A
Compendium of Research Articles by Prospective Researchers
Volume-IV
2021-22

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

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Compendium of Research Articles by Prospective Researchers

2021-22

Under the

Researchers' Prospective 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

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FOREWORD

It is indeed a matter of great pleasure to me that KCE Society's Post Graduate College of Science, Technology and Research, Jalgaon is doing a commendable job in inculcating the scientific attitude amongst the students by publishing ***A Compendium of Research Articles by Prospective Researchers*** under **Prospective Researchers' Scheme** (PRS) for the students.

It is an appropriate platform for the researcher students of the college for creation of innovative ideas on various research activities. Such venture will go a long way to create a conducive research environment in the college. I am sure that this volume touches upon latest research in the field of science and technology which is encouraging for all those who are aspirant in scientific research.

I take this opportunity to congratulate the principal, Co-ordinator, supervisors and students for their contribution in such a activity. My best wishes to the **volume IV**, ***A Compendium of Research Articles by Prospective Researchers.***

(Mr. N. G. Bendale)
Hon'ble President, KCE Society, Jalgaon

KHANDESH COLLEGE EDUCATION SOCIETY ★ JALGAON

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PREAMBLE

I am pleased to mention that, KCE society's Post Graduate college of Science, Technology and Research, Jalgaon is publishing the fourth 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. During the year 2021-22, this scheme is continued in which **23** projects (research articles) have been completed by **80** 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

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From the Desk of Editor

I am pleased to present to you the Fourth edition of ***A Compendium of Research Articles by a Prospective Researchers*** under the activity of "Prospective Researchers' Scheme" for the year 2021-22. This volume presents the seventeen articles from students and teachers of four Science Departments of college. **'Prospective Researchers' Scheme** is unique research activities of the college in which students undertake researchprojects 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 thirteen research projects were completed from four Departments of Organic Chemistry, Microbiology, Biotechnology and Statistics. The research/review papers based on their articles is published in a separate volume as **"*A Compendium of Research Articles by a Prospective Researchers* Volume IV"** 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 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.

Dr. Ravindra M. Patil
Chief Editor

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*Corresponding author and Supervisor

CHEMICAL SCIENCES

Solvent free Synthesis of Biginelli Dihydropyrimidinonesusing Glycine as catalyst

Nikita S. Gavale, Dhanashree K. Patil., Vikrant B. Khangar, Jayesh R.Khairnar, Rupali A. Chaudhari *

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Abstract

The synthesis of various substituted 3,4-dihydropyrimidinone has been achieved in presence of glycine as catalyst under solvent-free condition. The improved Biginelli reactions not only feature a simple procedure, but also give high yield and easy purification of procedure.

Keywords*: Biginelli dihydropyrimidinone, one pot condensation reaction, solvent-free condition, Glycine catalyst.*

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Introduction

Green Chemistry technique continues to grow in important. It is an alternative process to help to conserve resources and can reduce costs. The replacement of convention solvents with green solvent ethanol, which is harmless to health and available in large quantities, ison in trusting basic approach along this lines[1-3].

A multicomponent reaction plays an important role in modern organic chemistry, because they generally exhibit higher atom economy and selectivity as well as produce fewer by-product compared to classical multistep synthesis[4]. Furthermore, MCRs are easy to perform, quick, consuming less energy and involves simple experimental procedures [5]. The first multicomponent reaction was described in 1850 by striker[6], and there after many such reactions has been reported in the literature. They improve selectivity, reduce reaction time and simplified separation and purification of product than the chemistry has posed a new challenge for organic synthesis in that new reaction condition need to be found which reduced the emission ofvolatile organic solvents and the use of hazardous toxic chemicals[7].

In 1893, Italian Chemist Pietro Biginelli reported on the acid catalyzed cyclocondensation reaction of Ethyl acetoacetate, Benzaldehyde and Urea[8]. The reaction was carried out simply byheating a mixture of the three components dissolved in ethanol with a catalytic amount of HCl at reflux temperature. The product of this novel one pot, three component synthesis that precipitated on cooling of the reaction mixture was identified correctly by Biginelli as 3,4-dihydropyrimidin-2[1H]-one[9-10].

Since the late 1980 is, a tremendous increase in activity has again occurred, as evidenced by the growing number of publication and patents on the subject [11]. This is mainly due to fact that the multifunctionalized dihydropyrimidine scaffold [DHPMs, "Biginelli compound"] represent a heterocyclic system of remarkable pharmacological efficiency. In the past decades, a broad range ofbiological effects, including antiviral, antitumor, antibacterial and anti-inflammatory activates has been described to these partly reduced pyrimidine derivatives. More recently, appropriately functionalized DHPMs have emerged as e.g orally active antihypertensive agents. Or α_{1a} adrenoceptorselective antagonists. Most notably among these are the batzelladine alkaloids, which inhibit the binding of HIV envelope protein GP-120 to human CD4 cells and therefore, are potential new leads for AIDS therapy[12].

Result and discussion

Optimized reaction conditions:

Initially we performed reaction without catalyst the yield ofproduct is only about 50%. The time required

was also more (about 4 hours) to complete the reaction but we used glycine as catalyst the timewas reduced.

To investigate the role of glycine, reaction is carried out inpresence of glycine catalyst. It was observed the 3,4- dihydropyrimidinone formation timing was decreases in presence of glycine. While the same reaction occurred slowly in Lewis acid and organic acid.

Hence, by using glycine catalyst we performed the model reaction with different mole % of glycine to optimized reaction condition as shown in **table-1**.

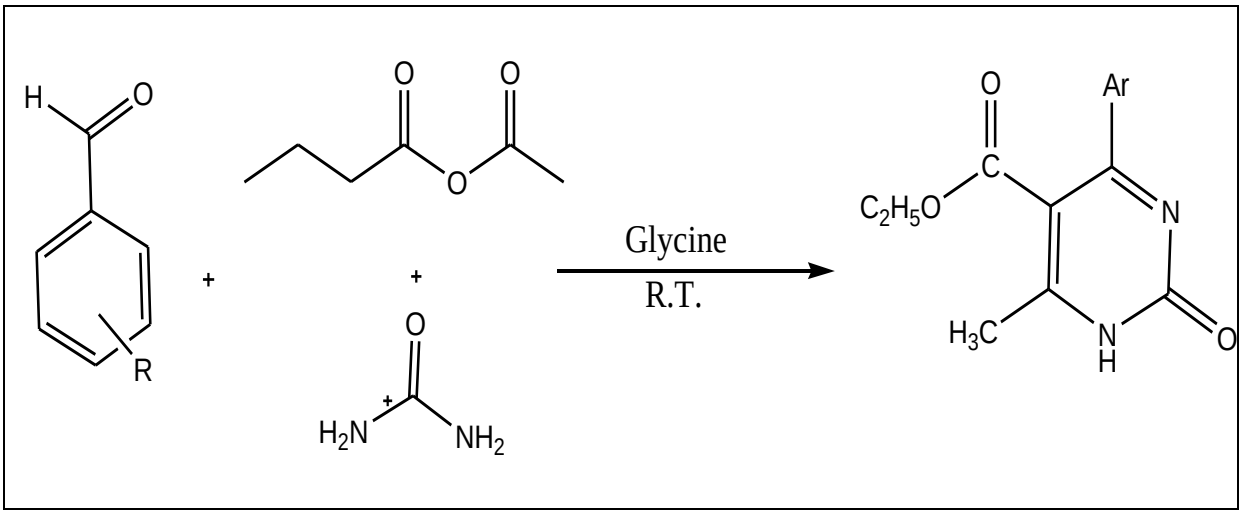
Table 1: Optimized amount of catalyst loaded

Entry	Catalyst(mole %)	Time (min)	Yield(%)
1	5	20	80
2	10	15	44
3	**15**	**10**	**96**
4	20	10	96
5	25	8	92

Here we find 15 mole% catalyst is sufficient to push the reaction. Hence the reaction can perform with 15 mole % as catalyst by optimized the reaction condition.

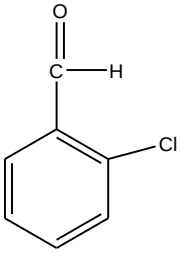
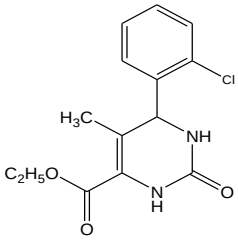
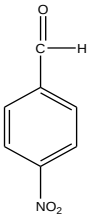
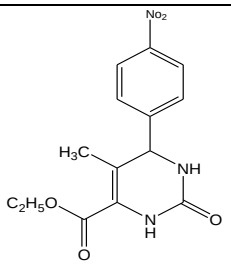
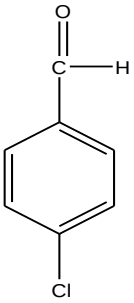
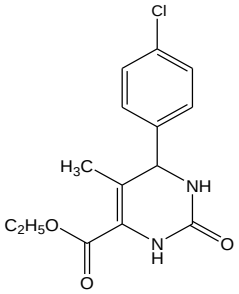
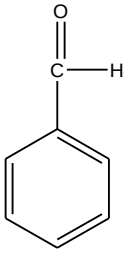
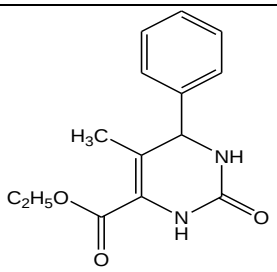
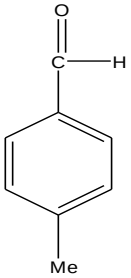
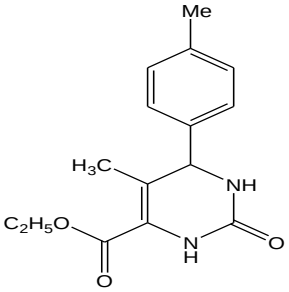
Synthesis of biologically active Dihydroprimidinone compounds we use readily available, inexpensive and environment friendly reagent. The reaction of various substituted benzaldehyde with ethyl acetoacetate and urea carried out using glycine as catalyst.

After the study of above optimized reaction condition were explored for the synthesis of series of dihydropyrimidinone derivatives from various substituted benzaldehyde, ethyl acetoacetate and urea using glycine as catalyst as shown in **scheme-1** and the results are summarized in **Table-2**.

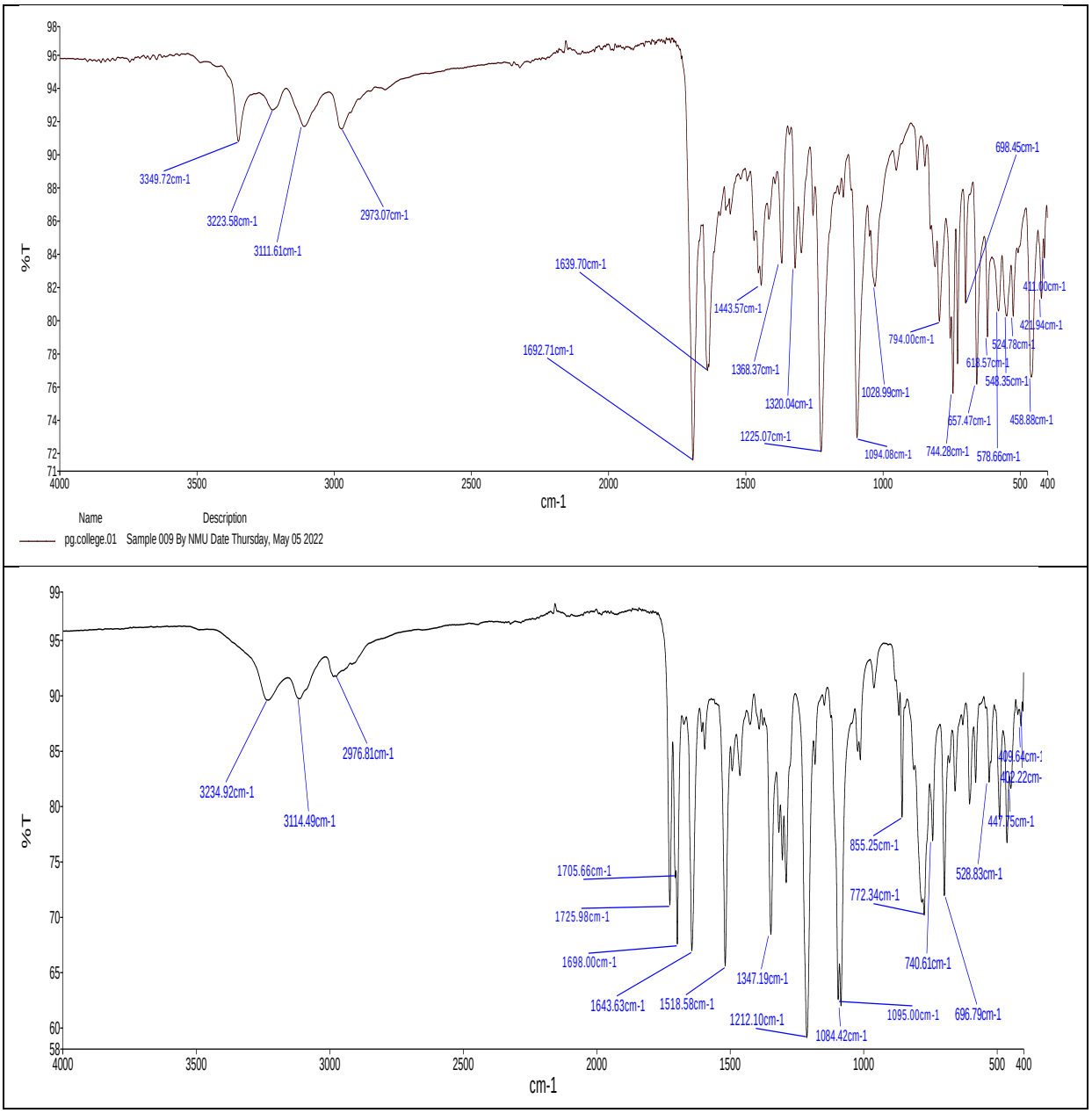


Scheme 1: Synthesis of dihydopyrimidinone by using glycine as catalyst

Table-2: Synthesis of Dihydropyrimidinone

Sr. No.	Substituted benzaldehydes	Product	Time (min)	Yield (%)	Melting Point (^oC)
1.			10	81	220
2.			20	70	176
3.			50	14.63	170
4.			8	84	160
5.			20	80	152

IR Spectra of compound (1&2)



Spectral data of compounds (1-4)

1.YellowSolid,IR(cm^1):1730.51(C=O),1450.21(C=N),3214.28(NH),3064.89(C=C),2962.12(C=O)

2.OrangeSolid,IR(cm^1):1730.51(C=O),1450.21(C□N),3214.28(NH),3064.89(C□C),2962.12(C=)

3.WhiteSolid,IR(cm^1):1730.51(C=O),1450.21(C=N),3214.28(NH),3064.89(C□C),2962.1(C=O)

4.BrownSolid,IR(cm^1):1730.51(C=O),1450.21(C□N),2962.12(C=O),3214.28(NH),3064.89(C□C)

Conclusion:

Solvent free synthesis is a highly efficient procedure for the preparation of substituted Dihydropyrimidinone derivatives, using glycine as a catalyst. Moreover, the procedure offers several advantages including high yield,

operational simplicity, cleaner reaction, minimum environmental impact and low cost; which make it a useful attractive process for the synthesis of the compound.

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Preparation and Physicochemical Analysis of Wine from Banana Fruit

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Department of Chemistry, KCE Society's Post Graduate College of Science, Technology and Research, Jalgaon

Abstract

Jalgaon district in Maharashtra, India is world famous for its banana (Musa Sapientum) fruit. Bananas have contain a lot of sugar, these fruit have a suitable substrate for making wine so it is easy to make alcohol from thisfruit. Wine is a major source of alcohol. Out of it Ethanol was also produced. Therefore, these study focuses on making wine from bananas and its physiochemical evaluation has also been done.

Keyword: *Banana Wine, Saccharomyces Cerevisiae, Hydrometer, Musa Sapientum.*

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Introduction

The idea of Rubisi (traditional banana wine) making in Tanzania has record of 300 years ago. It started wine making of banana juice (mulamba). Mulamba was becoming sour after two days and had no alcohol content. By that time farmer were drinking beer made from sorghum only and it was somehow bitter [1]. Banana (MUSA SAPIENTUM) is an important staple starchy food in Nigeria good source of sugar and fibers content making it a good sourcesof energy. The fruit has a long history in Nigeria as a staple food and seasonal crop with short shelf life under the prevailing temperature and humidity condition in tropical countries. It is a seasonal and highly perishable fruit, which can be available all year round. To forestall huge economic loss due to rapid determination of ripe banana, production of banana juice from pulp of ripe banana become a subject of research banana wine is a fruit wine madeexclusively from banana [2]. Wine is considered to be the one of the oldest alcoholic beverages produced by process of fermentation. Fermentation is relatively low energy preservation process which increases the self-life and decreases the need of refrigeration or any other forms of food preservation technology. Tropical wine are subjectively perceived as inferior quality on the basis of flavour,aroma, odour and colour [3]. Highly acceptable wines can be made from practically all fruits. There are some soft fruits from both temperate and tropicalregions whose pigment stability and flavour profilesmatch those of any wine from grapes, but suffer from the lack of intensive research and developmentgiven to grape wine. Banana is a seasonal and highlyperishable fruit, which can be available all yearround [4] In the wine-making process or the quality of the wine obtained have been reported, including improved primary and secondary flavours, malic acid decarboxylation by yeast, increased resveratrol, lactic acid, or glycerol contents, and improved survival properties [5]. Traditional process for the production of Urwagwa involves generally five mainly steps: ripening of green banana in warm pit covered with banana leaves and/or eucalyptus leaves (called Urwina in Rwandan language), peeling, banana juice extraction (mixing of ripe banana with spear glass, squeezing the mixture with their feet or hands), filtration of juice through grass held in calabash funnel and spontaneous fermentation. The fermentation process requires the addition of coarsely-ground, roasted sorghum malt and/or granulated sugars into the diluted banana juice [6]. Ripe bananas are consumed raw as a desert fruit. Banana serves as good nutritional sources of carbohydrates, minerals such as potassium and vitamins such as B1, B2, B3, B12, C and E. Following the high nutritional content of banana, it is consumed in large quantity in a variety of ways inAfrica. The banana fruit can be eaten raw or cooked(e.g. deep fried, dehydrated, baked in its skin or steamed), processed into flour or fermented for the production of beverages such as banana juice, beer

[7]. Banana have a lots of nutritional benefits and a better choice for people suffering from potassium deficiency because of its impressive potassium content. Potassium is an important component of cell and body fluid that helps control heart beats and blood pressure countering bad effect of sodium. Banana wine is an excellent health tonic that also aids in digestion. Medicinal uses of banana have positive contribution toward successful treatment of heartburn, temperature control, blood pressure, stroke risk, depression, anaemia, stress, ulcers, constipation, and diarrhea [8]. Jalgaon district in Maharashtra, India is world famous for its banana crop. However, for some time now, due to various weather conditions, the banana crop has not received adequate guarantees, which has resulted in economic losses to the farmers, leading to an increase in farmer suicides. That is our main objective as bananas are a perishable fruit, they are more likely to be damaged by the enzyme system. This creates many financial difficulties for the farmers. Also, some climate change and crop diseases cause severe damage to the banana crop. Therefore, the farmer has to bear the loss of his crop production. Therefore, it is necessary to store and preserve fruits after harvest after they have matured. One way to prevent damage to ripe bananas is to keep the banana crop fermented and make wine from it. Because wine is a value-added product for wine production. Therefore, there can be economic benefits in making wine from banana fruit. So bananas contain a lot of sugar, these fruit have a suitable substrate for making wine so it is easy to make alcohol from this fruit. Wine is a major source of alcohol. Out of it Ethanol was also produced. Therefore, these study focuses on making wine from bananas and its physiochemical evaluation has also been done.

