25
		Fix-2	1.48	0.85	0.98	1.28
		Fix-3	2.73	1.45	2.9	1.52
		Fix-4	1.51	0.86	0.99	0.57
		Fix-5	3.01	1.57	2.92	1.54
		Fix-6	3.63	1.93	3.16	1.68

**Overall conclusion and suggestion for caliper:**

- 1) The process was not under control for some parameters and not capable for some parameter.
- 2) Overall we say the process is not well settle. We need control process parameters.

- 3) The bracket production was stopped because of some reason so we have not got the data of new settle process. Then we not observed the next process parameter.

### **Overall conclusion and suggestion for wheel cylinder**

- 1) Observing the process we can conclude that our process is shifted from target and capability is very good for depth 7.5 but it's not capable for bore dia. 19.06 so we need to minimize the variation between bore dia.19.06.
- 2) In wheel cylinder the all processes are shifted from target. We want to shift process at target for further improvement.
- 3) Overall we say the process is not well settle for parameter bore dia. 19.06. We need to observe this parameter further for process improvement.

### **Some suggestions for machine handler for process improvement of products are:**

- 1) After every cycle production machine burr must be cleaned.
- 2) Focus on casting (raw material) adjustment.
- 3) Operator should handle each machine smoothly
- 4) During changing shifts machine setup will be checked regularly.
- 5) For more stability and improvement of process regular inspection is needed during process.

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## Statistical Analysis on Different Types of Harassment

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### Abstract

This paper explores sexual harassment in the workplace and travelling mode. A questionnaire has been developed and circulated for that purpose. This information is considered for the study as it reflects the current position in Jalgaon. The expected outcome of this paper is the development of policies and creation of awareness, which build on the findings of this research.

**Keywords:** Hypothesis testing, Time series based graphs and other statistical tools.

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### Introduction

Sexual harassment is a recurring problem around the globe. Different nations have taken measures to deal with the consequences of such a problem. Nations or companies are dealing with the topic by either reacting or pro-acting to the salient situations. India is facing the problem of increasing number of cases of sexual harassment at workplace despite the fact that there are numerous laws to curb the menace. Irrefutably, it hampers women's constitutional and fundamental rights to equality, justice and dignity. The paper begins with defining the concept of sexual harassment and then looks into the various aspects allied to this brutal issue.

### Objective

- To observe the frequency of woman and child abused due to harassment in Jalgaon district.
- To find the essential causes which are responsible for harassment.
- To analyse the locality is responsible to harassment or not.

### Tables and Graphical Representation of the collected data

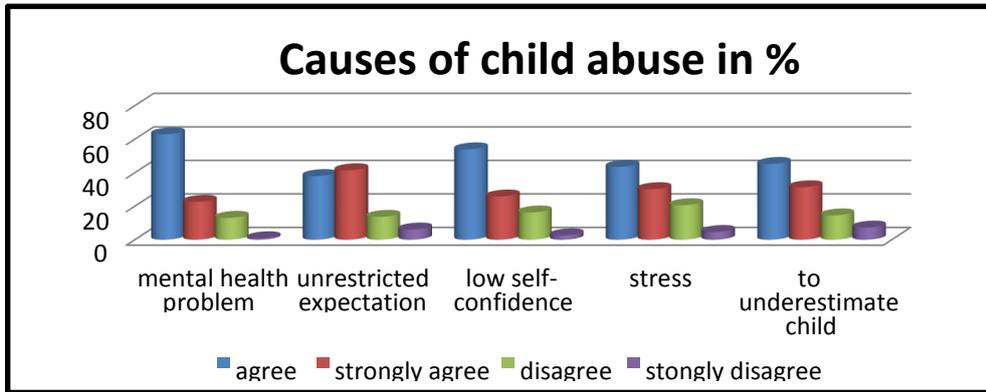
**Table 1: Number of student locality in percentage**

student	Female	Male
Urban	28.4058	26.08696
Rural	21.73913	23.76812

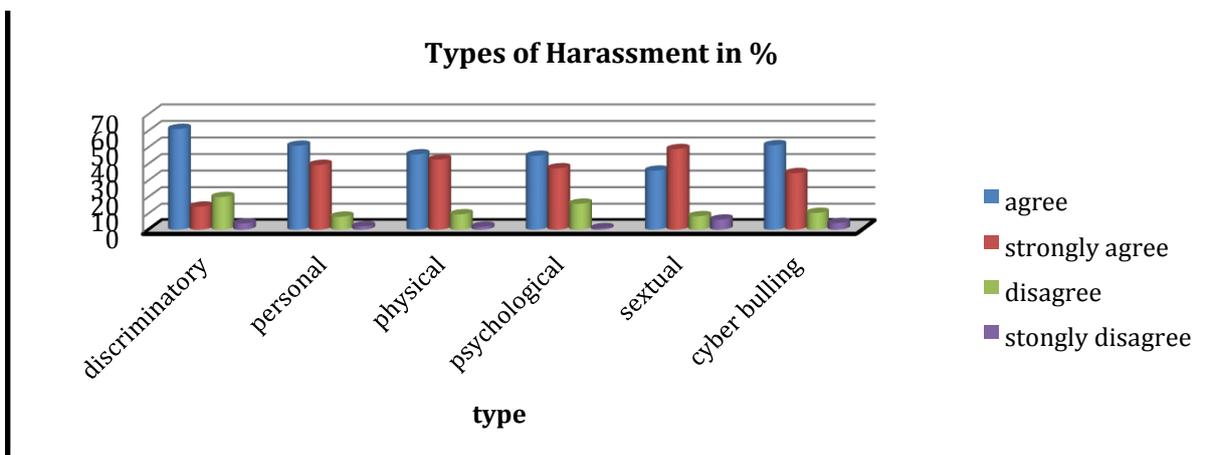
**Table 2: No. of response of students on Nirbhaya Pathak in percentage**

Response	Urban	Rural
Yes	20.57971	26.37681
No	23.2	27.82609

From table 1 and 2, it has been observe that the proportion of response of both male and female are same.