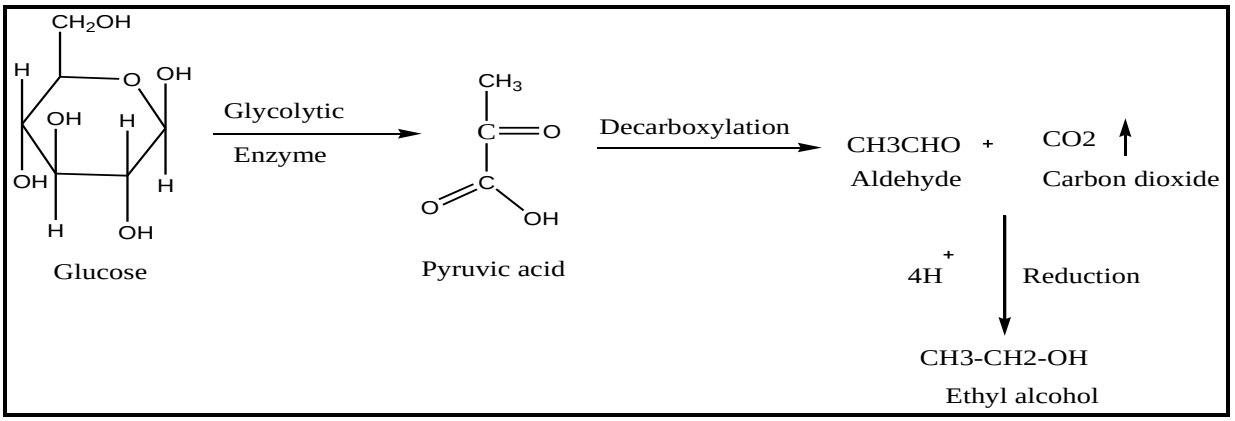
Material:

Material use for study were stainless steel mixing bowl, stainless steel pots and spoons, measuring cylinder, 2 big size glass jars, big size funnel, muslin cloth for filter, 1.5 lit. fresh water, 1.5 gm of white sugar, saccharomyces cerevisiae (Baker yeast), 3 fresh lemons, 100gm yellow and brown raisins, 20-20gm cinnamon and cardamom, purchased at Bhasker market, Jalgaon in March, 2022. Raw bananas we brought from the field without any artificial or chemical treatment are kept for 10 to 15 days ripening in a simple natural environment and the ripe bananas are we used.

Process for Banana Wine Preparation:

Washed Banana Fruit → Banana Pulp → Pectinase treatrnent (0.25% for 45 min) → pH 5 Add 5% Sugar(Glucose) → Add baker yeast (Saccharomyces Cerevisiae) → Fermentation (15 days at 32°C) → Filtering and clarify → Bottling → Storing in bottles for aging → Alcohol content (8-12%)

- **The main chemical conversion reaction of wine:**



Experimental section

1. Test for flavonoids (Ammonia test):

The wine sample was mixed with 1 ml. of 1% ammonia solution. The developing yellow colour indicates the presence of flavonoids.**Figure-1**

2.Test for glycosides (Sulphuric acid test):

The wine sample was added to a mixture of glacial acetic acid, 5% $FeCl_3$ and concentrated sulphuric acid was added. The developing brown ringindicates the presence of glycosides.**Figure-2**

3. Test for terpenoids:

The wine sample was added to a mixture of chloroform and a conc. solution of sulphuric acid carefully from the side of the test tube to form a layer. The developing reddish- brown coloration at the interface indicates the presence of terpenoid.**Figure-3**

4. Determination of organic acids:-

Qualitative detection of organic acids in the winewas carried out using paper chromatography. Standards (lactic, malic and tartaric acids) and banana wine were spotted on the commercially available Whatman Filter Paper No.1 Placed in apre-saturated chromatography chambercontaining the solvent mixture of butyl alcohol, formic acid and water solvent system (16:2:5). Paper chromatography was used to confirm the organic acids**.**

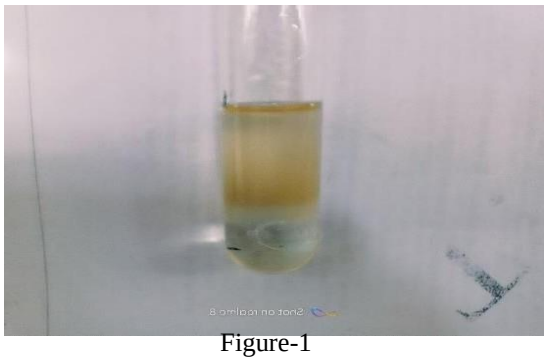


Figure-1

Figure-2

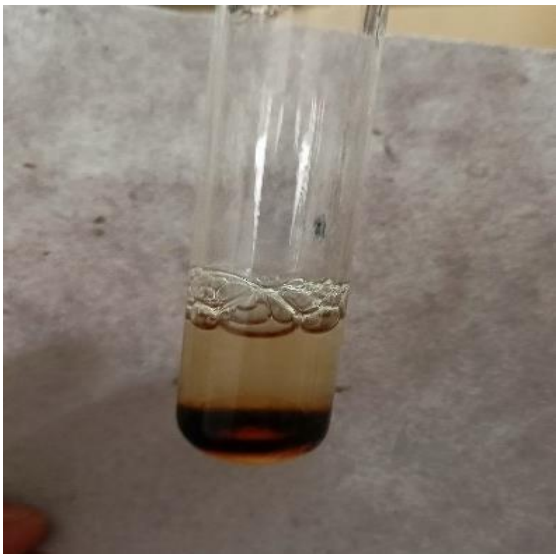


Figure-3

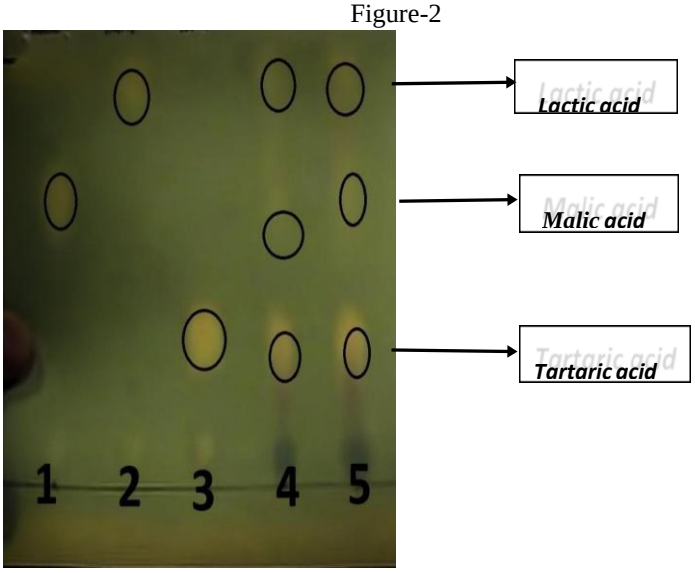


Figure-4

Test for alcohol contain in wine:-

Test for ethanol: Add few drops of iodine solution to alcohol (Original solution). Add 10 drops of Sodium hydroxide solution to alcohol. Gently swirl the testtube a few times. The dark colour of iodine should be start to fade.

Test for methanol: Add few drops of iodine solution to alcohol (Original solution). Add 10 drops of Sodium hydroxide solution to alcohol. Gently swirl the test tube for few seconds. A yellow colour precipitate of CHI_3 (iodine form) will indicates the presence of Methanol. But in our reaction there is no ppt. has formed so there Methanol is absent.

P^H Determination:-

The pH meter was standardized with buffer solution. The electrode of the pH meter was immersed in a glass beaker containing the sample and readings were obtained from the photo-detector of the pH meter. **Table-1**

- **Count Specific Gravity (Kg/m^3) by usingHydrometer :-**

  (Formula of ABV = (IG – FG) x *131.25)* **Table-1**

- **Count % of Alcohol present in the wine / ABV:-**

  Calculated Initial gravity of banana wine – 1.095 kg/m^3

  For example- our starting / initial gravity reading is 1.095 and our final gravity reading is 1.008 the math would look like this: (1.095 – 1.008) x 131.25 = 11.397, our alcohol by volume would be approximately 11.3%. (**Table-1)**

- **Temperature optimization**

  Variation in % of alcohol production during Banana Wine fermentation at different surrounding room temperature using baker yeast (Saccharomyces cerevisiae).

Table-1(Must fermentation day, pH, Specific gravity, % of alcohol, Room temperature)

Sr. No.	Must Fermentation period (Day)	pH	SpecificGravity (Kg/m^3)	% of alcohol/ ABV	Room Temperature (^{o}C)
1	0	3.96	1.040	7.2	36
2	3	3.64	1.032	8.2	37
3	6	3.41	1.027	8.9	40
4	9	3.20	1.020	9.8	38
5	12	3.09	1.014	10.6	41
6	15	2.98	1.008	11.3	41

- **Result and Discussion**

We used Saccharomyces cerevisiae (baker's yeast) to make wine and during that time the room temperature was slightly higher during the fermentation period. This reduced the fermentation period

of wine making and during this period we get excellent wines. Our banana wines alcohol percentage was also good.

Physicochemical parameters of banana wine

TEST	RESULT
Alcohol content	11.3%
pH of wine	3.0
Gravity of wine	1.008 Kg/m^3
Temperature for fermentation	37^0C
Total fermentation days	15 days.

- **Conclusion**

Banana wine has good flavour and aroma and high nutritional value. Its production could be way of reducing economic losses due to rapid deterioration of ripe banana as process is easy and does not require expertise and sophisticated equipment. Banana wine has lots of nutritional benefits vitamins including B_5, B_6, C, A are all present in banana wine and this makes it one of the high ranking beverages over other alcoholic once, vitamins A helps in restrains of eye sights. The over ripen banana can be used for wine preparation and easily be stored for a longer time. In addition to a storage benefits, banana winealso posses medicinal properties.

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Study of Effect of Particle Size and Catalyst on Chemical Recycling of PET Waste

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Abstract

PET waste taken from post-consumer soft drink bottles was subjected to alkali hydrolysis using sodium hydroxide. The product obtained in de-polymerization of PET waste is a value added product such as terphthalic acid (TPA). The reactions were studied using different particle size ranging from 1 to 5 cm^2 at specific constant temperatures. The purified product was characterized by recording its IR and melting point. Modified shrinking core model focused the light on depolymerization of the PET into TPA by fragmentation due to formation of pores and cracks.

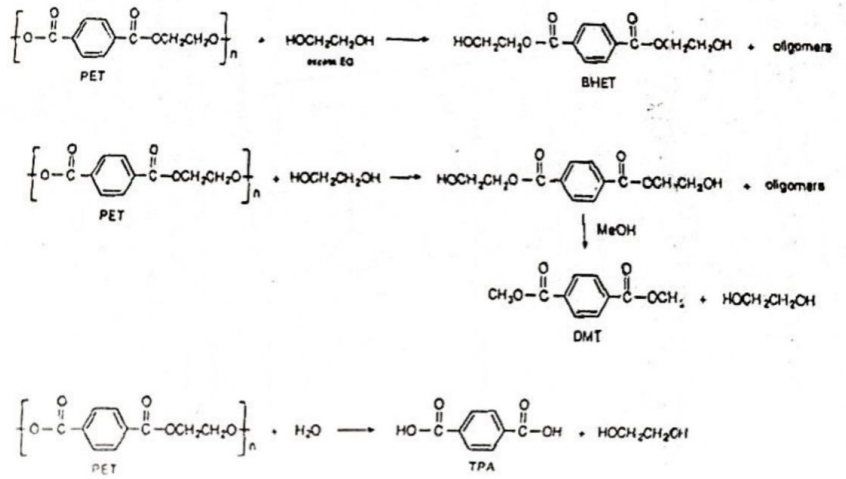
Key words: Depolymerization, PET , TPA, Chemical recycling, particle size.

corresponding author

Introduction

Polyethylene terephthalate (PET) exists both as a semi-crystalline and an amorphous thermoplastic polyester mainly used in textiles and packaging due to its good physicochemical properties such as lightweight, good heat resistance and dimensional stability resistance towards chemicals among others[1]. PET is best known as a monolayer, clear plastic widely used for (carbonated) beverage containers. Beside monolayer bottles, PET is extensively laminated with other types of polymers such poly olefines and ethylene vinyl alcohol (EVOH) especially in food packaging application, as it has a limited properties related to permeation of oxygen and sealing[2]. Generally, post-consumer plastics have very low recycling rates. However PET is one of the most recycled materials. PET can be recycled or recovered via four pathway namely primary recycling, secondary recycling, chemical recycling and incineration. This process is common practice for closed-loop recycling of PET bottles or recycling bottles to fibers. Although and this process is simple and requires relatively low investment, the generation of cyclic and linear oligomers during melting point processing causes around 30% reduction in PET melt viscosity [3]. Among the chemical methods glycolysis which is a method of alcoholysis using ethylene glycol is the oldest and most common method in Industry[5-6]. Although Chemical recycling of PET is increasing to enable for the production of PET designated for high-end applications, even at industrial scale, little fundamental information is available in the scientific literature related to these type of reactions. Especially the kinetics of chemical recycling on real plastic waste streams is not commonly understood among PET degradation method kinetic of PET glycolysis has been studied vastly[7-9]. According to these studies, after glycolysis, monomers recoveries are low (~25%) even in the presence of catalysts. Moreover the purification of monomers is problematic due to the occurrence of oligomers during degradation[8]**.** On the other hand high recoveries (~90%) can be obtained through methanolysis, but generally very harsh degradation condition are used and purification step is not always straight forward due to complex mixture of glycols. Phthalate derivatives and alcohols are in the reactions medium[10-11]. In addition the presence of water in the methanolysis process causes deterioration of the catalyst and formation of various azeotrope resulting in a decrease in the monomer purity [12-13]. Therefore, as an alternative alkaline hydrolysis of PET has been studied. In many of these studies either extreme degradation conditions eg:- high temperature and pressure are applied or catalysts are used [14,15,16,17,18] to the best knowledge there is detailed kinetic study available on the alkaline hydrolysis of PET in mild degradation condition. Further more in many scientific studies pure PET pellets are used related to chemicals degradation of PET, Which Typically different compared to more complex real plastic waste stream,

that differ in composition and shape, amongst others.



A) GLYCOLYSIS

B) METHANOLYSIS

C) HYDROLYSIS

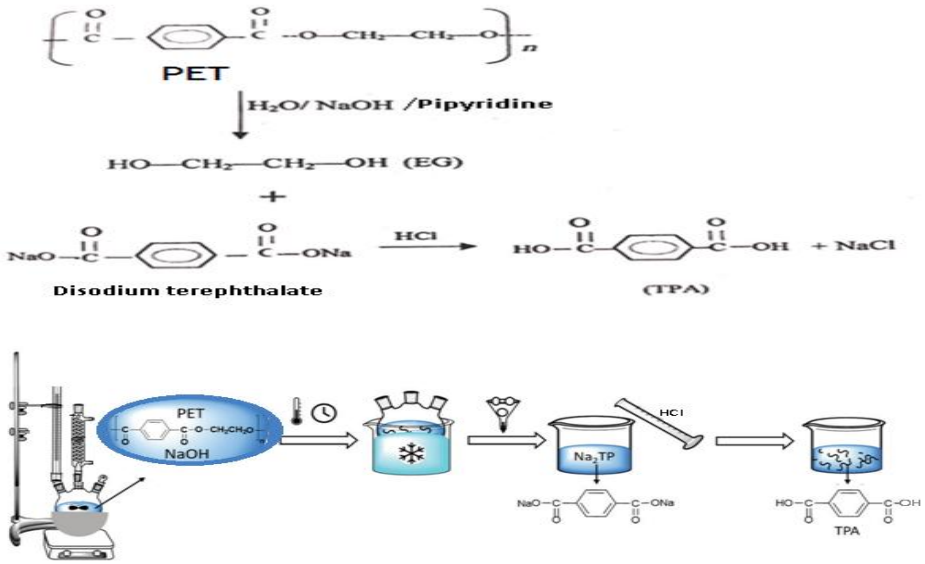
Global plastics production was estimated to be 367 million metric tons in 2020. Production in 2020 decreased by roughly 0.3 percent compared with the previous year due to COVID-19's impacts on the industry. The world now produces more than 380 million tones of plastic every year, which could end up as pollutants, entering our natural environment and oceans. In 2018, landfills received 27 million tons of plastic. This was 18.5 percent of all MSW land filled. The truth is that only a fraction of plastic is actually recycled. According to the most recent data estimates available from the Environmental Protection Agency, just 8.7 percent of the plastic that was discarded in the U.S. in 2018 was recycled. This will likely come as no surprise to longtime readers, but according to National Geographic, an astonishing 91 percent of plastic doesn't actually get recycled. This means that only around 9 percent is being recycled.

Bottled water accounted for 34.6 percent of global polyethylene terephthalate (PET) packaging consumption in 2019. This was followed by carbonated soft drinks, which accounted for slightly over a quarter of PET packaging consumed that year. More than 82 million metric tons of polyethylene terephthalate (PET) is produced globally each year to make single-use beverage bottles, packaging, clothing, and carpets, and it is one of the largest sources of plastic waste.13-Oct-2021. Virtually all single-serving and 2-liter bottles of carbonated soft drinks and water sold in the U.S. are made from PET. It is also popular for packaging salad dressings, peanut butter, cooking oils, mouthwash, shampoo, liquid hand soap, window cleaner, even tennis balls.

Apparatus & Chemicals: Two-necked round bottom flask, condenser, thermometer, beaker, funnel, distilled

water, NaOH, PET, pipyridine.

Methodology: PET alkaline hydrolysis was carried out by using two-necked round bottom flask equipped with condenser and agitator for stirring. 250 ml flask was charged with specific amount of sodium hydroxide, 4 ml pipyridine and 10 gram of PET of particular size in 150 ml of water. It was reflux for 3 hours using water condenser. After the specific time interval burner was removed to stop further heating. The reaction mixture was cooled and filtered to separate residual un-reacted PET. The filtrate of the reaction mixture was reprecipetated by adding concentrated hydrochloric acid till it is acidic. The milky white precipitate of TPA obtained was dried and weighed. PET was hydrolyzed in an aqueous alkaline medium with and without catalyst at atmospheric pressure to yield disodium terephthalate (Na_2 TP) salt. The solution mixture was then reprecipetated by concentrated hydrochloric acid, the TPA obtained was filtered dried and weighed. The chemical reaction and experimental set up are as shown below:



Characterization of TPA: The TPA obtained in depolymerization reaction was characterized by recording FTIR of Shimadzu make. The 1693.5 cm^{-1} frequency indicates presence of carbonyl group, 2555.6 to 3064.8 cm^{-1} frequency indicates presence of hydroxyl group, 783.1 cm^{-1} indicates Para substituted benzene and also comparing the spectra with control, TPA has been confirmed (figure 1 and 2). The Melting Point of TPA obtained was recorded for its characterization it was sublime above 300°C.