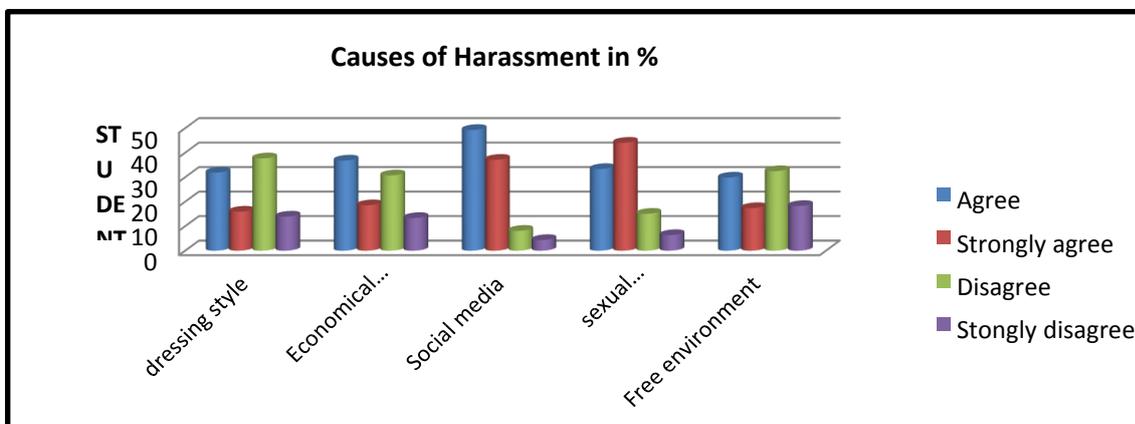


**Fig 1: No. of response of causes of child abuse**



**Fig 2: Responses of student on Types of Harassment**

**Conclusion:** From Fig 1 and 2.it can observe all factors considered under study are highly responsible for child harassment and harassment respectively.



**Fig 3: Determine response of cause of harassment in percentage**

**Conclusion:** From Fig 3.it can observe all factors considered under study are highly responsible for all types of harassment.

**Statistical tools used for assumption and hypothetical Responses:**Bartlett's test of Sphericity, Chi-square test for association, Forecasting of Data by using time series analysis

### 1) Bartlett's test of sphericity:

Null hypothesis  $H_0$ : Population correlation matrix is an identity matrix

Alternative hypothesis  $H_1$ : Population correlation matrix is not an identity matrix.

If the hypothesis that the population correlation matrix is an identity matrix is accepted because the observed significance level is large, the use of the factor model should be reconsidered. Test statistic is, under  $H_0$ , it follows a  $(k-1)$

**Decision rule:** If the value of  $\chi^2$  statistics is greater than  $\chi^2$  table value then these leads to rejection of null hypothesis.

#### **Conclusion:**

The Bartlett's Test of Sphericity is significant (0.0000). i.e., *value* is Less than 0.05.i.e. the significance level is small enough to reject the null hypothesis. This means that the correlation matrix is not an identity matrix. That is the variables under Consideration are correlated. Therefore, we can perform the factor analysis using these variables.

### 2) Chi-square test for association:

The test is applicable only when you have two categorical variables from a single from a single population. It is use to determine whether there is a significant association between two variable.

Null hypothesis  $H_0$ : There is no association between the two variables

Alternative hypothesis  $H_1$ : There is association between the two variables

Test Statistics is given by,  $\chi^2 = \sum \left( \frac{O_{ij} - E_{ij}}{E_{ij}} \right)^2$

Where, i is the number of levels for one categorical variable and j is the number of levels for another categorical variable.

**Aim:** to test the association between any two pairs of categorical variables in

#### **Open communication with parents:**

- **For student responses**

1) To test independency of gender of parents with respect to communication:

Test Statistics	Degrees of freedom	p-value	Result
4.643	1	0.031	Reject Null Hypothesis

2) To test independency of gender of parents with respect to age:

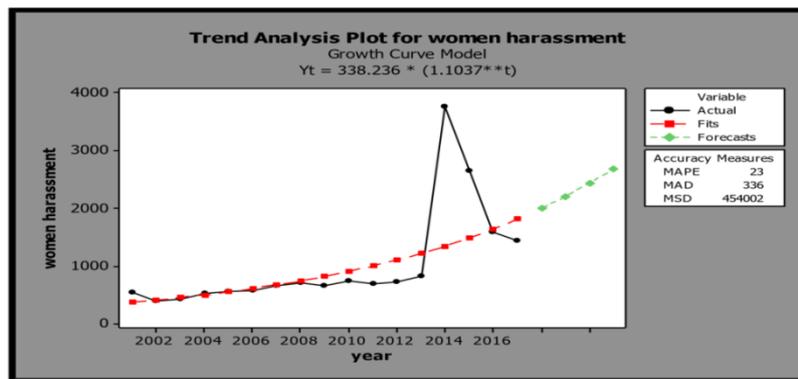
Test Statistics	Degrees of freedom	p-value	Result
8.975	3	0.030	Reject Null Hypothesis

**Interpretation:**

It has been observed that the association between factors under consideration is statistically significant.

**3) Forecasting of Data by using time series analysis**

**One-way Analysis of variance for women harassment:**



**Conclusion:** The maximum cases of cruelty is reported in police station during 2001 to 2007

**Overall conclusion:**

We have seen maximum female not secure in travelling. In Jalgaon district assault on women is maximum. People who harassed by someone still they do not share this things with their friends or family. To reduce harassment people must have share this problems with family, complaint to police.

**References**

1. www.ncrb.com
2. Introduction to Statistical quality control by Douglas C. Montgomery, John Wiley and sons.

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## **The Statistical Analysis of onion production (2003-2019)**

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### **Abstract**

Onion production has great importance in crop in recent years because of its very high export prospective. The cultivation of onion depends on, temperature; photoperiod etc. and it may suffers from several biological and non biological factors. Therefore one has to study the various factors which may affect on production of onion. For this we use statistical techniques such as Pareto chart, ANOVA and t-test.

**Keywords :** Pareto chart, one way ANOVA, two sample t-test

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---

### **Introduction**

Onion (*Allium cepa*) has been cultivated on Earth for the last 5000 years. It is the largest vegetable produced and consumed not only in India but also in the world. In India, onion is an extremely important commercial vegetable, having both food and medicinal values and grown almost across the country, mainly by small and marginal farmers, for domestic consumption as well as export. Indian onions are famous for their pungency and are available around the year, although with varying supply volumes, which sometimes creates volatility in prices. India produces all three varieties of onion – red, yellow and white.

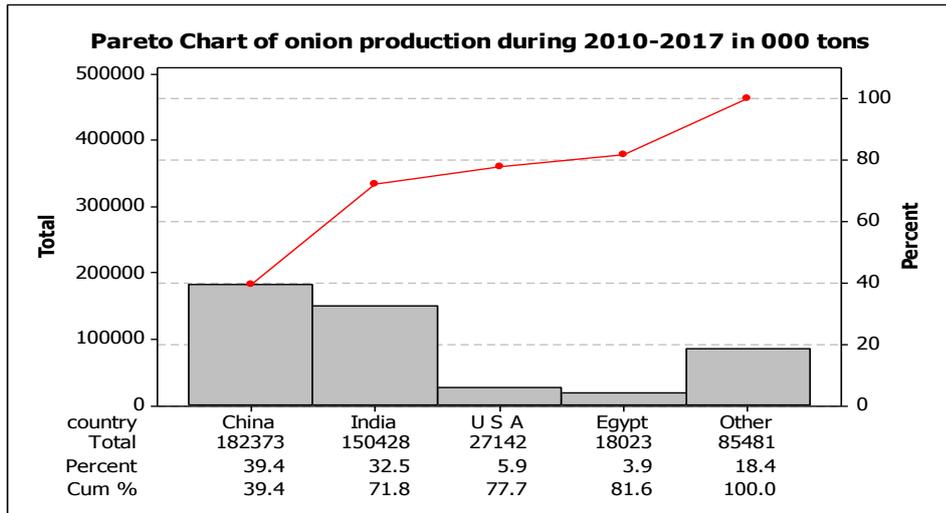
### **Objective**

- To study about the onion production, onion types, climatic conditions, import, export and consumption of onion and uses of onion.
- Our main objective is to use some statistical tools.
- To apply time series analysis for forecasting data.

### **Scope**

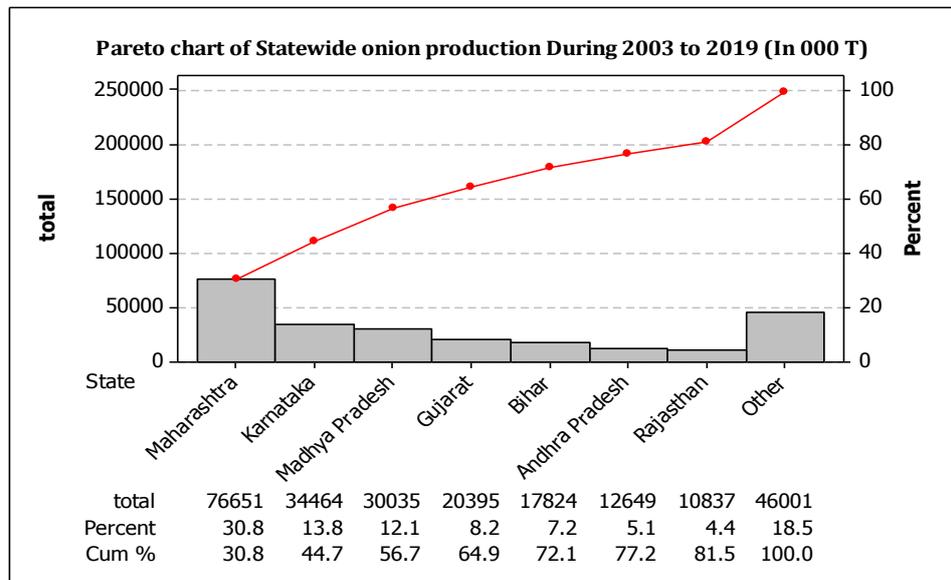
The project was conceived with the objective of understanding the informative part of onion plant and onion production and studies the other aspects of onion. The project output may helpful to find future forecasting.