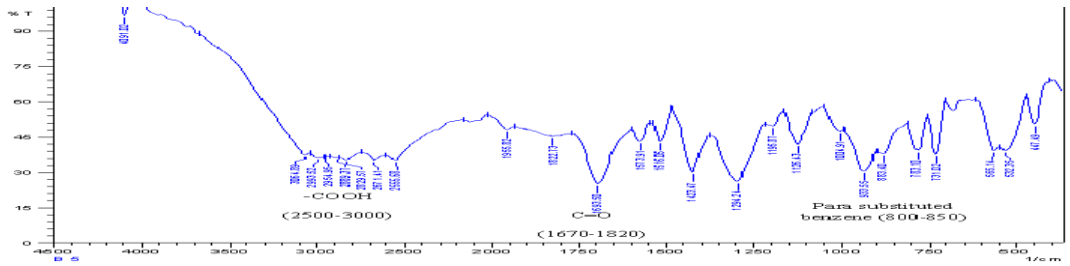


Fig.1: FTIR spectra of TPA (sample)

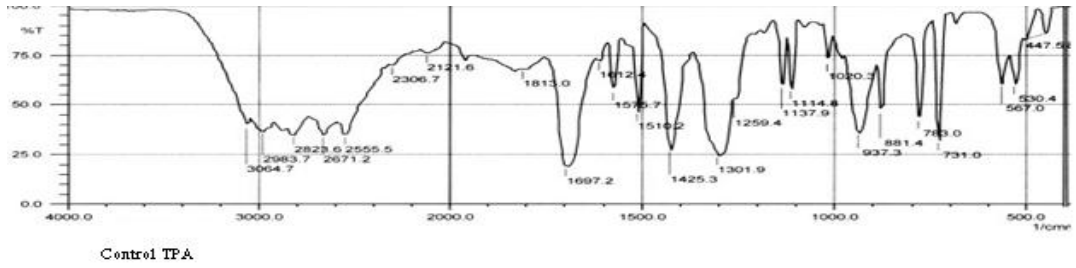


Fig.2: FTIR spectra of control TPA (Controlled)

Optimizations of amount of NaOH: It is evident from the Fig-3 that for 1 and 2 gram of NaOH conversion of PET into TPA is very low with of yield from 1.1% & 4% After that due to increase in rate of reaction with increase in amount of NaOH increase drastically and reaches to maximum with maximum yield of Chemical reaction that is 23%. Again with increase in amount of NaOH, percentage yield of reaction decrease. This is due to increase in viscosity of reaction, which retard the rate of reaction. Hence 4g NaOH is found to be optimized amount of NaOH required for chemical recycling PET waste of given experimental conditions.

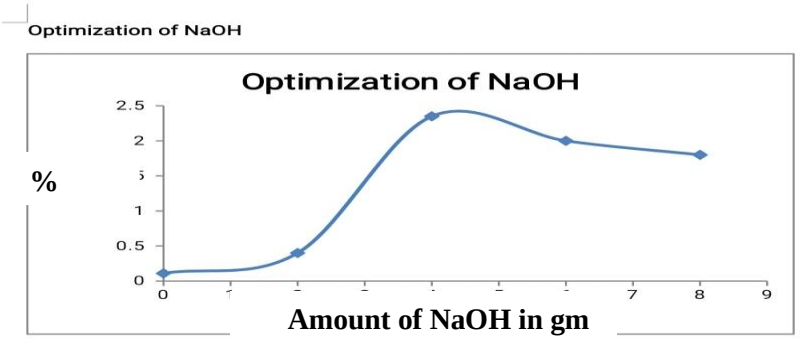


Fig 3: Optimization of NaOH

Effect of Particle size: Fig-4 shows effect of particle size as well as catalyst on the yield of depolymerization of PET into TPA. If is evident from the graph that the behaviors of with and without catalyst is same. Initially the 1cm² particle size % yield of low and as the particle size increase % yield increase reaches to maximum at 3 cm² particle size. After 3 cm^2 particle size again % yield decrease. Hence for 10g of PET optimum particle size at which maximum % yield is 3cm² It was also found that there is significant increase in % yield 3.5% to 28% using catalyst. Thus the reaction is in alkaline condition due to Sodium Hydroxide catalyst increase the pH up to 14 hence with maximum yield of reaction.

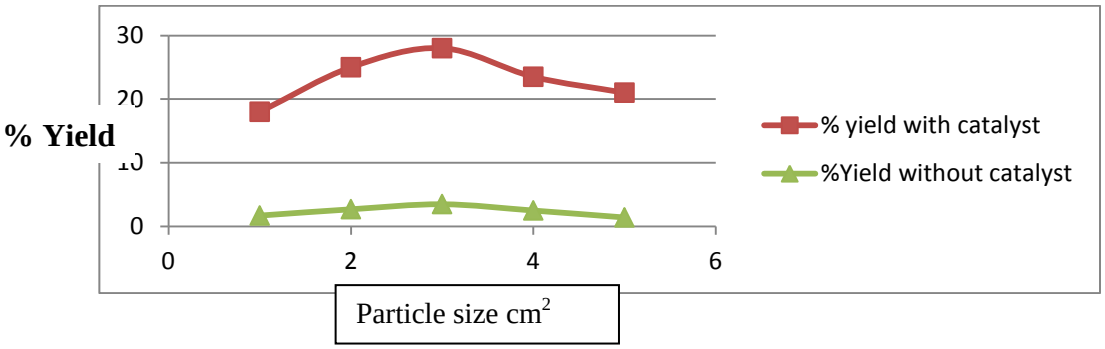


Fig.4: Effect of Particle size

A modified shrinking model: Yashioka et a[19 – 20] where the effective surface area was proportional to the degree of hydrolyzed PET due to growth of formation of pore and cracks on PET powder is shown in Fig A. We amend the model, as shown in Fig B, since besides the formation of pores and cracks PET powder simultaneously gets fragmented into the oligomers and TPA (figure 5). With increase in reaction time the oligomers are further depolymerised into TPA. This is proved by the increment in acid value with increase in reaction time from 338 to 644 for 10 to 60 min respectively.

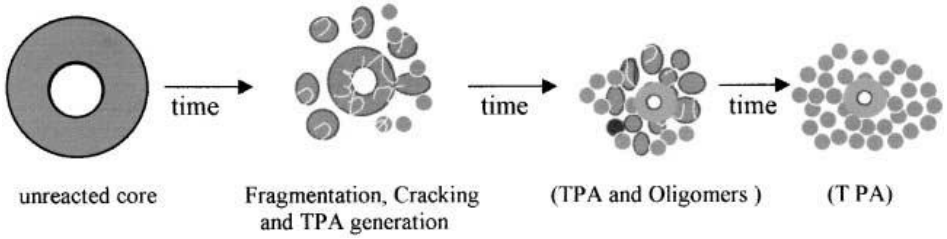


Fig.5: Shrinking core model

Conclusion:

1. Chemical recycling of PET waste into TPA is maximum at 4gm of NaOH. Hence optimized amount of NaOH For the reaction is 4 gm.
2. There is significant increase in % yield of the chemical recycling reaction using Pi- Pyridine catalyst.
3. For both with and without catalyst a % yield is maximum at 3 cm^2 particle size.
4. TPA do not have a sharp melting point, it sublimes above at 300 ^0C
5. The chemical recycling (Depolymerization is also due to simultaneously fragmentation of PET into TPA and Oligomers.

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One pot multicomponents synthesis of polysubstituted pyridines by using cerium(IV) carboxymethyl cellulose as an efficient and reusable catalyst

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Abstract

We report simple, efficient and one pot multicomponent protocol for the synthesis of the highly substituted pyridines, via the reaction of various aldehydes, malanonitrile, cyclic ketones and ammonium acetate in the presence of catalytic amount of cellulose based cerium(IV) as reusable catalyst at room temperature and using ethanol as green solvent and considering green chemistry approach

***Keywords**: Multicomponent reactions, Ce(IV)CMC, Pyridines.*

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Introduction

In the recent years, all the chemist being focused on green chemistry by using environmentally benign reagent, prevent waste, design safer chemicals, no toxicity to human and environment. Particularly those reactions are performed by using green solvent, eco-friendly catalyst, maximize atom economy as well as reaction perform at room temperature, to increase energy efficiency[1]. Multicomponent reactions (MCRs) have becomes an important tool for the efficient synthesis of wide variety of organic molecule[2-3].

Heterocycles and their derivatives have attracted the attention of chemist mainly because of broad spectrum biological and pharmacological activities associated with this class of compound specially having Nitrogen, Sulphur and Oxygen heteroatoms[4].

The term Pyridine was given by the Scottish chemist "*Thomas Anderson"* in 1849. He discovered this compound by boiling some bones of animals at high temperature and he obtained the colourless liquid harbouring a very disagreeable fish like odour highly flammable and soluble in many organic solvents as well as in water. The first major synthesis of pyridine derivatives was described in 1881 by "Arthur Rudolf Hantzsch"[5].

Many pyridine derivatives possess a wide range of biological and pharmacological activities and are already used as Dopamine transporter inhibitors, anti-inflammatory agent[6], antimicrobial agent[7]. The 2-amino-3-cyano-pyridine derivatives have medicinal applications as such as analgesic and anti-pyretic properties[8]. Pyridine containing compounds have medicinal applications as anti-viral, anti-malarial and anti-cancer[9].

Due to their wide range of biological advancement, synthesis of title compounds are still of intrigue. The preparation of 2-amino-3-cyano pyridine derivatives has been reported in the literature via a reaction of four components under reflux condition[10-11]. However many of these methods suffer from drawbacks such as such as expensive catalyst, long reaction time, low ordinary yield and environment pollution.

There is no attention has been paid to using biopolymer and its derivatives as a carrier in the preparation of support catalyst for this synthesis. The biopolymer carboxymethylcellulose (CMC) supported Ce^{IV} metal particles provided good surface area, by cationic absorption of CMC makes it Active catalyst. Smaller particles are dispersed onto a high surface area refractory support. Nano-particles (NPs) of catalyst are dispersed on surface and make it more active[12].

Considering these facts, we report simple, efficient and one pot multicomponent protocol for the synthesis of the highly substituted pyridines, via the reaction of various aldehydes, malanonitrile, cyclic ketones and

ammonium acetate in the presence of catalytic amount of cellulose based cerium(IV) as biodegradable catalyst at room temperature and using ethanol as green solvent and considering green chemistry approach.

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^{IV}) Catalyst

The Ce(IV)carboxymethylcellulose (CMC−Ce^{IV}) catalyst was prepared by metathesis reaction of cerric 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^{IV} was dried at 60^0C in the oven till constant weight.

Synthesis of Polysubstituted pyridine by using CMC-Ce(IV) Catalyst

In 150 ml round bottom flask, a mixture of ketone (10 mmol), malononitrite(10 mmol) and ammonium acetate (15 mmol), as ammonia source, and CMC-Ce^{IV}(20mg) were added to 30ml ethanol solution of aromatic aldehyde (10 mmol) and stirred for appropriate times at room temperature (Table 3). The progress of the reaction was monitored by TLC. After completion of the reaction, catalyst was recovered simply by recrystallization of crude product in hot ethanol. Catalyst is insoluble in ethanol and by using this method catalyst was easily recovered and to get corresponding pure product.

Result and discussion:-

Optimized reaction conditions:

a) Effect of the Solvent

It is well known that the reaction medium plays an important role on the reaction rate. Hence, we performed the reaction by using various solvent and without solvent as shown in table (1). From the above table it clear in pure ethanol get high yield of product & minimum time required for the completion of reaction.

Table 1: Effect of solvent

Entry No.	Solvent	Time(Min)	Yield(%)
1.	Solvent Free	150	71
2.	**Ethanol**	**120**	**85**
3.	Water	180	40
4.	CH_2Cl_2	180	53
5.	CH_3CN	180	68
6.	n-Hexane	180	62
7.	Toluene	180	55

b) Effect of catalyst:

Initially we performed reaction without catalyst the yield of product only about 60%. The time required was also more to complete the reaction. To optimize the reaction condition; we performed the model reaction with different amount of CMC− Ce^{IV} catalyst loaded as shown in **table-2.**

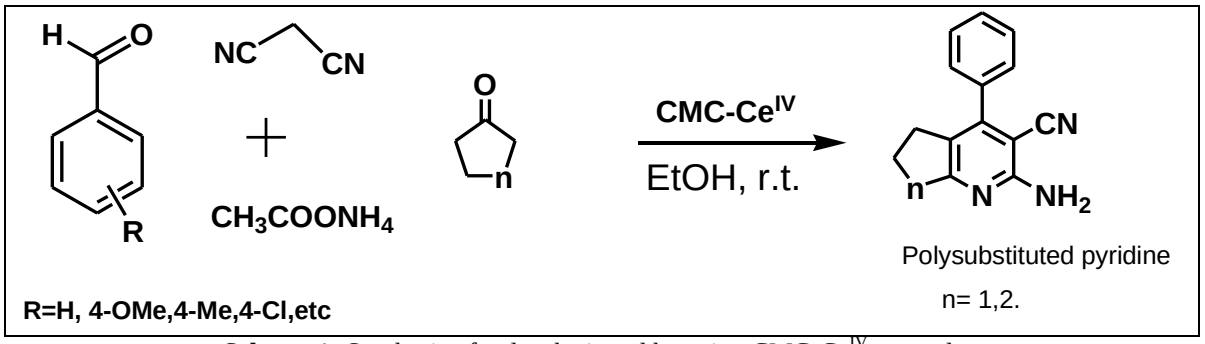
Table 2: Optimized amount of catalyst loaded

Entry	Catalyst (mg)	Time(Min)	Yield (%)
1.	0	150	60
2.	5	120	71
3.	**10**	**120**	**93**
4.	15	120	93

It was found that, the 20 mg catalyst is sufficient to push the reaction forward. Hence the reaction was

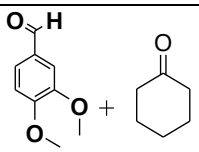
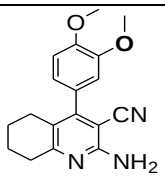
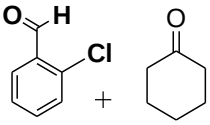
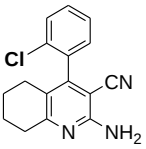
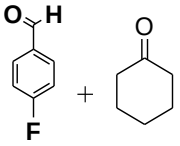
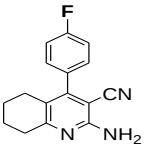
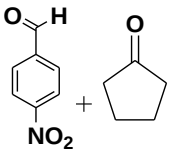
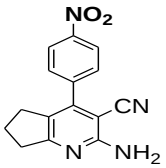
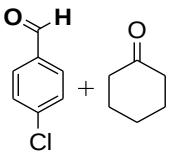
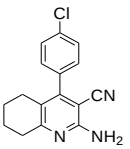
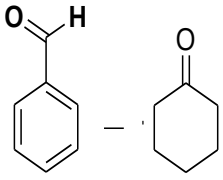
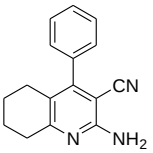
perform with 10 mg catalyst by optimized the reaction condition.

After the study of above optimized reaction condition were explored for the synthesis of series of polysubstituted pyridines derivatives from various substituted benzaldehyde, ketone, malononitrite, , and ammonium acetate using CMC-Ce^{IV} as catalyst as shown in **scheme-1** and the results are summarized in **Table-3**.

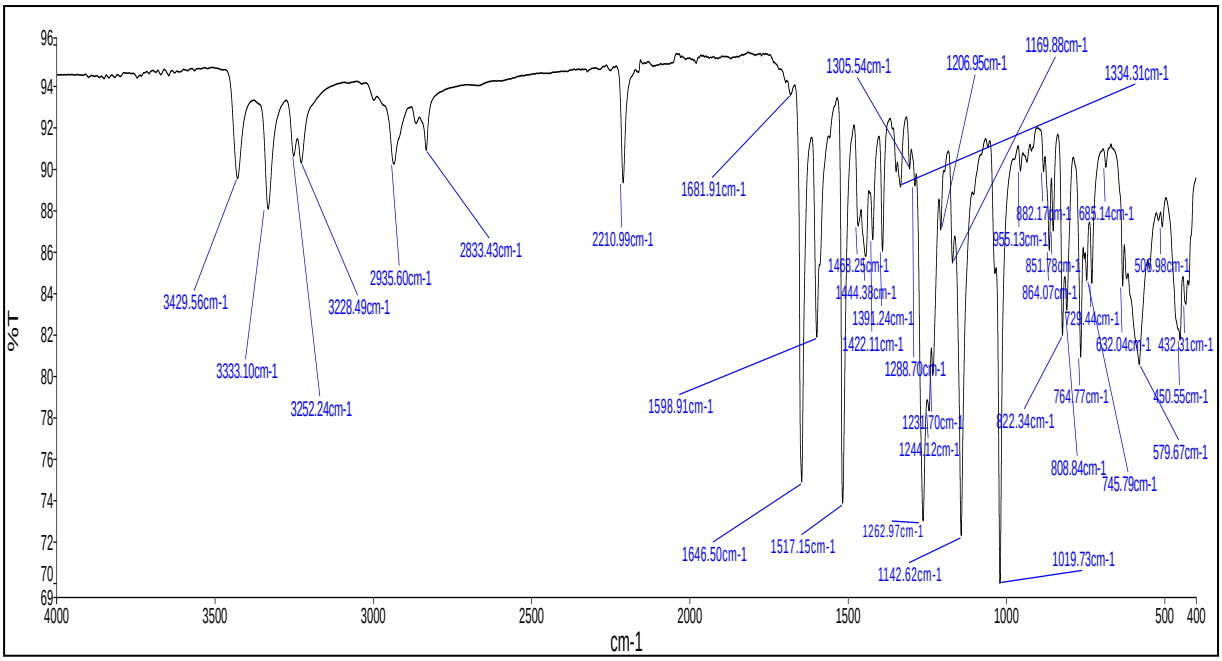


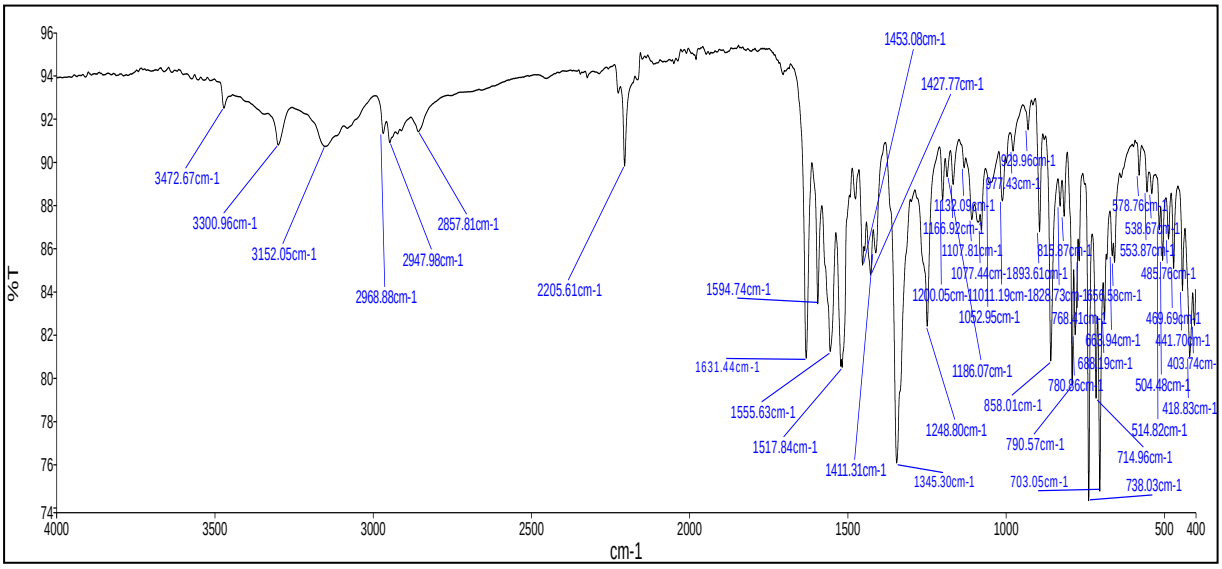
Scheme 1: Synthesis of polysubstituted by using CMC-Ce^{IV} as catalyst