**Statistical tools: Pareto chart:** A Pareto chart is simply frequency distribution of attribute data arranged by category. A special types of bar chart where the plotted in which values are arranged from largest to smallest.



**Fig. 1: Pareto analysis for countrywide total production of onion (2010-2017)**

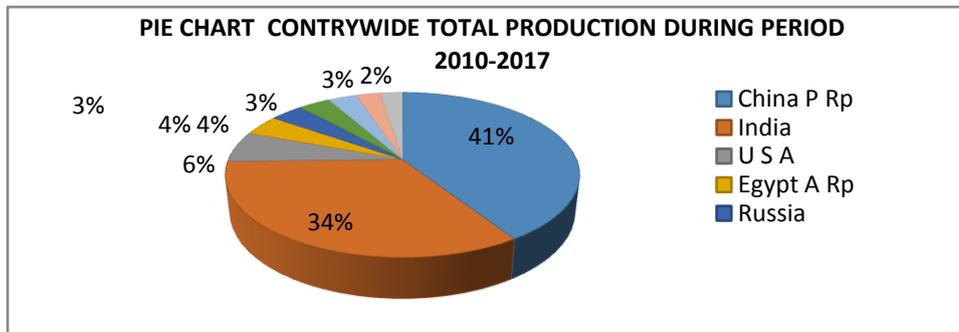
**Conclusion:** According to the Pareto chart, 80% production of onion is contributed by China, India, USA, Egypt and 20% production of onion contributed by other countries



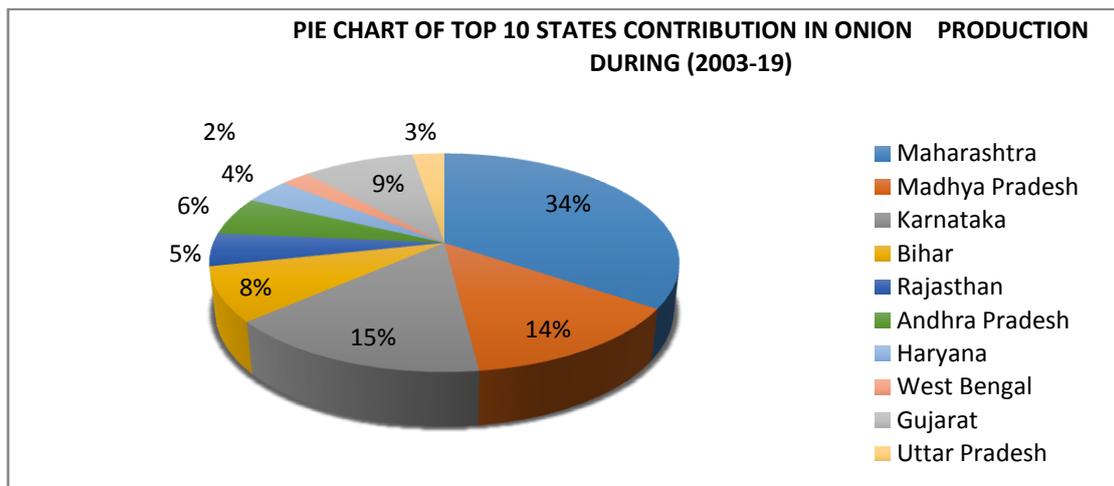
**Fig. 2: Pareto analysis for statewide total production of onion.(2003-2019)**

**Conclusion:** According to the Pareto chart, 80% of production of onion is contributed by Maharashtra, Karnataka, Madhya pradesh, Gujarat, Bihar, Andhra pradesh, Rajasthan.

**Worldwide statewide rank according to Production of Onion:** China has 1<sup>st</sup> rank, India has 2<sup>nd</sup> rank and U S A has 3<sup>rd</sup> rank of production of onion from 2010 to 2017. In India, Maharashtra has the first rank in onion production during the period 2003 to 2019.



According to above pie chart, China has largest contribution in onion production which is 41% while India has second largest contribution in onion production which is 34% then U S A , Egypt, Russia, Turkey, Pakistan, Netherland, Bangladesh have contribution in onion production 4%, 3%, 3%, 3% and 2% respectively.



**Conclusion:** According to above pie chart, Maharashtra has largest contribution in onion production which is 34% .then Madhya Pradesh has second largest contribution of onion production and Karnataka(15%), Bihar(8%), Rajasthan(5%), Andhra Pradesh(6%), Haryana(4%), West Bengal(2%), Gujarat(9%) and Uttar Pradesh(3%).