Table-3: synthesis of pyridines derivatives

Entry	Starting compounds	Product	Time (min)	Yield (%)	Melting point(^{0}c) Obs.	Melting Point(^{0}C)Lit.
1.			180	74	214	Present work
2.			120	78	218	Present work
3.			150	78	256	Present work
4.			120	83	228	Present work
5.			150	75	224	258-259
6.			150	93	234	234-235

IR Spectra of compounds (1&2)





IR Spectral data of compound (1-6):

1)Cream white IR (cm^{-1}): 3333.1(-N-H); 2210.9 (-CN), 1646.5(C=N), 1589.2(C=C), 1330 (C-N),

2) White solid, IR (cm^{-1}): 3300.9(-N-H); 2205.6(-CN), 1631(C=N), 1594.74(C=C), 1311 (C-N), 790.57(C-Cl)

3) White solid, IR (cm^{-1}): 3339.9(-N-H); 2235.99 (-CN), 1603.3(C=N), 1598.74(C=C), 1344 (C-N),

4) Brown solid, IR(cm^{-1}): 3349.0(-N-H); 2211.3(-CN),1638(C=N), 1589.2(C=C), 1311 (C-N),1346.86(NO_2)

5) Pale Yellow solid, IR (cm^{-1}): 3338.0(-N-H); 2227.(-CN), 1661(C=N), 1589.2(C=C), 1311 (C-N),

6) White solid, IR (cm^{-1}): 32909.9(-N-H); 2205.99 (-CN), 1623.3(C=N), 1578.74(C=C), 1304 (C-N),

Conclusion

The CMC-Ce^{IV} NPs were prepared by the ion exchange reaction. The inclusion phenomenon of sodium carboxymethyl cellulose with cerric ammonium nitrate was successfully characterized by FT-IR techniques. We have developed a simple and efficient protocol for one-pot synthesis of pyridines

derivatives via the reaction of various aldehydes, malanonitrile, cyclic ketones and ammonium acetate in the presence of catalytic amount of cellulose based cerium(IV) as reusable catalyst at room temperature and using ethanol as green solvent and considering green chemistry approach. The high catalytic activity of CMC-Ce^{IV} was accounted due its Lewis acid sites. The advantages of procedure includes simplicity of operation, wide substrate scope, good yields, no chromatographic separation technique, an easy recovery of the catalyst and recyclability of catalyst.

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Preparation and application of the CMC-Fe^{II} nanocatalyst for the Synthesis of Schiff's Bases

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***Abstract**: CMC-Fe^{II} 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 substituted aniline and various aromatic aldehyde 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.*

***Keywords:** CMC-Fe^{II}; Schiff"s Bases; Metathesis Reaction; Multi-component reaction.*

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Introduction

Green Chemistry is the branch of chemistry that involves tools techniques & technologies. It is helpful to chemists & engineers in research, development and production for development of eco-friendlier and efficient Product which may also have significant financial benefits[1]. The aim of green chemistry involved the use of raw material obtained from renewable sources, environmentally benign catalyst and reagent[2]. Multi-component reactions (MCRs) are eco-friendly process as they obey green chemistry principles.[3]

The interest in metal nano particles (NPs), attributable to their high surface area, high biocompatibility, incredible availability and low harmfulness, has developed significantly. In addition, the high catalytic activity of metallic NPs can be accounted due to its Lewis acid site.[4] 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.

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[5.] Many biologically important Schiff base have been reported which possess antibacterial,[6] antifungal,[7] antimicrobial[8] and anti-HIV[9] activities. Owing to their wide range of biological advancement, synthesis of title compounds are still of intrigue.

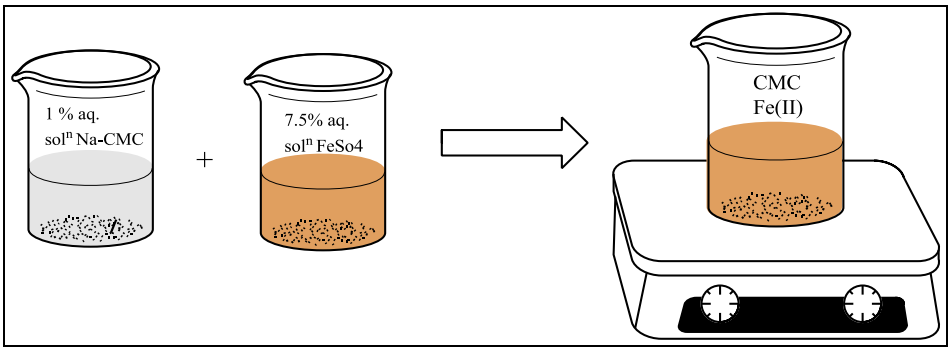
Considering these facts, we have decided to synthesize Schiff bases of various substituted benzaldehydes and aromatic amines efficiently using CMC-Fe^{II} as a recoverable and reusable catalyst in ethanol as a solvent via. Multi-component reactions **(Scheme 2)**.

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 Fe(II)carboxymethylcellulose (CMC− Fe^{II}) Catalyst

The Fe(IV)carboxymethylcellulose (CMC−Fe^{II}) catalyst was prepared by metathesis reaction of ferrous sulphate and Na-CMC. The brown solid was precipitated which was left to equilibrate in a solution for 15 hour. The resulting brown solid was separated from the solution and washed thoroughly with distilled water. The wet CMC− Fe^{II} was dried at 60^0C in the oven till constant weight.**(Scheme 1)**.



Scheme 1: Synthesis of Fe(II)carboxymethylcellulose (CMC− Fe^{II}) Catalyst

Synthesis of Schiff's Bases by using CMC-Fe(II) Catalyst

In 100 ml beaker, a mixture of substituted benzaldehyde (10 mmol), substituted aniline (10 mmol) and CMC-Fe^{II}, (15 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**.

Result and discussion:

Optimized reaction conditions:

a) Effect of the Solvent

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 1**.

Table 1: Effect of solvent

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.	**Pure Ethanol**	10	**5**	**94**
6.	Toluene	10	25	52

From the above table it clear in pure ethanol get high yield of product & minimum time required for the completion of reaction.

b) Effect of catalyst:

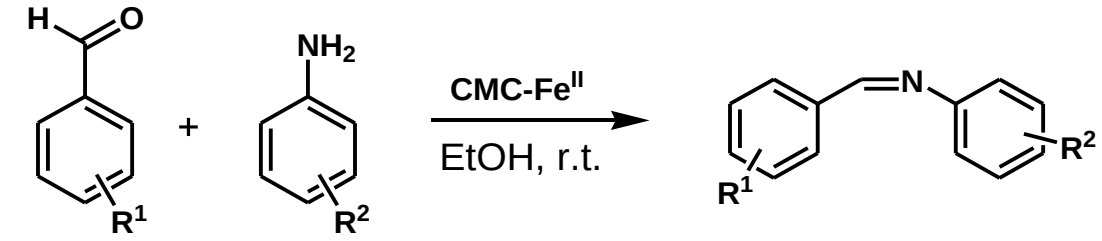
Initially we performed reaction without catalyst the yield of product only about 60%. The time required was To optimize the reaction condition, we performed the model reaction with different amount of Fe^{II}-CMC catalyst loaded as shown in **Table 2**.

Table 2: Optimized amount of catalyst loaded

Entry	Catalyst (mg)	Time (min)	Yield (%)
1	5	10	68
2	10	5	90
3	**15**	**5**	**94**
4	20	5	94

Here we found that, the 15 mg catalyst is sufficient to push the reaction forward.

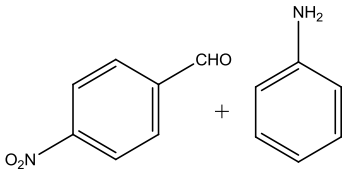
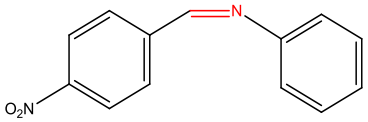
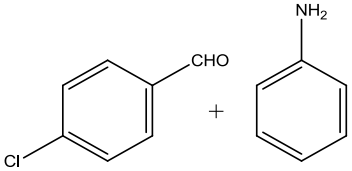
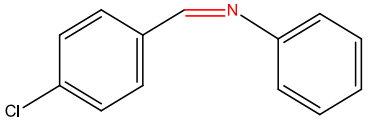
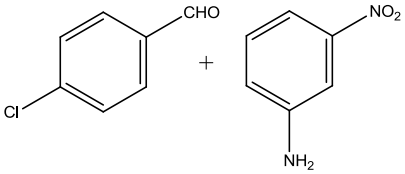
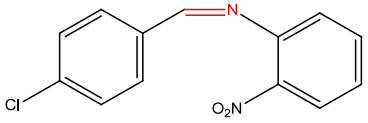
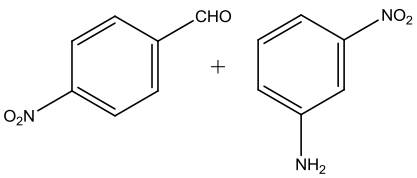
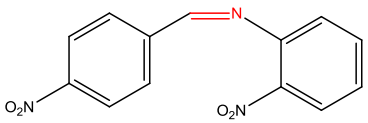
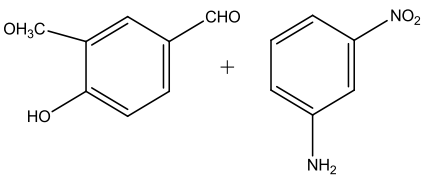
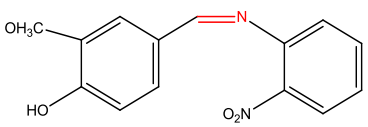
After the study of above optimized reaction condition were explored for the synthesis of series of Schiff bases of various substituted benzaldehydes and aromatic amines efficiently using CMC-Fe^{II} catalyst in ethanol as a solvent via. Multi-component reactions **(Scheme 2)** and the results are summarized in **Table3.**



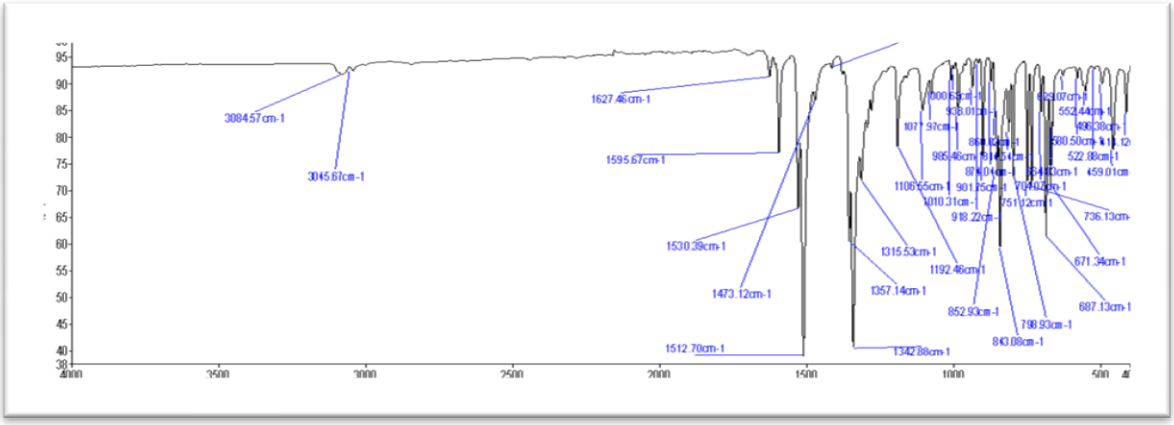
R=H, 4-OMe,4-Me,4-Cl,etc

Scheme 1: Synthesis of Schiff's Bases by using CMC-Fe(II) Catalyst

Table-3: Synthesis of Schiff's base derivatives (1-5)

Sr. No.	Starting compounds	Product	Time (Min)	Yield (%)	Melting Point (^{0}C)
1.			29	92	98
2.			25	83	80
3.			30	91	110
4.			20	85	140
5.			22	79	168

IR Spectra of compounds (1)



Spectral data of compounds (1-4)

1) Olive green solid, **IR (**cm^{-1}): 1595(C=N), 1342(C-N), 1132 (C-O), 1512 (C=C aromatic), 3064(C=C-H), 3054(C-C-H).

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

Conclusion

The CMC-Fe^{II} NPs were prepared by the ion exchange reaction. The inclusion phenomenon of sodium carboxymethyl cellulose with cerric ammonium nitrate was successfully characterized by FT-IR techniques. We have developed a simple and efficient protocol for one-pot four-component reaction of substituted aniline and various aromatic aldehyde to the synthesis of Schiff‟s bases in the presence of catalytic amount of cellulose based Fe(II) as reusable catalyst at room temperature and using ethanol as green solvent and considering green chemistry approach. The high catalytic activity of CMC-Fe^{II} was accounted due its Lewis acid sites. The advantages of procedure include simplicity of operation, wide substrate scope, no chromatographic separation technique, good yields, and an easy recovery of the catalyst and recyclability of catalyst.

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One pot synthesis of 2-Amino-3,5 Dicarbonitrile-6-Ethoxy-Pyridine by using WEB

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Abstract

One pot multicomponant synthesis of 2-amino-3, 5dicarbonitrile-6-ethoxy-pyridines derivative using WEB (water extract of banana peel ash) as green catalyst is describe. A variety of aromatic aldehyde (with electron donating & electron withdrawing groups) in conjunction with aromatic & aliphatic ethanol are known to tolerate this reaction condition using WEB

Keywords*: WEB (water extract of banana peel ash), Solvent free condition ,one pot multicomponant synthesis*

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Introduction

Among the nitrogen containing heterocycles the pyridine nucleus as a key structural sub unit in a range of bioactive compound. Both naturally occurring and synthetic is of considerable interest. [1] The pyridine ring system represent a major class of heterocycles and there analogous exhibit diverse biological and physiological activities[2].

In particular 2-amino-3-5 dicarbonitrile 6-ethoxy pyridine serve as "privileged scaffold" due to their potential therapeutic application.[3] These is one pot multicomponent synthesis of 2-amino-3-5 dicarbonitrile 6-ethoxy pyridine employing WEB as a natural feedstock without help of any toxic reagent, external base, transition metal catalyst. External additive and organic solvent. The water extract of Banana has been prepared by an aqueous extract of banana peels. Which supplied long term economic and environment impact in the near future.

Many synthesis methods have been developed for the construction of 2- amino-3-5dicarbonitrile 6-ethoxy pyridine derivatives. "K. Niknam & A. R. Hosseini "[2016]. These reported method three component condensation reaction between aldehyde, malanonitrile and ethanol catalyzed by Lewis-Bronsted base is DBU [4] pottasium per magnet etc. Lewis-Bronsted acid nanoparticle, ionic liquid such as zinc chloride [5] etc.

They are important biological activities such as anti hepatitis [6], antibacterial [7], anticancer [8], non nucleoside agent of human adenosine-A [9], anti-inflammatory agents etc. This compound are recognize as potential target for new drug development in the treatment of 'Parkinson's disease, asthma, hypoxia etc In our present work we have chosen WEB as a catalyst. Our main purpose is to make the reaction cost effective with used of readily available catalyst. we synthesized 2-amino-3-5dicarbonitrile 6-ethoxy pyridine from malanonitrile, aldehyde and ethanol.

Result and discussion:

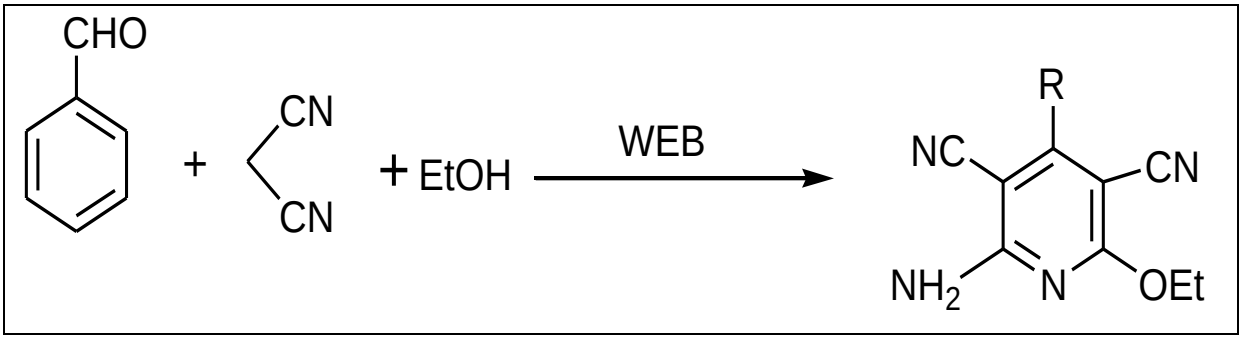
Optimized reaction conditions:

Initially we performed reaction with solvent and without solvent. But we used ethanol in reaction scheme there is no extra need of solvent. The quantity of ethanol which is used for the synthesis is sufficient for the reaction. We also check with the solvent reaction, but there is no more difference in % of yield of product between with solvent and without solvent reaction. Hence we perform of project in solvent free condition. Hence by using solvent free reaction, we performed the model reaction with different in ml. of WEB to optimized reaction shown in table below.

Table-1 Effect of amount of WEB

Entry	Catalyst(ml)	Time (min)	Yield (%)
1.	5	15	40
2.	10	15	50
3.	15	15	75
4.	**20**	**15**	**94**
5.	25	15	95

Here we find the 20 ml catalyst is sufficient to push the reaction. Hence the reaction can perform with 20 ml as catalyst by optimized the reaction condition. Thus in the synthesis of biological active pyridine compounds we use readily inexpensive and environment friendly reagent. The reaction of various substituted aldehyde with malanonitrile carried out WEB as catalyst in ethanol.