**One-way ANOVA: China, India, U S A, Iran, Egypt**

Method

$H_0$  : All means are equal for given countries, Vs  $H_1$  : At least one mean is different, Significance level  $\alpha = 0.05$

**Analysis of Variance:**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	4	3249347623	812336906	608.42	0.000
Error	35	46730527	1335158		
Total	39	3296078150	-	-	-

**Model Summary and Mean:**

Model Summary		Means				
S	1155.49	Factor	N	Mean	Std. Dev	95%CI
R-sq	98.58%	China	8	22797	958	(21967, 23626)
R-sq(adj)	98.42%	India	8	18803	2314	(17974, 19633)
R-sq(pred)	98.15%	U S A	8	3392.7	226.8	(2563.4, 4222.1)
		Iran	8	2170.2	206.9	(1340.8, 2999.5)
		Egypt	8	2253	555	(1424, 3082)

Pooled Standard Deviation = 1155.49

**Decision:** P-value (0.000) < alpha(0.05) hence, reject null hypothesis.

**Conclusion:** At least one mean is different.

**One-way ANOVA: Maharashtra, Madhya Pradesh, Karnataka, Bihar, Rajasthan**

$H_0$  : All means are equal Vs  $H_1$  : At least one mean is different, Significance level  $\alpha = 0.05$

**Analysis of Variance:**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	4	164574183	41143546	26.87	0.000
Error	75	114852937	1531372		
Total	79	279427120			

**Model Summary and Mean:**

Model Summary		Means				
S	1237.49	Factor	N	Mean	St. Dev	95%CI
R-sq	58.90%	Maharashtra	16	4791	2239	(4174, 5407)
R-sq(adj)	56.70%	Madhya Pradesh	16	1877	1283	(1261, 2493)
R-sq(pred)	53.23%	Karnataka	16	2154	924	(1538, 2770)
		Bihar	16	1114	142.8	(497.7, 1730.3)
		Rajasthan	16	677.3	351	(61.0, 1293.6)

Pooled Std Dev = 1237.49

**Decision:** P-value (0.000) < alpha (0.05). We reject null hypothesis

**Conclusion:** Average production of onion in given states are not same.

### **Two-Sample T-Test and CI: Production 1, Production 2**

Two-sample T for Production 1 vs Production 2

	N	Mean	StDev	SE Mean
<b>Production 1</b>	4	54478	2102	1051
<b>Production 2</b>	4	61384	3008	1504

Difference = mu (Production 1\_1) - mu (Production 2\_1), Estimate for difference: -6906

95% CI for difference: (-11623, -2190)

T-Test of difference = 0 (vs not =): T-Value = -3.76 P-Value = 0.013 DF = 5

**Decision:** P-value (0.013) < alpha (0.05). We reject null hypothesis.

**Conclusion:** There is significance difference between mean production of onion Period-1 and Period-2

### **Overall Conclusion:**

1. In India, Maharashtra is largest producer of onion production and in world china is largest producer of onion production.
2. In world and in India 80% of onion production contributes by China, India, USA, Egypt ,and Maharashtra, Karnataka, Madhya Pradesh, Gujarat, Bihar, Andhra Pradesh, Rajasthan respectively.
3. The forecasting values of onion production in world are 24796.00, 25689.20, 26550.00, 27378.40, 28174.5 And in India are 24796, 25689.2, 26550, 27378.4, and 28174.5
4. According to Rank table, In world China has 1<sup>st</sup> rank of onion production in period 2010-2017. In India Maharashtra has 1<sup>st</sup> rank of onion production in period 2003-2019.

### **References:**

- 1) <http://agriexchange.apeda.gov.in/>
- 2) Regression Analysis by Douglas C. Montgomery, John Wiley and sons.

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## **Statistical Analysis of Higher Education Quality in India by NAAC**

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### **Abstract**

As of 2020, India has over 1000 universities, with a break up of 54 central universities, 416 state universities, 125 deemed universities, 361 private universities, 7 Institute under State Legislature Act, and 159 Institutes of National Importance which include IIMs, AIIMS, IITs, IIITs, IISERs and NITs among others. There are 39931 Colleges affiliated to these universities. The students are confused about in which university/ college they should take admission where there is good quality of teaching staff, Excellent infrastructure, research culture etc. To solve such type of problem about educational standard, National Assessment and Accreditation council assesses the quality of college and university and award the grade with CGPA. Using the grade and CGPA we have some statistical technique to check and compare the quality in various universities in Maharashtra and India. Secondary data of CGPA obtained from NAAC web site has been used to analyze the data using some statistical technique for comparison of universities.

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### **Introduction**

India has one of the largest and diverse education systems in the world. Privatization, widespread expansion, increased autonomy and introduction of Programmes in new and emerging areas have improved access to higher education. At the same time, it has also led to widespread concern on the quality and relevance of the higher education. To address these concerns, the National Policy on Education (NPE, 1986) and the Programme of Action (PoA, 1992) spelt out strategic plans for the policies, advocated the establishment of an independent National accreditation agency. Consequently, the National Assessment and Accreditation Council (NAAC) was established in 1994 as an autonomous institution of the University Grants Commission (UGC) with its Head Quarter in Bangalore. The mandate of NAAC as reflected in its vision statement is in making quality assurance an integral part of the functioning of Higher Education Institutions (HEIs). The NAAC functions through its General Council (GC) and Executive Committee (EC) comprising educational administrators,

policy makers and senior academicians from a cross- section of Indian higher education system.