Scheme 1: Synthesis of dihydopyrimidinone by using glycine as catalyst

Spectral data of compounds (1-4)

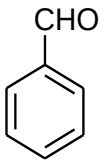
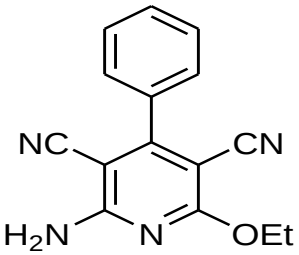
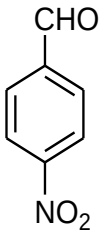
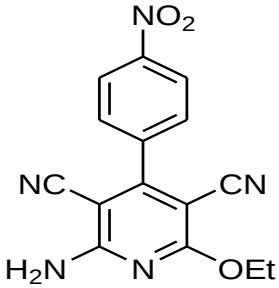
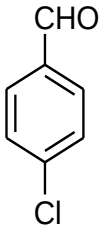
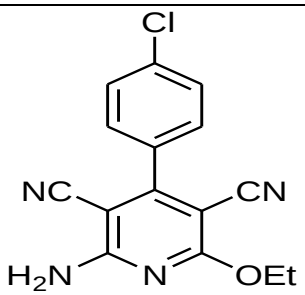
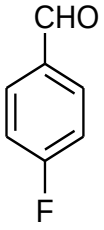
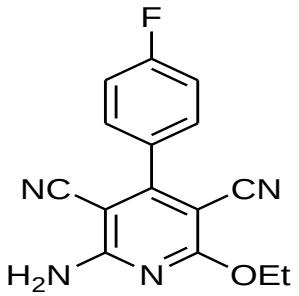
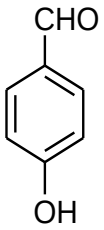
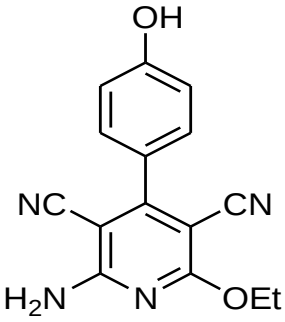
1) Creamy White,Solid 2224 cm-1(CN) 2979.95 cm-1(Ar-CH) 1187.70 cm-1(COC) 3463.59 cm-1(-NH2) 1621.05cm-1(C=C)3320.10cm-1

2) Brown, Solid 2231.01cm^{-1}(C≡N) 1580.24cm^{-1}(NO_2)1109.11cm^{-1}(C-O-C)1520.00cm^{-1}(C=C) 3463.59cm^{-1}(-NH)

3) White Solid 2229cm-1(CN) 3036.55 cm-1(Ar-CH) 3463.59cm-1 (NH2) 1163.98cm-1(COC) 1594.34 cm-1 (C=C) 716.86cm-1 (Cl)Ar

4) Pearl White,Solid 2223.82 cm-1(CN) 3099.09 cm-1(Ar-CH) 3463.59cm-1 (NH2) 1298 cm-1(CO) 1344 cm-1 (C=C) 1406.29cm-1(F)

Table-2: Result Table

Sr. no	Starting compound	Product	Time (min)	Yield (%)	Melting point (Ref. M.P) (^{0}C)
1.			15	42	210 [216]
2.			15	80	288 [286]
3.			15	53	200 [224]
4.			15	60	218
5.			15	46	310 [317]

Conclusion:

We have reported efficient synthetic method for 2-amino-3-5 dicarbonitrile 6-ethoxy pyridine derivatives by successive one-pot multicomponent reaction of various aldehyde with malononitrile, ethanol using WEB as catalyst. It has been shown that the yields are high and reaction completion time is within 10-15 min. Short reaction time, good to excellent yields, safe process and simple work-up make this method an attractive and useful contribution to the present organic synthesis for the preparation of 2-amino-3-5 dicarbonitrile 6-ethoxy pyridine derivatives.

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Reactivity of Tyrosine and Tryptophan for synthesis of azo dyes

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Abstract

Azo dyes are synthesis of starting from primary aromatic amines by diazotization and coupling with phenol or primary aromatic amines, amino acid. These compounds have numerous application such as antioxidant, the polymeric biodegradable prodruges and many pharmacological uses.

Keywords*: Azo dyes, coupling reaction, Diazotization.*

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Introduction

Green Chemistry technique continues to grow in important. It is an alternative process to help to conserve resources and can reduce costs. The replacement of convention solvents with green solvent ethanol, which is harmless to health and available in large quantities, ison in trusting basic approach along this lines[1]

Azo dyes contain at least one nitrogen–nitrogen double bond (N=N); however, many different structures are possible.[6] Mono azo dyes have only one N=N double bond, while diazo and triazo dyes contain two and three N=N double bonds respectively. The azo groups as generally connected to benzene and naphthalene rings, but can also be attached to aromatic heterocyclic or enolizable aliphatic groups. These side groups are necessary for importing the colour of the dye with many different shades and intensities being possible.[6]

The history of dyeing can be divided into two great periods, the pre-aniline extending to 1856 and the post aniline period. Aniline (C_6H_5-NH_2) becomes available from coal tar in19th century and in 1856, William Henry Perkin at the age of 17 used it in the synthesis of maveine. In 1897 the scale industrial of indigo started and rapidly reached 10,000 tones a year, completely replacing the production.[6]

Azo compounds are well known for their medicinal importance and are recognized for their applications as antidiabetic,[7] antiseptics,[8] antineoplastic,[9] antibacterial,[10-11] and antitumer,[12] They are involved in a many biological reactions such as inhibition of DNA, RNA, carcinogenesis protein synthesis and nitrogen fixations,[13-14] The azo compounds viz Evans blue and Congo red are being studied as HIV inhibitors of viral replications[15].

Owing to the scheme, we synthesized azo dyes by using different aniline and phenols and catalyst in continuation of our efforts to the eco-friendly synthetic approach towards. Synthesis of azo dyes by the reaction of aniline derivative and phenol derivative using amino acids as a catalyst in ethanol as a solvent using MCR’s gives high yield of products.

Result and discussion

Optimized reaction conditions:

Initially we performed reaction without solvent, the yield of product is only about 50% the time required was also more (about 30 min) to complete the reaction but when we used water as a solvent the yield of product increases up to 90% and time also reduced about 10 min.

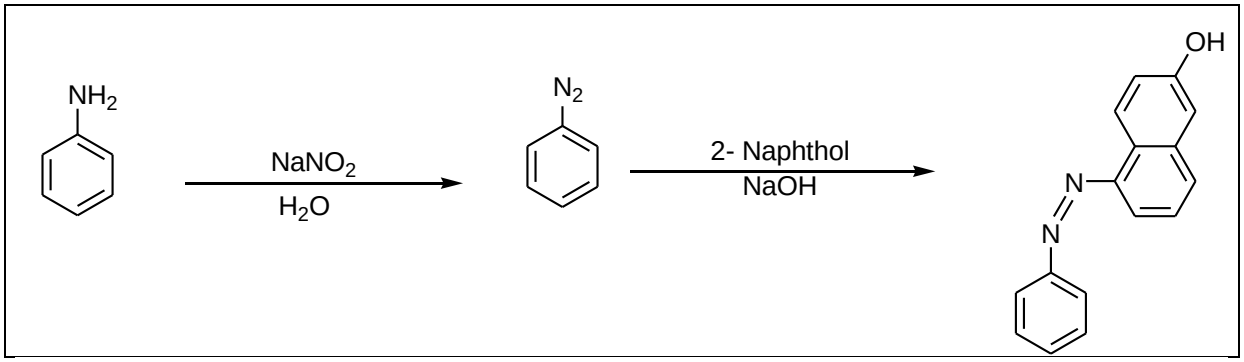
To investigate the role of water, reaction was carried out in water or another organic solvent it was observed that azo dye formation was increases in water while same reaction occurred slowly in other organic solvent.

Table-1 Efeect of amount of catalyst loaded

Sr. No	Catalyst (Mole %)	Tryptophan		Tyrosine	
		Time (min)	% Yield	Time (min)	% Yield
1	5	35	49	25	75
2	10	25	82	20	88
3	**15**	15	86	**15**	**98**
4	20	15	90	15	98

Here we find that 15% of 10 mmole catalyst is sufficient to push the reaction. Hence, the reaction can perform with 15% catalyst by optimized the reaction condition.

Thus, in the synthesis of biologically active azo compound we use readily available, inexpensive and environment friendly reagent the reaction of various phenols and amine carried out using amino acids (as catalyst) in water (as solvent).



Scheme 1: synthesis of Azo dyes

Spectral data of compounds (1-4)

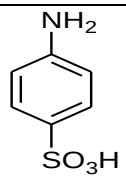
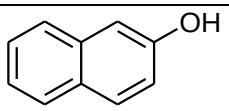
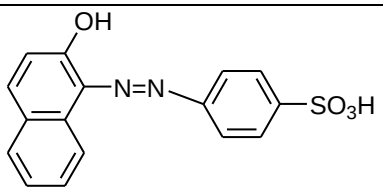
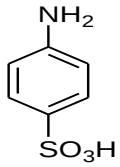
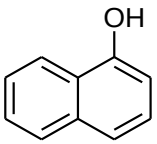
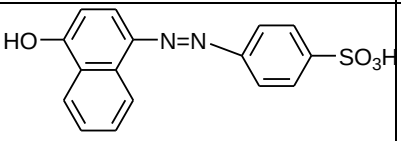
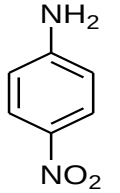
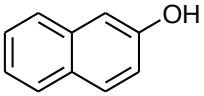
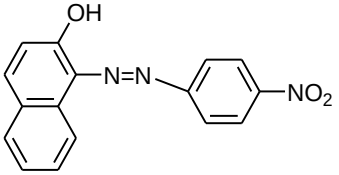
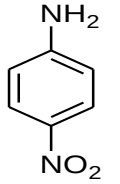
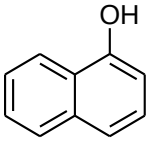
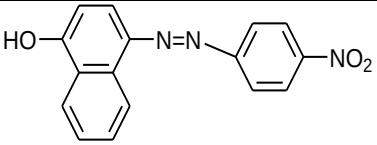
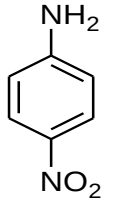
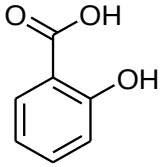
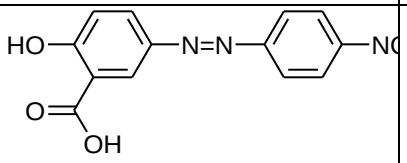
1) Red, Solid **IR**(cm-)1629.28 cm $^{-}$ (C=C Aromatic) 1363.32 cm $^{-}$(N=N)1582.83 cm $^{-}$ (C-NO_2) 3034.35 cm $^{-}$ (O-H)

2) Orange, Solid **IR**(cm^{-})1628.91cm^{-} (C=C Aromatic)1363.71cm^{-} (N=N)3371.49cm^{-} (O-H)

3) Brown, Solid **IR**(cm^{-})1581.91cm^{-} (C=C Aromatic)1295.16cm^{-} (N=N)3034.41cm^{-} (O-H)1581.91cm $^{-}$ (C-NO_2)

4) Black, Solid **IR**(cm^{-})1599.61cm^{-} (C=C Aromatic)1298.77cm^{-} (N=N) 3745.90cm^{-} (O-H)

Table-2 Synthesis of product

Sr. No.	Starting Compound	Coupling Compound	Product	Time (min)	Yield %	Melting Point (^{0}C)	Ref. M. Pt. (^{0}C)
1				40	72	202	200
2				35	82	270	270
3				30	71	230	240
4				40	75	248	-
5				45	82	290	-

Conclusion: -

The reaction under study is efficient, simple and eco-friendly method for synthesis of azo dyes by employing their component one-pot condensation reaction using water as solvent. This method has advantages of simple reaction work up, easy isolation of product, and high yield of product. It is better to prevent waste than to treat or clean up waste after it is formed. Synthetic material should be designed to maximum the incorporation of all material used in the process into final product.

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MICROBIOLOGY

Study on Antimicrobial Properties and Phytochemical Analysis of *Pistacia integerrima*

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Abstract

The plant Pistacia integerrima is the medicinal plant belongs to the Anacardiaceae family. In this present study, the galls part of plant material was collected and prepared methanol and ethanol extract. The concentration of 14.7 g and 23g per 150 ml of solvent was obtained from methanol and ethanol extraction respectively. The galls of the plant shows antimicrobial activity on pathogenic microorganisms such as Staphylococcus aureus, Escherichia coli, Salmonella typhi, Bacillus, Bacillus subtilis and Streptococcus and the test organisms shown MIC of 200 mcg fot both the extracts. The analysis of photochemical reveals the presence of flavonoids, saponins, steroids, terpenoids, resins and tannins.

Key words: *Antimicrobial activity, Phytochemicals, Pathogenic microorganisms etc*

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Introduction

Pistacia integerrima is important medicinal plant. It is commonly known as Karktashringi in sanskrit. The plant is associated with aromatic, astringent and high medicinal properties as per ayurvedic medication system. *Pistacia integerrima* belongs to the cashew family Anacardiaceae comprised of 500 different plant species distributed all over the world. The genus name Pistacia is originated from the percian name 'pesteh' which indicates the meaning of green almond. It comprises twenty different evergreen or deciduous plant species mainly shrubs and small trees with food, medicinal and ornamental significance. On the leaves and petioles of this plant, are found peculiar gall like excretions, which give the appearance of horns from a distance galls are produced on the tree in response to attack by insects and are expectorant and toxic. The genus is characterized by its high amount of terpenoids. The plant is known for its galls as it is considered as the storehouse of secondary metabolites such as steroid, flavonoid, tannins, saponins and phenols.

Classification of *Pistacia integerrima*:

Kingdom–Plantae

Phylum – Trachiophytes

Order – Sapindales

Family – Anacardiaceae

Genus – pistaciaspecies – integerrima

Method and Material

Collection of gall part of *Pistacia integerrima*

The gall part of *Pistacia integerrima* was collected from local market area of Jalgaon. The gall part was grinded into fine powder and store in bottle until when required for use. The laboratory culture of pathogenic microorganisms *S. typhi, E. coli, Bacullus, B. subtilis, S. aureus, Streptococcus* were used for antmicrobial study of extract

Extraction of Methanolic and Ethanolic extract of gall part of *P. integerrima*

For extraction, the gall powder was placed into Soxhlet thimble with the help of filter paper and methanol/ethanol was added in thimble. Solvent is heated under reflux. Condensation and extraction with fresh solvent. Solutes were transferred from the extraction chamber into the reservoir. The extract is then dried in oven.

Antimicrobial sensitivity

Antimicrobial sensitivity of extract was tested on Sterile Muller-Hinton agar plates. The suspension (dilutions) of microorganisms were prepared spread on to the Muller Hinton agar plates. The wells were punched on each plate with the help of cork borer. The extract was added in each plate and incubated at 37℃ for 24 hours.

Minimum Inhibitory Concentration: The suspension of cultured microorganisms was added into the Muller Hinton broth and OD was adjusted to 0.01nm.The concentration from 10 mcg to 200 mcg per ml of Methanolic and ethanol extract were adjusted in broth. The tubes were incubated at 37^0 C for 24 hours and Growth compared with control.

Qualitative analysis of phytochemicals

Table.1 Analysis of Phytochemicals *P. integerrima*

Phytochemical analysis was done as per the protocol,

Test	**extract**	**Contents**		**Observation**	**Result**
		1	**2**		
Tannins	1g	10 ml D/W	2drops of 5% FeCl3	Greenish ppt.	Positive
Saponins	0.2g	5ml D/W	-	Small bubbles	Positive
Flavonoids	0.2g	5-6 drops NaOH	0.5 ml Conc. HCl	Yellow to colourless	Positive
Steroids	1mg	10ml Chloroform	10ml Conc. H2SO4	Formation of red ring, and lower portion turns yellow green florescence	Positive
Terpenoids	1mg	1ml Chloroform	1.5ml Conc. H2SO4	Reddish brown ring formation in middle	Positive
Resins	1mg	1ml Alcohol	5-10drops D/W	Shown turbidity	Positive

Result and Discussions

Collection of gall part of *P. integerrima*

Fig. 3. Gall part of *P. integerrima*

fig 4. Powder of galls of P. integerrima

Soxhlet Extraction Of Methanolic And Ethanolic Extract

From the 80gm of dry gall powder 14.7g Photochemical extracts from methanol and 23gm Photochemical extracts from ethanolic solvent were obtained.

Antimicrobial sensitivity testing

The test strain of Gram negative *E. coli, S. typhi, S.aureus, and gram positive Bacillus, B. subtilis, Streptococcus* were found to be sensitive to ethanolic and Methanolic extract and shown zone of inhibition against Methanolic and Ethanolic extact. In this testing the ethanolic extract gives better sensitivity than methanolic extract.

Minimum Inhibitory Concentration

The tset organisms *Salmonella typhi, Esherichia coli, Staphylococcus aureus, Bacillus subtilis, Bacillus, Streptococcus* spp shown MIC of 200 mcg.

Qualitative analysis of photochemical

The photochemical such as tannins, steroids, flavonoids, terpenoids, saponins, and resins were detected in the both (methanolic and ethanolic) extracts of plant.

Conclusion

The antimicrobial activity of gall part of *P. integerrima* were studied against the pathogenic microorganisms such as *E. coli, Bacillus, B. subtilis, S. aureus, Streptococcus* and *S. typhi* from which it can be concluded that both the extracts of the plant shows the antimicrobial activity against all these microorganisms and the highest antimicrobial activity obtained against *E. coli.* The phytochemical testing were revealed that the presence of phytochemicals such as tanins, terpenoids, resins, steroids, flavonoids, saponins in the methanolic and ethanolic extracts of the plant.

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Studies on Plant Growth Promoting Ability of Rhizobium in Fenugreek Root Nodules

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Abstract

PGPR are beneficial microorganisms that colonize rhizosphere and help in promoting plant growth, promoting from biotic and abiotic stresses and significantly increasing soil fertility. The present project carried out to find out the effect of plant growth promoting Rhizobium from Fenugreek root nodules on plant growth enhancement like phosphate solubilisation, Siderophores production, biological nitrogen fixation, rhizosphere engineering, and production of phytohormone. The isolated organism was able to produce Siderophore, IAA and was also able to solubilize phosphate and potassium. The isolate after treated with seeds shown more plant growth as compare to non treated seeds. The germination rate of treated seeds was found more as compare to non-treated seeds. Maximum growth was exhibited by the Mungbeans.

Keywords: *PGPR, Rhizobium, Fenugreek etc.*

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Introduction

Plant growth promoting rhizobacteria have been identified in influencing the growth and yield of many plants. The effects of PGPR on plant growth can be mediated by direct or indirect mechanisms (Glick 1995). The direct effects have been most commonly attributed to the production of plant harmones such as auxins, gibberellins and cytokinins; or by supplying biologically fixed nitrogen. Interaction of plant growth promoting rhizobacteria (PGPR) with host plants is an intricate and interdependent relationship involving not only the two partners but other biotic and abiotic factors of the rhizosphere region. The PGPR may promote the plant growth either by using their own metabolism (solubilizing phosphates, producing hormones or fixing nitrogen) or directly affecting the plant metabolism (increasing the uptake of water and minerals), enhancing root development, increasing the enzymatic activity of the plant or "helping" other beneficial microorganisms to enhance their action on the plants; or may promote the plant growth by suppressing plant pathogens. In this study attempt has been made to use plant growth promoting microorganisms to increase plant nutritional status .

Materials and Methods

Collection of sample

Root nodules from leguminous plant were collected in sterile polythene bags. The roots were first washed thoroughly with sterile distilled water for 10 seconds and then surface sterilized in ethanol of 95% and again washed in distilled water for few seconds. By the help of glass rod the root nodules were smashed. The isolation was done on Yeast Extract Mannitol Agar Congo red agar plate.

Pot Assay

Pre-soaked seeds were added into enriched YEMA broth and incubated it for 24 hours at 30 ^{0} C. These seeds were incorporated into sterile soil which was kept as test. Seeds without coating of the isolated bacterial cultures were also incorporated into sterile soil as control.

The sown seeds in cups/pots were allowed to grow for 10 - 15 days. Then the plantlets were observed for comparison of shoot, root length and nodulation.

Siderophore Production

All of the glass ware were immersed in acid water overnight to remove the contaminating iron and then washed with detergent and finally with deionized water. Iron free Succinate medium was prepared and its pH was adjusted to (7.0) with NaOH and sterilized.Bacterial culture was inoculated in the succinate medium with single colony transfer and incubated at 28°C for 48 hours at 120 rpm on shaker. After incubation, the cultures were centrifuged at 7000 rpm for 15 minutes. A volume of 1 ml of supernatant was taken in a small glass tube to which 1 ml of CAS reagent was added. Then after 2 - 5 minutes it was observed for colour change from blue to orange.

Indole Acetic Acid Production (Auxin Production)

The amount of indolic compounds secreted by bacteria into the culturemedium was estimated by cultivating bacteria in Czapek Liquid medium supplemented with L-tryptophan (Merck).

Of the bacterial suspension5 to 10% was transferred to 30 ml flask containing 5 ml of the same medium supplemented with 200ug ml-1 of tryptophan and maintained at 28°C in dark under constant agitation of 140rpm. After growth for 48hrs, liquid cultures were centrifuged at 7000rpm at 4°C for 10 min(cool centrifuge).Collect supernatantAdd 1ml of culture supernatant to 2ml of Salkowaski's reagent.Keep mixture in dark environment for 30 min till reddening dark red indicate higher amount of indolic compounds. The colour intensity was measured at absorbance of 530nm .Auxin concentration was estimated using standard curve prepared with known amounts (1,5,10,25 and 50ug mL^{-1}) of filtered IAA

Phosphate Solubilization

Pikovaskaya's agar medium containing calcium phosphate as the inorganic form of phosphate was used in assay.Loopful of bacterial culture was spottedin the center of the plates and kept for incubation at 28°C for 4-5 hrs.Appearance of transparent halo zone around the bacterial colony indicated the phosphate solubilizing activity of the bacteria.

Potassium Solubilization

GYCaA agar medium containing5% fixed potassium substanceas organic form of potassium was used in assay. Loopful bacterial culture was spotted in the center of petri dishes containing GYCaA agar.

Plates were incubated for 4 days at 28°C. The appearance of clear zone around the bacterial colony indicated the potassium solubilizing activity of bacteria.

Hydrogen Cyanide Production

King's medium was amended with glycine. Bacteria were streaked on agar plate. Filter paper soaked in 0.5% picric acid and 2% sodium carbonate solution was placed at the top of the plate and sealed with parafilm. Plates were incubated at 37°C upto4 days. Development of yellow to red on filterpaper colour indicated the positive HCN production.

Pot Assay

The treated seeds after sowing have shown significant increase in shoot length as compared to non treated seeds. Maximum growth was exhibited by the Mungbeans.

Table.1 shoot growth of Rhizobium Treated and Non treated seeds

	NT	T	NT	T	NT	T	NT	T	NT	T
Shoot growth/ Days	Wheat	Wheat	Fenugreek	Fenugreek	Black Gram	Black Gram	Chickpea	Chickpea	Mungbean	Mungbean
5	9	10.5	6.3	4.9	8.6	9.9	9.5	9	18	19.5
10	20	19.8	8.3	7	17.7	20.5	22	26.7	29.1	33
15	23	25.8	9.3	8.6	22.7	25	26	31	35.5	42

The isolate shows the positive tests for production of Indole Acetic Acid Test, Siderophores The isolate also exhibited Phosphate and Potassium Solubilisation activity. The isolate shows negative Hydrogen Cyanide Production activity.

Conclusion

The isolated organism was able to produce IAA, which is a phytohormone and help as biocontrol for plant diseases. The organism was also able to solubilize phosphate and potassium which are most important nutrients required by plants. Siderophore Production activity was detected by isolated organism; as Siderophores are iron chelators they fulfil the iron requirement of plants.The isolate after treated with seeds shown more plant growth as compare to non treated seeds. The germination rate of treated seeds was also better as compare to non-treated seeds. The isolated organism wasn't able produce HCN, its good as HCN can inhibit plant growth on higher production.

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Isolation and Characterization of Phosphate Solubilizing Microbes from Agricultural Soil

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Abstract

Plant acquires phosphorus from soil. It is least mobile element in plant and soil contrary to other macronutrient. In this study role of Phosphate solubilising microbes in increase in phosphate uptake by plants and use as biofertilizer for agricultural crops was studied

The three different microbial strains were isolated from soil sample that is capable of phosphate solubilising was isolated and screened. The isolated species were identified by based on colony character, Gram staining Biochemical tests and carbohydrate fermentation test. The phosphate solubilization index measurement was done by using the formula and the solubilization index. Role of Bacillus species, Pseudomonas species and the Rhizobium species in phosphate Solubilization was studied. Highest Phosphate Solubilization Index/ efficiency was found between 35-40^{0}C temperature. Use of phosphorus solubilizing bacteria as inoculants increases phosphate uptake. These bacteria also increase prospects of using phosphatic rocks in crop production. Greater efficiency of phosphate solubilizing bacteria has been shown through co-inoculation with other beneficial bacteria

Keywords: *Soil phosphorus, Solubilization, Mineralization etc.*

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Introduction

Phosphorous is an essential nutrient required by plant. Phosphorous deficiency results in the leaves turning brown accompanied by small leaves, weak stem and slow development. Use of chemical fertilizers for improving soil fertility is the common approach of increasing agricultural production; a large portion of inorganic phosphates applied to soil as fertilizer is rapidly immobilized after application and becomes unavailable to plants. Phosphate solublizing microorganisms substantially influence the soil productivity by solubilizing insoluble Phosphate through their metabolic processes Plant can absorb phosphate only in soluble form. A considerable number of bacterial species are able to exert a beneficial effect upon plant growth. Mostly they are associated with the plant rhizosphere, so they are called as rhizobacteria. This group of bacteria has been termed plant growth promoting rhizobacteria. They are used as biofertilizers or control agents for agriculture improvement, and there are numerous researchers for the area with the agricultural environment conservation.

Material and Methods

Isolation and Screening of Phosphate solubilizing bacteria

The Soil samples were collected from rhizosphere of plant depth of 6-15cm from the agricultural land. Phosphate solubilizing bacteria (PSB) were isolated from each sample by serial dilution and spread plate method on Pikovskaya's agar medium (PVK) containing insoluble Tricalcium phosphate and incubated at 25 ^{0}C for 3-5 days. Colonies showing halo zones were picked and purified by subculture method on Pikovskaya's (PVK) agar

medium.

Diameter of clearance zone was measured successively after 3-5 days. The Phosphate Solubilization Efficiency (PSE) is the ratio of total diameter .i.e. clearance zone including bacterial growth and the colony diameter.

$$PSI = \frac{\text{Colony diameter + Holozone diameter}}{\text{Colony diameter}}$$

Effect of Temperature and pH on phosphate solubilization efficiency

The phosphate solubilization efficiency (PSE) of the isolates was studied at 25^0C,30^0 C, 35^0 C, 40^0C temperature and effect of pH was studied by Pikovskaya's agar adjusted at different pH values 5,6,7and 8 with incubation temperature 37ºC.

Bioassay for Plant growth promoting activity.

To study the plant growth promoting activity of inoculum, healthy seed each of wheat, cotton and tomato were selected and pot assay was carried using sterile soil.

The seeds were sterilized with 0.1% $HgCl_2$ and wash with sterilize D/W and were added into inoculums .After seed treatment sowed seed into pots with respective controls were kept and label it. Pot were irrigated daily in all pots with sterile D/W and kept in light. After 10-15 days the proper growth of plant measured and compared with respective controls

Result and Discussion.

Screening of Phosphate Solubilizing Microbes

Fig1. Isolate showing zone of Phosphate Solubilizing

The three isolates exhibiting zone of clearance on Pikovaskaya's were selected.

The isolated species were identified by colony character, Gram staining Biochemical tests and carbohydrate fermentation test were perform for isolate 1, 2 and 3. After matching the result with literature isolate 1 identified as *Bacillus* species, isolate 2 as a *Pseudomonas* species and isolate 3 as a *Rhizobium* species.

Analysis of phosphate solubilizing activity

1. Qualitative Method

All the selected isolates were found to be potent of phosphate soubilizers showing clear zone around their colonies. The phosphate solubilization index measurement was done by using the formula and the solubilization index shown in the table. Comparing with PSM 1 and PSM 2 the PSM 3 showing highest phosphate solubilization index that is 3.25.

Fig.1 Zone of clearance around isolate

Table.1 Qualitative analysis of phosphate solubilizing activity

Sr. No	**Isolate of PSM**	**Solubilization Index (SI)**
1	PSM 1	3
2	PSM 2	3.3
3	PSM 3	3.25

Table 2. : Effect of growth temperature on phosphate solubilisation

Sr. No.	Temperature (0 C)	Solubilization Index (SI)		
		PSM 1	PSM	PSM 3
1	25	3	2	3
2	30	2.5	1.5	2.7
3	35	2.8	4	3
4	40	3.6	3	1.5

The phosphate solubilisation of isolates was studied at different temperature using solubilisation index. No profound effect on phosphate solubilizing was observed for isolates

Table 3 : Optimisation of physiological condition pH range

Sr. No.	**pH range**	**Solubilization Index (SI)**		
		PSM 1	**PSM 2**	**PSM 3**
1	5	3	2.2	2.3
2	6	3	2.5	2.11
3	7	2.6	2.22	2.18
4	8	2.3	3	2.3

The phosphate solubilisation of isolates was studied at different pH as 5, 6, 7 and 8 by using solubilisation index. The optimum pH for phosphate solubilizing for isolates was found between 6 to 7.

Pot Assay for plant growth promoting activity.

Initially the fast growth was recorded for the seeds growing in control but after some days the seeds treated with PSM 3 inoculum shows highest root length and shoot length as compare with the inoculums of the PSM 1 and PSM 2

Conclusion:

The three different microbial strains were isolated from soil sample that is capable of phosphate solubilising was isolated and screened**.** The isolated species were identified by based on colony character, Gram staining Biochemical tests and carbohydrate fermentation test identified as *Bacillus* species, *Pseudomonas* species and *Rhizobium* species. The phosphate solubilization index measurement was done by using the formula and the solubilization index. Comparing with *Bacillus* species and *Pseudomonas* species the *Rhizobium* species showing highest phosphate solubilization index that is 3.25.Highest Phosphate Solubilization Index/ efficiency between 35-4^{0}C temperature The *Rhizobium* species inoculum shows bigger aerial height, high root length and shoot

length with Wheat, Cotton and Tomato seeds .

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Studies on Protease Producing Microorganism from Hot Water Spring

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Abstract

A protease is an enzyme that catalyzes proteolysis, breaking down protein into smaller polypeptides or single amino acids, and spurring the formation of new protein products. They do this by cleaving the peptides bonds within protein by hydrolysis, a reaction where water breaks bonds. Present research work was carried to isolate protease production bacteria from hot water spring. Five bacterial isolates were screened on casein agar plate for proteolytic activity. UPD1 shows highest proteolytic activity. So it were further analyse for morphological, biochemical, quantitative estimation, dialysis and immobilize enzyme activity. This isolate shows excellent protease production at pH 8 and at temperature 55^{0}C. Maximum protease production was found to be 504.4 U/ml. Free and immobilized enzyme activity of protease was compared. The immobilize enzyme shows highest enzyme activity as compared to free enzyme.

Key words: *Hot water, Thermophiles, Protease etc.*

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Introduction

Thermophiles is descended from the Greek: thermostat, meaning heat, and Thermophiles, meaning heat-loving organisms, are organisms with an optimum growth temperature of 50 °C or more, a maximum of up to 70 °C or more, and a minimum of about 40°C, but these are only approximate. (*Doolittle, W. F., 1999).*Enzymes is protein that helps speed up metabolism, or chemical reaction in our bodies. They build some substance and breaks other down. A protease is an enzyme that conducts proteolysis, that is, begins protein catabolism by hydrolysis of the peptide bonds that link amino acids together in the polypeptide chain forming the protein. Proteases are broadly classified as endopeptidases or exopeptidases enzymes. Exopeptidases cleave the peptide bond proximal to the amino or carboxy termini of the substrate. Based on the site of action at the N or C terminus, they are classified as aminopeptidases and cabroxypetidases. Endopeptidases cleave peptide bonds distant from the termini of the substrate. Based on the functional group present at the active site, endo-peptidases are further classified into four prominent groups, i.e., serine proteases, aspartic proteases, cysteine proteases and metallo-proteases (Raghunath et al 2010) .Based on the pH optima, they are referred to as acidic, neutral, or alkaline proteases(Singh K 20212, Wilson et al 2010). Presently, the worldwide auctions of industrial enzymes are assessed at about $4.2 billion in value . Proteases signify one of the 3 largest groups of enzymes and is projected to reach a global market of approximately $ 2.21 billion in terms of value by 2021 at a compound annual growth rate (CAGR) of 6% from 2016 to 2021. Out of proteases produced, microbial proteases account for the largest share in the market in terms of value, next the animal and plant source one to one.Very few reports are available on the bacterial enzymes showing both thermophilic and alkalophilic nature. Therefore, the present work aims to study the biotechnological and industrial applications of a thermophilic alkaline protease isolated from hot water spring. (Balsam et al 2017)

Materials and methods

Enrichment and Isolation of Microorganism

Samples were collected from the hot water spring at Unapdev, Jalgaon and transported to the laboratory and kept at room temperature. Microorganisms were isolated from the collected water sample using Horikoshi and Nutrient agar plate by incubating at 37^0c and 50^0c for 24-48 h.The isolated bacterial colonies were picked up, purified and preserved on nutrient agar slants at 4^0C for further use.

Screening of of protease producing bacteria

Protease activity of isolated bacteria detected on skimmed milk agar. The clear zone appeared around the spot inoculation indicated protease activity.

Identification of bacterial isolates:

The isolates were subjected to a set of morphological and biochemical tests for the purpose of identification.

Protease Assay

Protease activity was determined by following the regular assay method using casein as a substrate. The protease activity was assayed by incubating 1 ml of the enzyme with 2.5 ml of 1% (w/v) casein (prepared in 100 mM sodium phosphate buffer; pH 7.0 and 100 mM Tris-HCl buffer; pH 9.0) at 37°C for 30 minutes. Reaction was terminated by adding 2.5 ml tricarboxylic acid (TCA). TCA soluble fraction containing soluble peptides was measured using the Lowry method by referring the standard curve of tyrosine (0-100 μg/ml)

Effect of pH on enzyme production

The effect of variable pH on the production of protease was analyzed by growing the selected bacterial isolate in the production medium by varying the pH (6.0, 6.5, 7, 7.5 and 8.0).

Effect of Temperature on enzyme Production

0.1ml culture of freshly developed isolates inoculated into test tube containing respective medium and incubated at temperature of 25^0C, 30^0 C, 35^0 C, 40^0 C, 45^0 C and 50^0 C. After 48hours the enzyme production was measured .

Purification of Protease

Enzyme supernatant was subjected to protein fractionation by differential ammonium sulphate precipitation. Fractionation of protein was done by addition of small increments of solid ammonium sulphate at 4°C with constant stirring.

Immobilization of Enzyme

Purified protease was immobilized on sodium alginate beads by entrapment method. Two ml of purified was suspended in 10 ml of 3% (w/v) sodium alginate solution (3gm dissolved in 100 ml warm distilled water).Alginate drops were solidified upon contact with $CaCl_2$ forming beads.The activity of the immobilized enzyme and activity of free enzyme were calculated.

Results and discussion

Screening of of protease producing bacteria

The isolation of thermophilic organisms was done by using s Horikoshi medium and Nutrient Agar.Protease activity of isolated bacteria was detected on skimmed milk agar. The isolate showing clear zone around the spot inoculation indicated protease activity. The isolated strains were characterized by morphological and biochemical tests.

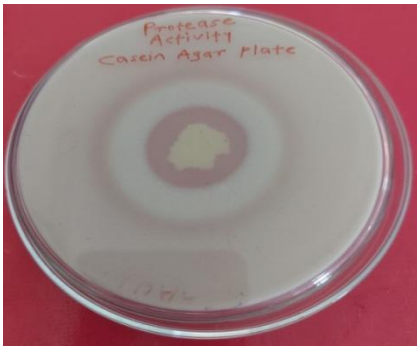

Fig 1. Zone of Hydrolysis

Production of Protease

Bacterial isolate that was subjected to the production of enzyme and its protease activity was determined for five successive days using spectrophotometric method. The protease activity was measured at 660 nm by using casein as substrate. Quantitative assay was determined by Lowry Method.

Table.1. Quantitative estimation of protease

Days	1	2	3	4	5
Enzyme activity(U/ml)	143.0	313.5	385.0	440.1	505.4

The maximum protease production was seen at day 5 of incubation. Protease assay was carried out by Follin Lowry method. After performing protease assay comparing with standard tyrosine enzyme activity of protease was found to be 505.417 µg/ml/min. The range of pH from 7.5 to 8 was found to be good for protease production. The protease production was studied at different temperatures such as 30^0c , 40^0c , 50^0c , 60^0c , 70^0c. The optimum temperature for protease production was found to be between 30 to 50^0c.

Purification of protesse

Protease was subjected to partial purification using ammonium sulphate precipitation method; protein content was precipitated at 60% of salt. Further purification was carried out using dialysis method.

Table.2 Purification of Protease Enzyme

Strain	**Purification Step**	**Volume (ml)**	**Total Activity**	**Total Protein (mg)**	**Specific Activity(U/ml)**
UPD 1	$NH_4(SO_4)2$ Precipitation	100	3877.32	6	646.22

Immobilization of partially purified enzyme

The dialyzed enzyme was immobilized by sodium alginate in chilled calcium chloride solution to obtain beads.

Table.3 Enzyme Activity of Immobilized protease

Free Enzyme Activity (U/ml)	**Immobilized Enzyme Activity(U/ml)**
646.22	914.56

As compared to Free Enzyme, Immobilized Enzyme shows more enzyme activity.

Conclusion

From the present study it can be concluded that that efficient microbial strains were isolated from water sample that is capable of producing protease. Morphological and Biochemical characteristics of organism reveals that it belongs to *Bacillus lichiniformis* and it able to produce protease enzyme. The isolate shows maximum protease production at pH 8 and 50^0c temperature.

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Comparative Study of Bioethanol Production From Banana Waste And Sugarcane Baggasse

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Abstract

Banana and sugarcane wastes are lignocellulosic agricultural waste that has the potential to produce bioethanol as a renewable form of energy. Pre-treatment and Hydrolysis are crucial steps in Bioethanol production. Present study determined the comparative efficiency of Banana and sugarcane wastes pre-treatment techniques namely acidic and alkaline pre-treatment's on the production of Bioethanol. Using an 8%, yeast inoculum, maximum ethanol production was completely achieved in 4 days respectively.

Key Words: *Bioethanol, Hydrolysis, Sugarcane baggasse, S. cerevisiae etc.*

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Introduction

The interest in study of waste management being increased worldwide presently as the pollution by various means are day by day. The waste utilization plays a vital role to overcome various pollutions and make possible to utilize the wastes. Banana and sugarcane wastes are lignocellulosic agricultural waste that has the potential to produce bioethanol as a renewable form of energy.Pre-treatment and Hydrolysis are crucial steps in bioethanol production. Such biomass include residues from agriculture or forest, industrial and municipal wastes and dedicated energy crops. The substances that have been used previously to produce bioethanol include sugarcane baggasse, corn curbs, news papers, saw dust, rice straw, wood wheat, ettc.