#### **VISION:**

- To arrange for periodic assessment and accreditation of institutions of higher education or units thereof, or specific academic programmes or projects
- To stimulate the academic environment for promotion of quality in teaching-learning and research in higher education institutions
- To encourage self-evaluation, accountability, autonomy and innovations in higher education
- To undertake quality-related research studies, consultancy and training programmes,
- To collaborate with other stakeholders of higher education for quality evaluation, promotion and sustenance.

The NAAC methodology for Assessment and Accreditation is very much similar to that followed by Quality Assurance (QA) agencies across the world and consists of self-assessment by the institution along with external peer assessment organized by NAAC.

#### **CORE VALUES:**

*(i) Contributing to National Development, (ii) Fostering Global Competencies among Students, (iii) Inculcating a Value System among Students, (iv) Promoting the Use of Technology and (v) Quest for Excellence*

#### **Revised Assessment and Accreditation (A&A) Framework**

The Revised Assessment and Accreditation Framework is launched in July 2017. It represents an explicit Paradigm Shift making it ICT enabled, objective, transparent, scalable and robust.

- Data based quantitative indicator evaluation with increased objectivity and transparency
- towards extensive use of ICT confirming scalability and robustness
- Simplification of the process drastic reduction in number of questions, size of the report, visit days and so on.

- Boosting benchmarking as quality improvement tool. This has been attempted through comparison of NAAC indicators with other international QA frameworks
- Introduction of Pre-qualifier for peer team visit, as 30% of system generated score
- Introduction of *System Generated Scores* (SGS) with combination of online evaluation (about 70%) and peer judgment (about 30%)
- Introduction of the element of *third party validation* (DVV) of data

Distribution of Qualitative metrics ( $Q_nM$ ), Quantitative metrics ( $Q_mM$ ) for universities and colleges along with the key indicators is in **Table 1**.

**Table 1:** Distribution of Metrics and KIs across Criteria

Type of HEIs	Universities	Autonomous Colleges	Affiliated/Constituent Colleges	
			UG	PG
Criteria	7	7	7	7
Key Indicators (KIs)	34	34	31	32
Qualitative Metrics ( $Q_nM$ )	36	35	35	36
Quantitative Metrics ( $Q_mM$ )	79	72	58	60
<b>Total Metrics (<math>Q_nM + Q_mM</math>)</b>	<b>115</b>	<b>107</b>	<b>93</b>	<b>96</b>

## Data Analysis and Interpretations:

### 1. QUALITY INDICATOR FRAMEWORK (QIF) - DESCRIPTION

The criteria based assessment forms the backbone of A&A process of NAAC. The seven criteria represent the core functions and activities of a HEI. In the revised framework not only the academic and administrative aspects of institutional functioning but also the emerging issues have been included. The seven Criteria along with their weightages to serve as basis for assessment of HEIs are as above tables.

### 2. Statistical Analysis of CGPA of colleges various Universities in Maharashtra:

Statistical Analysis of CGPA scores of Universities in Maharashtra valid up to March 2019 is studied for statistical parameters such as range, mean, variance, standard deviation, coefficient of variation and standard errors. These results are given in table 2.

**Institutional CGPA has been calculated by the equation**

$$\text{Institutional CGPA} = \frac{\sum_{i=1}^7 (CrWGP_i)}{\sum_{i=1}^7 (W_i)}$$

Where, Criterion-wise weighted Grade Point : ( $CrWGP_i$ ) and Weightage : ( $W_i$ )

### 3. Statistical Analysis of GRADES of colleges various Universities in Maharashtra:

Grades awarded to the colleges of various universities is studied which is given in table 3.

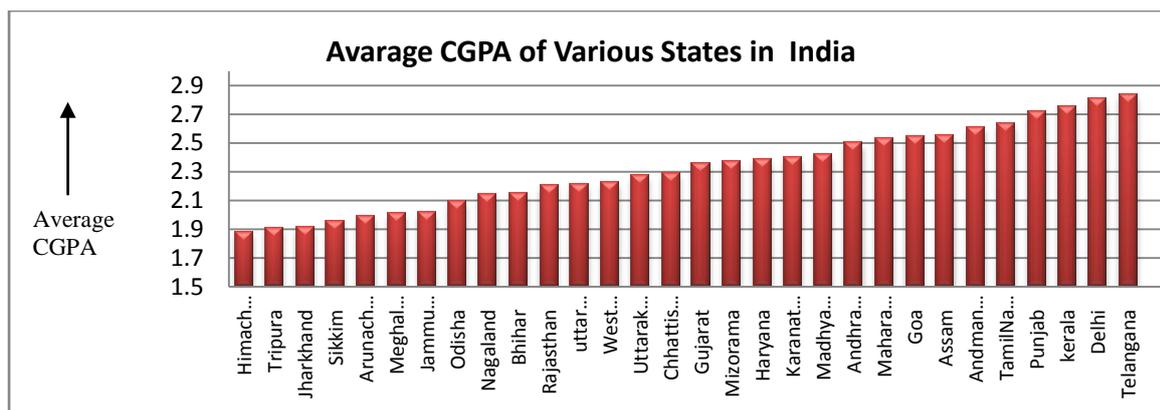
**Table 2: Statistical Analysis of CGPA SCORES of Universities in Maharashtra valid up to March 2019**