Materials And Methods

Collection of Banana wastes and Sugarcane baggasse.

Lignocellulosic residues of banana wastes were obtained from the Banana farm located in Khedi near Jalgaon and Sugarcane baggasse were obtained from the Sugarcane juice center located near Aakashwani, Jalgaon.

Acid hydrolysis

Fifty gram of sample was Pre-treated with 0.5% H_2SO_4, at 121°C for 30 min. Thereafter, the residues washing by deionised water to neutral pH generated. The pH was 4.

Alkaline hydrolysis

The Pretreated sample with 0.5% H_2SO_4, then treated with 2N NaOH to adjust the pH to 5. Autoclaved at 121°C for 30 minutes.

Analytical tests for detection of Flavonoids, Glycosides, Terpenoids were carried as per the standard protocol.

Isolation and Enrichment of Yeast

The Yeast *S. cerevisiae* was isolated from the raw liquor sample.

Determination of organic acids

Qualitative detection of organic acids in a bioethanol was carried out using paper chromatography. Standards (malic acid and tartaric acid) and bioethanol sample of banana and sugarcane were spotted on the commercially

available Whatman filter paper no. 1 placed in pre-saturated chromatography chamber containing the solvent mixture of butyl alcohol, formic acid and water solvent system (16:2:5).

Effect of Concentration on bioethanol production

The percentage of ethanol was calculated by specific gravity method.

Results and Discussion

The isolation of yeast from liquor sample was done by using YPD Agar medium. **Production, Distillation and Dehydration of Bioethanol**

After 3 days of fermentation the raw fermented media was then distilled using Distillation assembly. The evaporating temperature was kept constant during Distillation for 3 hours. The distillates condensed and collected in the flasks.

Analytical Tests

Effect of pH on bioethanol production

The bioethanol production at different pH was studied. As the pH increases to basic pH, the production of Bioethanol decreases and finally no distillate obtained.

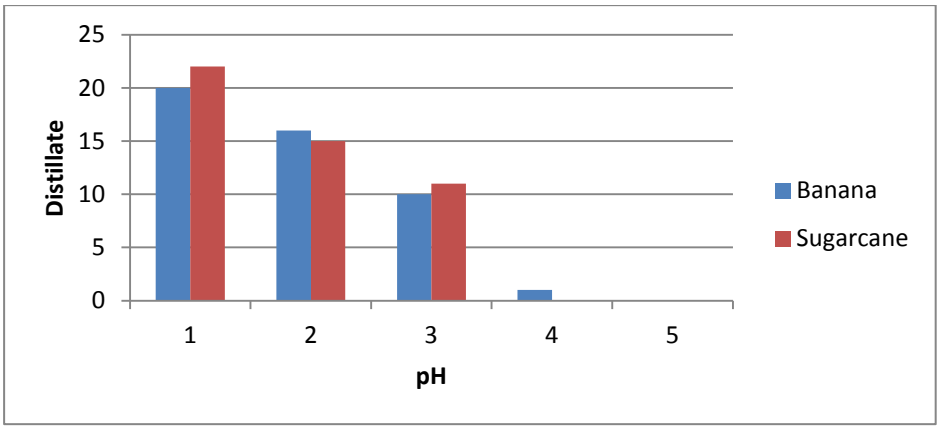

Fig.2. Effect of pH on bioethanol production

Effect of temperature on bioethanol production

The optimum temperature for bioethanol production was found to be 30°C. As the temperature increases the production of Bioethanol decree

Effect of concentration of inoculum on Bioethanol Production

On the basis of concentration of inoculum, there was no profound resultsobserved. The slight increase in the amount of distillate was found. The maximum amount of distillate was found 23 ml in banana substrate and 25 ml in sugarcane substrate.

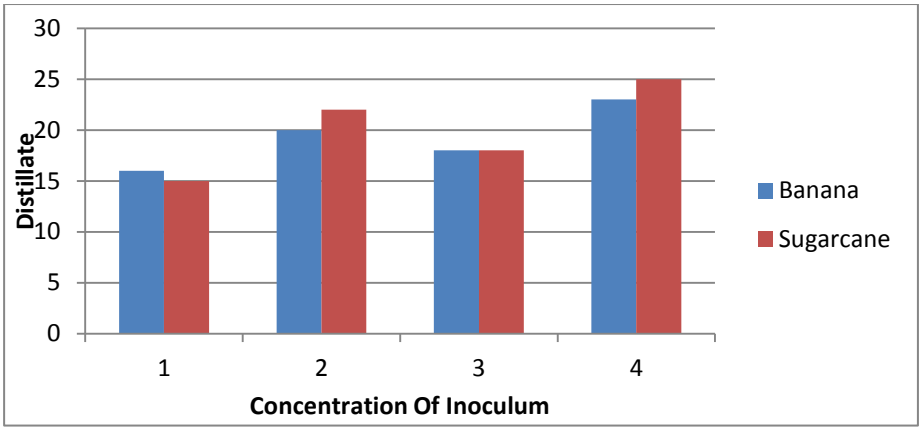

Fig.3. Effect of concentration of inoculum on bioethanol production

Effect of Substrate Concentration on bioethanol production

The percentage of ethanol was calculated by specific gravity method. By weight and volume percentage of ethanol was found using the chart. The maximum concentration of ethanol was found to be 10% & 12.39% by weight and volume in banana substrate and 10% & 24% by weight and volume in sugarcane substrate respectively.

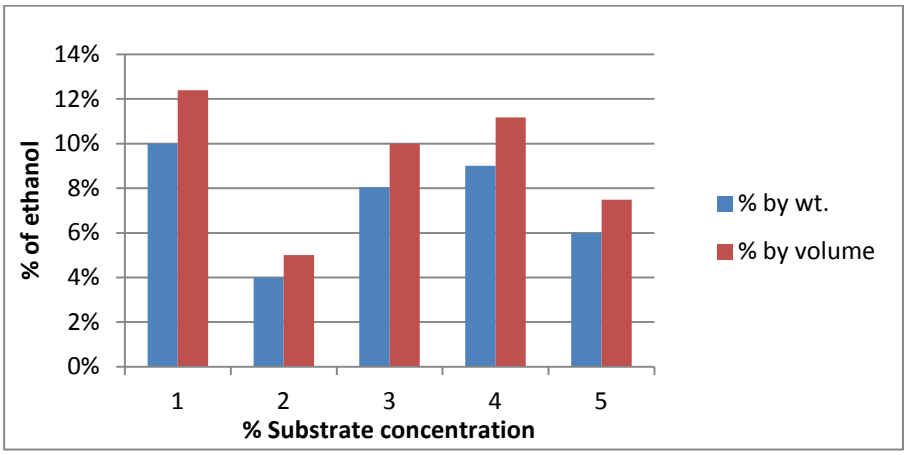

Fig.4. Effect of Banana Substrate Concentration

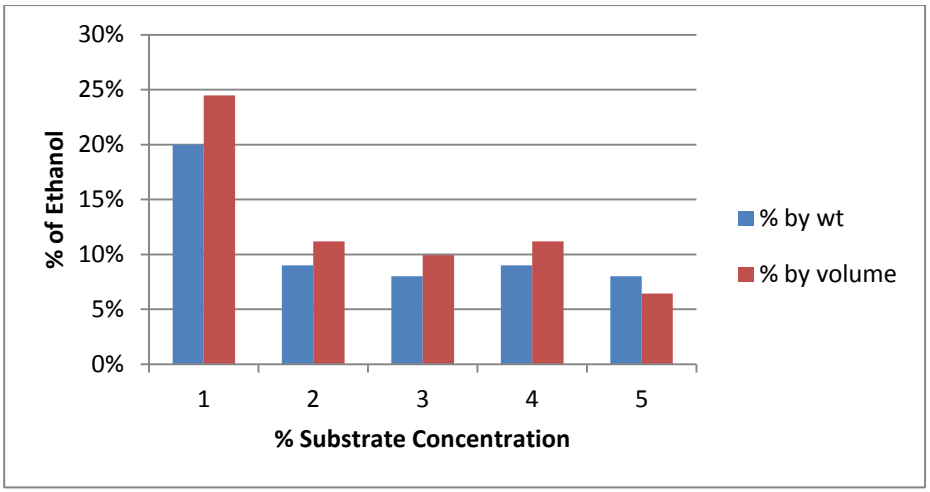

Fig.5. Effect of Sugarcane Substrate Concentration5.0.

Conclusion

From the present study it is concluded that,Bioethanol production from banana and sugarcane is pre-treatment dependant.The sugars released after hydrolysis were found to be 1.492 & 0.767 in banana and sugarcane baggasse respectively. The optimum pH and temperature for bioethanol production was found to be 5 and 30°C respectively.The glycosides content present in Bioethanol. By detection of organic acids using paper chromatography, the banana bioethanol contains Malic acid and sugarcane bioethanol contains Tartaric acid The concentration of Bioethanol by specific gravity method was calculated as 10% & 20% by weight and 12.39% & 24.47% by volume at its maximum in Banana and Sugarcane Bioethanol.

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Studies on Fruits Spoilage Causing Microorganisms

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Abstract

Microorganisms are major and important factor responsible for fruit spoilage. They survive in suitable temperature with the Presence of food and water, which caused changes in the appearance, colour and smell of the fruits. The present study was carried out to examine the presence of various bacterial species in spoiled fruit samples comprising of Apple (Malusdomestica), Banana (Musa paradisiaca L. and Papaya (Carica papaya). Bacteria were successfully isolated by serial dilution-agar plating method.

Key words: *Hot water, Thermophiles, Protease etc.*

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Introduction

Fruits are natural sources of minerals; vitamins besides carbohydrates and other essential substances. Fruits play a vital role in human nutrition by supplying Necessary growth factors such as vitamins and essential Minerals in daily diet which help to live a healthy life (AL Hindi et al., 2011). India is the fourth largest producer of Fruits in the world, yet due to losses in the field, during Storage, transit or trans-shipment, during handling Processes of the crop from the grower to the whole sale Dealer and to retailer and finally to consumers they become inadequate (Barth et al., 2013). Most microorganisms that Are initially observed on whole fruit or vegetable surfaces are soil inhabitants (Andrews and Harris, 2000; Janisiewicz and Kerstin, 2002). Spoilage refers to any change in the condition of food in which the food becomes undesirable or unacceptable for human consumption (Akinmusire, 2011). Bacterial Spoilage first causes softening of tissues as pectins are Degraded and the whole fruit may eventually degenerate Into a slimy mass. Starch and sugars are metabolized Next and unpleasant odours and flavours develop along With lactic acid and ethanol (Rawat, 2015). Some spoilage Microbes are capable of colonizing and creating lesions On healthy,

Methods and material

Sample collection

The unwashed and unprocessed spoiled fruit . Banana (Musa paradisiaca L.), Papaya (Carica papaya) and Apple(Malusdomestica), were collected in plastic zip bag from the local market Jalgaon

Isolation of microorganisms from spoil fruit

The bacteria were isolated from spoiled fruits by using Serial dilution method. The spoiled Fruits were crushed which was serially diluted from 10^{-1} to 10^{-7} dilutions. 0.1ml of each of diluted fruit suspension was streak over specifically labelled Nutrient agar medium ,Mannitol Salt agar & Sabaraoud dextrose agar medium plates. The inoculated Petri plates were incubated at 25^{0} C for 24 hours

Characterization of bacterial isolates

The selected, bacterial isolates were subjected to morphological and biochemical tests for the purpose of identification.

Results and discussions

A study was carried Out to examine the presence of various bacterial species in spoiled fruit samples. The microorganisms were isolated from spoiled fruits sample were isolated enumerated , purified and studied using standard microbiological methods.The microorganisms found associated with spoiled fruits samples were identified mainly belonging to *S. Aureus, Micrococcus luteus, Bacillus subtilis, Bacillus sp., Staphylococcus sp., Pseudomonas aeruginosa, Klebsiella pneumonia, E.coli, Proteus vulgaris*

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BIOTECHNOLOGY

Plant Tissue Culture Studies in Pomegranate *(Punica granatum)*

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Abstract

A reliable and reproducible protocol to get healthy and well-formed plants from nodal explants of the pomegranate (Punica granatum L) has been developed. Nodal segments were cultured on Murashige and Skoog. The media was prepared as a basal medium supplemented with 0.2 to 2 mg/L 6-benzylaminopurine (BAP), 0.1 to 1 mg/L 1-naphthalacetic acid (NAA). For proliferation stage, 0.1 to 0.5 mg/L BAP and NAA was tested. For rooting stage 0.0, 0.25 and 0.5 mg/L 3-Indolebutyric acid (IBA) and NAA on MSmedium were tested. The nodal explants grown on MS medium containing 0.4 mg/L BAP, 0.04 mg/L NAA, the highest proliferation rate (10 to 15 shoots/explants) in establishment stage. The plantlets grown on MS medium were found to have better survival compared to WPM medium. Treatment comprising MS media devoid of any plant growth regulator produced highest number of roots (5.60 ± 0.74) and root length (6.78 ± 0.52 cm). The plantlets with well-formed root systems weregradually acclimatized in greenhouse using cocopeat and later shifted to polyhouse.

Keywords: *Punica granatum, micropropagation, 6 - benzyl adenine, hyperhydricity*

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Introduction

Plants have been the source of food, cloth and shelter the basic needs of man for a long time. A large number of plants have medicinal properties, which have been exploited by man for curing various ailments / diseases. Over exploitation and changing climatic / environmental conditions are causing many of them to be endangered or at the verge of extinction. The role of plant tissue culture technology in conservation, micropropagation, secondary metabolite production and other applications has been discussed by Moriguchi, et al. 1987.

The regeneration of whole plants through tissue culture is popularly called as 'Micropropagation'. It allows the production of large number of plants from small pieces of the stock plant in relatively short period of time. Micropropagation may be achieved directly, indirectly or through somatic embryogenesis.

Punicagranatum L. belongs to the family Punicaceae, which comprises only one genus and two species, other being *Punicaprotopunica* ; Moriguchi et al. 1987; Guarino et al. 1990; Jbir et al. 2008). Punica had been classified as the only genus within its family because of the unique structure of the ovary and fruit. However, molecular phylogenetic studies conducted by Huang and Shi (2002) suggested that this genus be included in family Lythraceae. Pomegranate is native to Iran, Afghanistan, Baluchistan, and Himalayas in Northern India. It has been naturalized in Mediterranean regions and the Caucasus since ancient times and is widely cultivated all over India, Southeast Asia, Malaysia, the East Indies, tropical Africa, and the USA. Pomegranate is exploited for nutritional value of its fruit, the medicinal properties of different parts of the tree, and its use as an ornamental (Naovi et al. 1991;Jayesh and Kumar 2004; Johanningsmeier and Harris 2011).

Pomegranate (L.) is one of the oldest known edible fruits and is capable of growing in different agro-climates ranging from tropical to temperate regions of the world. It is presumed that pomegranate was domesticated in

the Middle East about 5000 years ago. Interestingly, it is considered to be one of the first five domesticated edible fruit crops along with fig, date palm, grape and olive. Botanical name of Pomegranate is *Punica granatum* L., which belongs to Punicaceae family. Pomegranate name is derived from "*Pomuni granatum*," in which Pomum means apple and granatus means grainy, which is also considered as "seeded apple".

In vitro propagation of pomegranate has been reported through axillary shoot proliferation from nodal segments (Zhang and Stolz 1991; Naik et al. 1999, 2000; Kanwaret al. 2004; Murkute et al. 2004), shoot tips (Murkute et al. 2004), and cotyledonary nodes (Sharon andSinha 2000). Regeneration of P. granatum plantlets *in vitro* can occur through organogenesis from callus derived from leaf segments (Murkute et al. 2002; Deepika and Kanwar 2010).

Materials and Methods

Materials

Analytical reagent (AR) grade biochemicals, growth regulators, components of tissue culture media were obtained from M/s. Hi-Media India Ltd., Mumbai, organic solvents from M/s. Qualigens and M/s. S D Fine-Chem Ltd, Mumbai, Borosil make glass ware and double glass distilled water were used for culture and other experiments.

Methods

Selection of superior mother plant

The choice of mother plant is vital for obtaining good quality clones and so need to ensure that it is healthy.

Media preparations

The growth, development and morphogenicresponse of an explant in culture depends on itsgenetic make-up, surrounding environment andcomposition of the culture medium. The last ofthese is the easiest to manipulate. The success of aplant tissue culture experiment largely dependson the selection of right culture medium. The clue for developing a basic culture medium seems tohave initially come from the nutritional requirementsof plants growing in soil, and later fromnutrient solutions used for whole plant culture.

Preparation of Murashige and Skoog medium

Preparation of medium is a time consuming and tedious process if each constituents are weight and added to distilled water, hence stock solution was prepared separately for macro nutrients, micronutrients, iron source and organic nutrients. Stock solution is a concentrated solution from which several dilutions can be made.

Preparation of explants

For callus induction, each leaf explant (10 x 10 mm) was cultured on 20 ml of MS medium solidified with 0.4% ClariGel (w/v) (Hi-Media) and supplemented with 0.2-2 mg/L concentration of the phytohormones namely, 6 - benzyl adenine (BAP), 2,4- diclorophenoxyacetic acid (2, 4 – D), Indole – 3 - acetic acid (IAA) and kinetin, α - naphthalene acetic acid (NAA) in glass culture tubes (25 x 100 mm, O.D. x length, Borosil make) and phytajars.

Results & Discussion

Effect of BAP on regeneration of shoots

For the nodal explants grown on MS medium with different concentration of BAP (0.2 to 2 mg/L), the highest average growth response (99 %) was recorded on MS medium containing BAP 1.8 mg/L, whereas 4 to 6 shoots per explants having highest shoot length (0.7 to 1.9 cm) was recorded at same concentration.

Effect of NAA on regeneration of shoots

Results for the nodal explants grown on MS medium with different concentration of NAA 0.1 to 1 mg/L indicated that the highest average growth response (97%) was recorded on MS medium containing 0.9 mg/L NAA, whereas two to three shoots per explants having highest shoot length (0.7 to 1.1 cm) was recorded at same concentration.

Effect of combination of BAP and NAA on multiplication of shoots

In the subsequent experiments, 16 combinations of BA and NAA concentrations were compared to optimize growth. The optimal BA concentration was 0.4 mg/L and the optimal NAA concentration was 0.04 mg/L. Among the various combinations tested, the highest number (5.3) of shoots per explant was obtained on ML medium containing 0.6 mg/L BA and 0.04 mg/L NAA. The maximum shoot multiplication rate (284.56%) was obtained on MLmedium containing 0.4 mg/L BA and 0.04 mg/L NAA, on which the shoot clusters were verdant green and thriving, without defoliation or hyperhydricity.

The basal medium can only guarantee the survival of the culture and minimal physiologicalactivities. The plant can initiate cell division, morphogenesis, organ differentiation, and development only when the medium is supplemented with appropriate plant growth regulators (Gaspar et al., 1996). Auxins and cytokinins are generally considered the most important growth regulations in in vitro propagation (George et al., 2005). A balance between auxins and cytokinins is necessary for the formation of buds and root (Gaspar et al., 1996). It has been reported that an optimal combination of BA and NAA in a culture medium could significantly enhance shoot proliferation (Perveen et al., 2011).