Statistics	BAMU	KBC NMU	Mumbai	Nagpur	Amravati	Pune	Kolhapur	Solapur	Nanded
Sample size (No. of colleges )	23	17	41	38	32	110	23	12	13
Range	1.73	2.03	2.05	1.56	1.53	1.66	1.29	1.66	1.48
Mean	2.5204	2.4906	2.5876	2.4724	2.4409	2.6211	2.4565	2.2033	2.3631
Variance	0.2024	0.2855	0.2152	0.1810	0.19452	0.1395	0.1056	0.2136	0.1976
Std. Deviation	0.4499	0.5343	0.46387	0.4255	0.44104	0.3735	0.3249	0.4622	0.4445
Coeff of variation	0.1785	0.2145	0.17927	0.1721	0.18069	0.1425	0.1323	0.2097	0.1881
Std Error	0.0938	0.1296	0.07244	0.0690	0.07797	0.0356	0.0677	0.1334	0.1233

**Table 3: Grade Analysis of Universities in Maharashtra as on March 2019**

Grade /No. of colleges (309)	BAMU 23	KBC NMU 17	Mumbai 41	Nagpur 38	Amravati 32	Pune 110	Kolhapur 23	Solapur 12	Nanded 13
A++ (2) (0.647 %)			2						
A+ (6) (1.942 %)		2				3		1	
A (44) (14.238%)	5	1	3	4	6	22	2		1
B++ (46) (14.885 %)	1	3	10	8	4	17	2		1
B+ (62) (20.06 %)	4	3	12	5	3	25	6	2	2
B (115) (37.214% )	11	5	9	15	15	37	12	5	6
C (34) (11.00 %)	2	3	5	6	4	6	1	4	3

#### 4. Statistical Analysis of CGPA of colleges various Universities in India:



**Fig. 1:** Average CGPA of Various States in India

**Conclusion:**

Only two college awarded A++ and 6 colleges awarded A+ grade in Maharashtra.. About 50% of the colleges in Maharashtra awarded either A or B++ grades. Most of the college i.e. 37.214 % of the colleges awarded B grade. Few colleges (11 % ) awarded C grade. Mean CGPA of Mumbai University, Mumbai is maximum i.e. 2.5876 and minimum for Solapur and it is 2.2033. Mean CGPA of KBC NMU is 2.4906. Telangana has highest average CGPA (2.8353) in India and Himachal Pradesh has lowest (1.8825). Maharashtra universities are among in first top ten in India in terms of average CGPA.

**References:**

1. www.naac.gov.in
2. Annual Report of NAAC 2017
3. Institution accredited by NAAC up to 4, March 2019.
4. NAAC News May 2020.
5. Manual for Affiliated / Constituent Colleges published in July 2017.

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# MATHEMATICS

## Some Magical Results of Various Numbers

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### Abstract

In this paper we express the different numbers in various forms and some of them are generalized. Also we find the one property related with the number 11.

**Keywords:** even numbers, odd numbers, squares, divisibility.

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### Introduction

A rapid progress of the world in the number theory, there are lots of magical theories are innovated. Number theory is a major tool in the mathematics and other sciences.

What is the magic number in mathematics? It is 1729(called Hardy-Ramanujan number) which was discovered by mathematician Srinivas Ramanujan. A number 1729 is called magic number because it can be expressed as the sum of the cubes of two different sets of numbers. From the same idea we developed such types of numbers, in which we expressed that numbers as like number 1729 and also add some mathematical approach. We find another numbers like as above and express that in interesting forms. There are lots of properties of various numbers which increase the beauty of the number theory. For example  $1^2 = 1$

$$11^2 = 121$$

$$111^2 = 12321$$

$$1111^2 = 1234321 \text{ and so on}$$

Also we introduced the one property of number 11.

### Methodology

Number 2017:

We express this number as

$$9^2 + 44^2 = 2 \times 7^3 + 11^3 = 2017$$

Number 2020:

2020 is the most memorable year. During this year the world suffering from Covid-19 virus. We express this number as sum of squares of two different numbers in two ways

$$16^2 + 42^2 = 2020$$

$$24^2 + 38^2 = 2020$$

Number 2025:

This number 2025 can be expressed in following different ways

$$(20 + 25)^2 = 27^2 + 36^2 = 2025$$

$$5^2 + 20^2 + 40^2 = 2025$$

$$10^2 + 20^2 + 25^2 + 30^2 = 2025$$

$$(2 + 0 + 2 + 5) \times 225 = 2025$$

Number 1458:

This is the amazing number. We can express this number as follows

$$(1 + 4 + 5 + 8) \times [(1 + 8) \times (4 + 5)] = 18 \times 81 = 1458$$

$$(1 + 4 + 5 + 8) + (1 \times 4 \times 5 \times 8) \times (1 + 8) = 1458$$

$$2 \times 27^2 = 2 \times 9^3 = 1458$$

$$[2(1 + 4 + 5 + 8)]^2 + \frac{(1+4+5+8)^2}{2} = 1458$$

$$2 \times (1^3 + 4^3 + 5^3 + 8^3) + 3 \times (1 + 4 + 5 + 8) = 1458$$

Number 1981:

This can be expressed as (1) the sum of the cubes of four numbers, (2) difference of cubes of two numbers.

$$1. \quad 11^3 + 7^3 + 6^3 + 4^3 + 3^3 = 1981.$$

$$2. \quad 13^3 - (6)^3 = 1981.$$

Number 1800:

This can be expressed as the sum of the cubes of first five even numbers

$$2^3 + 4^3 + 6^3 + 8^3 + 10^3 = 1800.$$

If we add these five numbers we get 30 and  $2 \times 30^2 = 1800$ .

In general  $2^3 + 4^3 + \dots + (2n)^3 = 2(2 + 4 + \dots + 2n)^2$ .

The result for the same is the sum of cubes of first  $n$  even numbers is equal to eight times the sum of cubes of  $n$  numbers.

$$2^3 + 4^3 + \dots + (2n)^3 = 8(1^3 + 2^3 + \dots + n^3)$$

Number 1225:

This can be expressed as the sum of the cubes of first five odd numbers

$$1^3 + 3^3 + 5^3 + 7^3 + 9^3 = 35^2 = 1225.$$

Also, it can be expressed as the sum of squares of three different numbers in two ways

$$10^2 + 15^2 + 30^2 = 1225$$

$$6^2 + 10^2 + 33^2 = 1225$$

Number 12345679:

If the number 12345679 multiplies by table 9 then we get magical answer

$$12345679 \times 9 = 111111111$$

$$12345679 \times 18 = 222222222$$

$$12345679 \times 27 = 333333333$$

.....

$$12345679 \times 81 = 999999999$$

Property of number 11:

Consider one number between 01 to 99 say  $ab$  and take  $ba$  reverse of that number.

Then

1.  $ab + ba$  is divisible by 11
2. The four digit number  $abba$  is divisible by 11.

**Conclusion:**

We can find such type of numbers and express in various forms. Also we can develop properties of some numbers.

**References:**

1. Hardy Ramanujan number, Wikipedia.
2. Elementary Number Theory, David M. Burton, Tata McGraw-Hill Publishing Company Limited.
3. Introduction to Analytic Number Theory, Tom M. Apostol.

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## **Some Results of Mathematics those are shown in Nature**

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### **Abstract**

In this paper we discuss about the terms of mathematics that are demonstrate in nature. The nature shows various patterns in trees, birds, flowers, animals etc in which we introduce the mathematics regarding with that.

**Keywords:** symmetry, hexagon, polygon, Fibonacci series

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### **Introduction**

Mathematics plays vital role in various fields. The nature filled with the full of mathematics. If we consider flowers there is one of the mathematics related term. There is mathematics on the animals. In the honey comb there is one type of mathematics like hexagonal symmetry. The great mathematician Euclid says, "The laws of nature are but the mathematical thoughts of God".

Mathematics in Nature is an excellent and undaunted introduction to the ideas and methods of mathematical modeling. To formulate and solve puzzles which are observed in nature and for the solution of it mathematics plays crucial role. The art of estimation and the effects of scale are also introduced by the mathematics. Mathematics is all around us. As we discover more and more about our environment and our surroundings we see that nature can be described mathematically. The beauty of a flower, the majesty of a tree, even the rocks upon which we walk can exhibit nature's sense of symmetry.

### **Methodology**

The various forms in the nature describe mathematically such as rainbows, river meanders, spider webs, honeycombs, and the markings on animal coats, etc. The beautiful patterns on hills, volcanoes, animals, butterfly, leaf, etc can be described mathematically.

#### **I. Geometrical shapes:**

There are different types of geometrical shapes observed in nature. Earth is one of geometrical shape with tremendous gravity on its outer edges. The Earth shows geometric shape called sphere. Other geometric shape hexagons fit most closely together without any gaps which is appeared in honey comb. In the honey comb, the hexagonal wax cells are used for what bees create to store their eggs and larvae. Hexagons are six-sided

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polygons, closed, 2-dimensional, many-sided figures with straight edges. Also geometrical shape cones are made by volcanoes. The formation of cones with the steepness and height of which depends on the runniness (viscosity) of the lava. If speed of runny lava is fast then it forms flatter cones and thick, viscous lava forms steep-sided cones. Cones are 3-dimensional solids.

## II. Fibonacci number:

If you construct a series of squares with lengths equal to the Fibonacci numbers (1, 1, 2, 3, 5, 8, 13, 21, etc) and trace a line through the diagonals of each square, it forms a Fibonacci spiral. Examples of the Fibonacci spiral can be seen in nature, including in the chambers of a nautilus shell. Fibonacci spiral can be also found in sunflower, Pineapple.

## III. Symmetry

Symmetry is everywhere in the Nature. Symmetry is when a figure has two sides that are mirror images of one another. Symmetry would then be possible to draw a line through a picture of the object and along either side the image would look exactly the same. This line would be called a line of symmetry.

There are two kinds of Symmetries: 1.Bilateral symmetry and 2.Radial symmetry

1. Bilateral symmetry: An object has two sides that are mirror images of each other are called bilateral symmetry. For example human body, butterfly, leaf, etc.
2. Radial symmetry: In the radial symmetry a center point and numerous lines of symmetry could be drawn. Radial symmetry is rotational symmetry around a fixed point known as the center. Radial symmetry can be classified as either cyclic or dihedral. For examples some flowers, spider comb, starfish, China rose, etc. The half orange internal structure is one of the examples of radial symmetry.

## Conclusion

Mathematics is everywhere in this universe. We hardly ever note it. We enjoy nature and study about what mathematical idea is in it. Mathematics expresses itself everywhere, in all most every facet of life- in nature all around us.

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