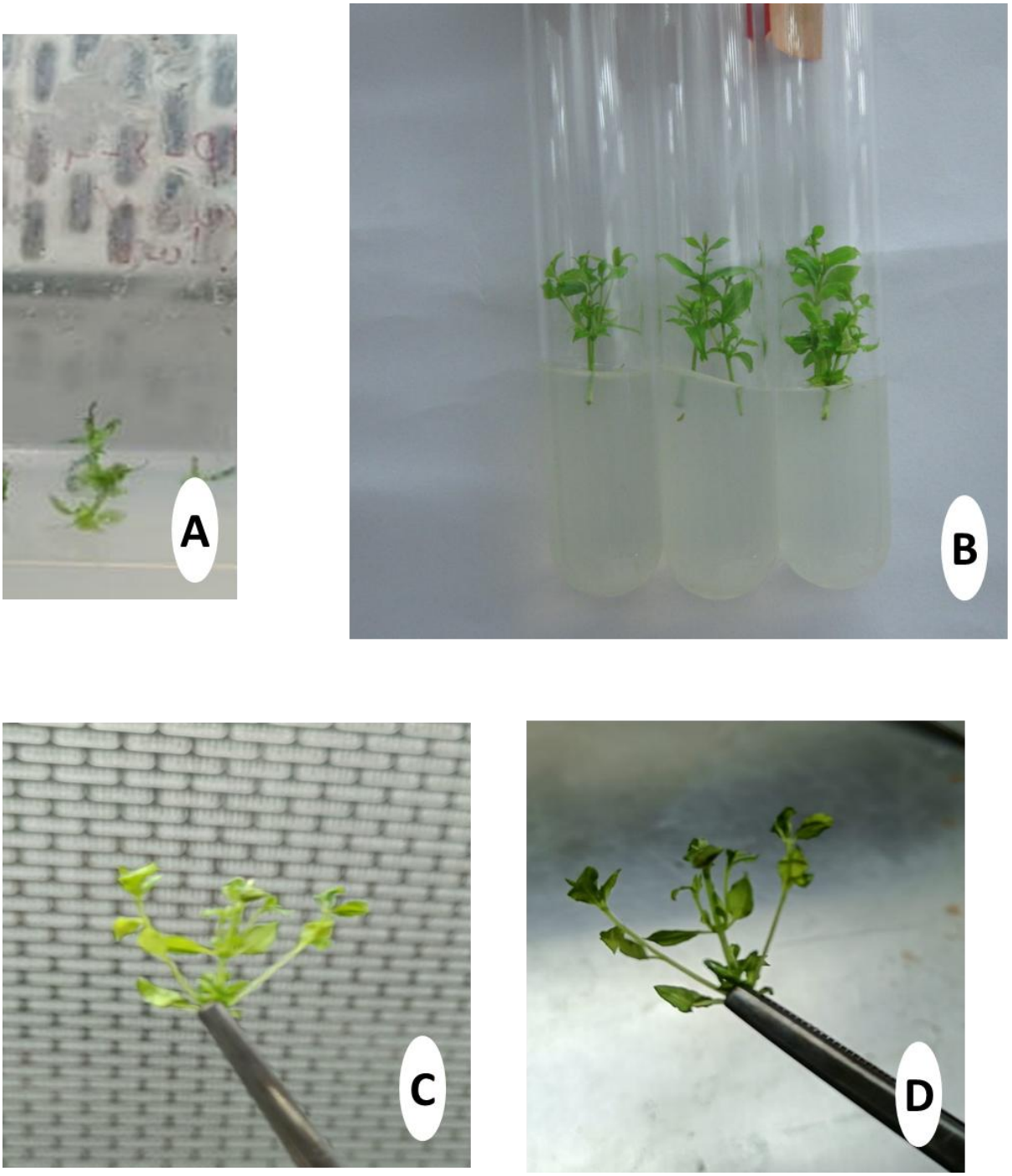

Figure: Multiplication of pomegranate nodal explants in MS medium fortified with BAP.

MS basal medium supplemented with 0.25-2.0 µM IBA or 2.0 µM NAA did not show rooting. The maximum rooting (89.00 %), average number of 6.57 roots per shoot and maximum average root length of 2.93 cm was achieved on half strength MS basal medium containing 500 mg l^{-1}activated charcoal followed by half strength MS medium supplemented with 0.50 µM NAA showing 83.00% rooting, 4.50 average number of roots per shoot and 2.12 cm of root length. The plantlets with well developed roots were obtained within four weeks of incubation on rooting medium. The plantlets were hardened in plastic cups containing cocopeat and

subsequently transferred to earthen pots containing soil and sand (1:1). The per cent survival of plantlets was 80 % after 60 days of transfer and remained constant thereafter. The plant height increased over time from 3.26 cm at 0 day to 12.48 cm at 360 days of transfer. The average number of leaves shoot diameter and number of nodes also increased over time, indicating successful establishment of tissue culture raised plantlets.

5. Conclusion

The maximum shoot multiplication rate (284.56%) was obtained on MS medium containing 0.4 mg/L BAP and 0.04 mg/L NAA, on which the shoot clusters were verdant green and thriving, without defoliation or hyperhydricity.Thus, the present study emphasizes the suitability of growth regulator-free MS medium with half strength and activated charcoal, for maximum rooting of *in vitro* raised microshoots. All the treatments showed 100% root induction. Treatment comprising MS media devoid of any plant growth regulator produced highest number of roots (5.60 ± 0.74) and root length (6.78 ± 0.52 cm). Callus induction, growth and frequency from leaf explants of *Punicagranatum* L. were standardized. Leaf explants on MS medium supplemented with 8.0 µM NAA and 9.0 µM kinetin was compact, yellowish green in colour and showed very good callus growth.

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Proteases of Euphorbian Garden Plant: *Euphorbia Leucocephala*

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Abstract

Euphorbia is a very large and diverse genus of flowering plants in the spurge family. Latex of Euphorbian plants is a good source of proteolytic enzymes, mainly cysteine, serine proteases. This is the first report on this plant regarding evaluation of proteolytic activity. The latex of them contain either cysteine or serine proteases sometimes both also. Evaluation of proteolytic enzymes, cysteine and serine in the latex has been carried out in particular criteria such as pH optima (3 and 8), temperature optima (30^0C and 50^0C) and their chemical behavior towards inhibitor and activator is studied. Latex proteases inhibited by $HgCl_2$ and PMSF at specific concentrations. However reducing agents such as cysteine- HCl and β- mercaptoethanol enhance the native activity. Other solvents fails to retard native state except ethanol. The results of present investigation led to conclusion that E. leucocephala is a new source of cysteine and serine protease. However, characterization of physicochemical properties of them is advocated.

Keywords: *Cysteine proteases, serine proteases, Euphorbia leucocephala, PMSF inhibitor.*

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Introduction

Euphorbia leucocephala native of southern Mexico. Its life cycle habitat is perennial. Proteases are a highly complex group of enzymes that differ in their substrate specificity, catalytic mechanism and active site chemistry. Peptidases, or peptide hydrolases, are enzymes able to hydrolyze peptide bonds. Some are used as used as ornamental in landscaping. Some of Euphorbian plants are cultivated as ornamental/garden plants in natural and international gardens. Most of member of these family synthesis proteases in different tissues. The order of amount of protease present in these plants are serine<cysteine<aspartic<metallo-proteases. In our previous communication we have reported various aspects of *Euphorbia leucocephala* crude enzyme (**E. L. CE**) study which has been carried out in our laboratory. Proteolytic enzyme of some laticiferous plants belonging to Khandesh region of Maharashtra, India is reported by Mahajan & Badgujar et al (2009). The comparative study of Cysteine proteases of four laticiferous plants and its characterization of euphorbian linn documented by Badgujar & Mahajan et al (2012), A detailed account of forty Ethanobotanical Euphorbian plants used for various application of North Maharashtra region is given by Mahajan and Adsul et al (2013). Recently Mahajan et al (2016) reported the detail account on twenty five garden euphorbian plants regarding their proteolytic activity. In the present investigation we have focused our study on characterization of least record on protease from *Euphorbia leucocephala.*

Materials and Method

Proteases appear to play key roles in the regulation of biological processes in plants, such as the recognition of pathogens and pests and the induction of effective defense responses. Various proteases found in latices of several plant families viz Apocynaceae, Asclepiadaceae, Asteraceae, Caricaceae, Convolvulaceae, Moraceae

and Euphorbiaceae. Here the prime reason for selection of Euphorbian plants is to search vegetables rennin like source.

Euphorbia leucocephala is identified by taxonomist, Dr. Tanveer A. Khan, Department of Botany, H. J. Thim College of Arts and Science College, Mehrun, Jalgaon, Maharashtra, India. The latex of *E. leucocephala* was collected early in the morning by superficial incision of stem or leaves of healthy plant and allowing the milky latex to drain in clean glass vial, brought to the laboratory and kept in refrigerator till the experiment started.

Preparation of crude enzyme

Latex was homogenized in a homogenizer under chilled condition and filtered through four folds of muslin cloth. Filtrate latex sample was centrifuged at 15,000 rpm for 45 minutes at 4^0C. The resulting supernatant of latex enzyme called "Crude enzyme" or "Centrifugal fraction" which was used for further investigation of protease enzyme assay.

Protease assay

Proteolytic activity of plant tissue was determined by the colorimetric assay using 1% casein as a substrate. The protease activity was expressed as amount of enzyme required to produce peptide equivalent to μg of tyrosine/min/mg protein at 370C and protein content was determined according to Lowry's method using Bovine serum albumin as the standard protein. One unit of protease activity is defined as the amount of enzyme to release 1 μg of tyrosine per minute at 37^0 C. A tyrosine standard curve was calibrated (10 to 100 μg/ml) using Folin Phenol reagent. Specific activity of the proteolytic enzyme is expressed as the number of units per milligram of protein.

Effect of pH on enzyme activity of E.L.CE

The effect of pH on proteolytic activity of crude enzyme was measured with casein as substrate (pH 2.0 – 3.0) using 0.1 M of Glycine- HCl buffer. The buffers used were acetate buffer (pH 4.0- 5.0) phosphate buffer (pH 6.0 – 8.0) and Glycine- NaOH buffer (pH 9.0 – 10.0).

Effect of temperature on enzyme activity of E.L.CE

The effect of temperature on enzyme activity was studied by using casein at constant pH 7. The crude enzyme was incubated at the desired temperature, in the range of 20-80^0C, for 15 minutes in phosphate buffer (pH 7) and an aliquot was used for the activity measurement at the respective temperature.

Effect of Surfactant and Oxidizing Agent on Proteolytic Activity of E.L.CE

The effect of different surfactants, Triton X-100, and Tween 80, and oxidizing agent (H2O2) at 1% (v/v) final concentration on enzyme activity was studied by pre incubating enzyme preparation for 60 minutes at 37^0C in the above surfactants and oxidizing agent before analysis. Then, the residual proteolytic activity was measured by using casein according to standard assay procedure relative to control (without chemical surfactants and oxidizing agent) and the resulting activity was taken as 100 %.

Effect of Organic Solvents on Proteolytic Activity of E.L.CE

The stability of enzyme activity in presence of different water miscible or immiscible organic solvents was treated by incubating the enzyme preparation with organic solvents of (70%, v/v) at 37^0C for 1 hour. After incubation, the residual enzyme activity was determined as discussed earlier. The enzyme activity of a control sample (without solvent), incubated under the same conditions, was taken as 100 percentage. For this assay, the solvents used were alcohol, butanol and methanol.

Effect of Substrate concentration on proteolytic activity of E.L.CE

The enzyme is very broad spectrum in its activity usually casein is preferred for analyzing proteolytic activity of biological source, rather than haemoglobin, albumin, gelatin. In the present investigation the different concentrations of substrate that varies concentration from 0.1 % to 2% and activity is determined by as usual method.

Effect of inhibitors on proteolytic activity of E.L.CE

Inhibition of the hydrolysis of casein by crude enzyme was performed using 10 mM PMSF (Phenyl methane sulfonyl fluoride), 10 mM EDTA (Ethylene diamine tetra acetic acid), and 10mM $HgCl_2$. The enzyme preparation was incubated with inhibitors individually at room temperature for 60 minutes. The residual proteolytic activity against casein was determined. A control assay of the enzyme activity was done without inhibitor and the resulting activity was taken as 100%.

Effect of Activators on proteolytic activity of E.L.CE

Activator hydrolysis the casein by crude enzyme was investigated using 10 mM cysteine- HCl, 10 mM β-mercaptoethanol, and 10mM Sodium metabisulfide. The enzyme preparation was incubated with activators individually at room temperature for 60 minutes. The residual proteolytic activity against casein was determined. A control assay of the enzyme activity was done without inhibitor and the resulting activity was taken as 100%.

Results and Discussion

Emphasis is given to evaluate of other industrial applications pertaining to agricultural, medicinal and environmental protection. In this laboratory proteolytic activity of Euphorbian plants i.e *Euphorbia leucocephala* taken for partial characterization of enzymes for two reasons (i) to search potential of possible proteases present in *E. leucocephala*. (ii) Partial characterization of proteolytic enzymes present in latex.

Characterization of Protease of E.L.CE

Effect of pH on enzyme activity of E.L.CE

The proteases of *E. leucocephala* latex exhibit high proteolytic activity within a pH range from 2 to 10, interestingly high activity of protease of E.L.CE were observed at pH 3 peak I and pH 8 peak II, that indicates the presence of two proteases viz acidic and alkaline protease (Figure 1). The reaction is carried out constantly at 37^0C.

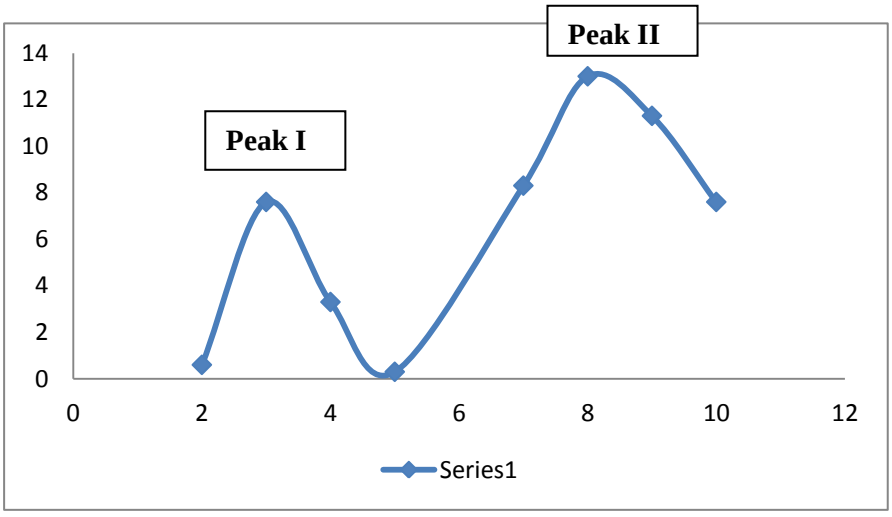

Figure 1: Effect of pH on enzyme activity of E.L.CE

Effect of temperature on enzyme activity of E.L.CE

The proteases of *E. leucocephala* latex exhibit proteolytic activity within a temperature range from 20^0 to 80^0C. Surprisingly here also it exhibit two peaks, peak I and peak II indicating that two enzymes have two optima i.e. at 30^0C and 50^0C (figure 2). The reaction is carried out at constant pH 7.4.

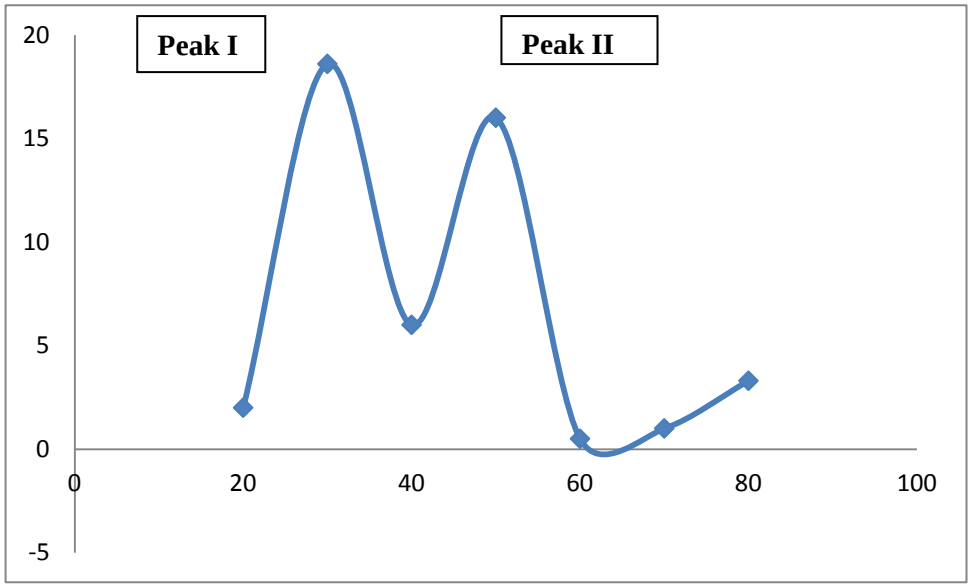

Figure 2: Effect of temperature on enzyme activity of E.L.CE

Table 1: Effect of Surfactant/Oxidizing Agent/organic solvent on Proteolytic Activity of E.L.CE

Sr. No	Name of surfactant/oxidizing agent/organic solvent	Proteolytic activity (TU/ml/min)
1	Tween 80	11.83
2	Triton X 100	9.16
3	H_2O_2	0.00
4	Ethanol	4.33
5	Methanol	0.00
6	Isopropanol	0.00

Table 2: Effect of Substrate concentration on proteolytic activity of E.L.CE

Sr. No	Concentration of Substrate (mg/ml)	Proteolytic activity (TU/ml/min)
1.	20	1.22
2.	40	2.56
3.	60	3.43
4.	80	4.43
5.	100	5.28
6.	120	5.74
7.	140	6.28
8.	160	6.61
9.	180	7.96

10.	200	7.11
11.	220	7.11
12.	240	7.12

From the Table 2 it is cleared that Vmax of the E.L. CE protease would be 7.1 as the substrate concentration increases there would be no increases the reaction velocity, and hence the Vmax/2 that is the Km 3.55, and apparent Km is 12.2 mg/ml. The reaction is carried out at pH 7.4 at 37^0C.

Partial Characterization of Protease of E.L.CE

Effect of inhibitors on proteolytic activity of E.L.CE

In order to identify the classes of protease enzymes of *E. leucocephala* plant latex, the effect of different proteases inhibitors have been evaluated. Table 3 shows the proteolytic activity of the protease enzyme of latex after its inhibitions with the following class specific inhibitors mercuric chloride (inhibitor of cysteine protease), PMSF (inhibitor of serine protease), EDTA (inhibitor of metalloprotease) using casein substrate. Maximum inhibition of proteolytic activity of plant latex containing enzyme occurs in presence of PMSF. Enzymes show remarkable inhibition in the presence of mercuric chloride, EDTA on the activity of latex enzymes.

Table 3: Effect of Inhibitors on proteolytic activity of E.L.CE

Sr. No	Inhibitors (mM)	$HgCl_2$ TU/ml/min	PMSF TU/ml/min	EDTA TU/ml/min
1	*	4.67	4.67	4.67
2	2	3	0.006	8
3	4	1.67	0.003	7
4	6	2	0.002	6
5	8	1.17	0.01	3
6	10	0.83	0.003	4.33

***** = Control without inhibitor.

Effect of Activators on proteolytic activity of E.L.CE

In order to further identify the classes of protease enzymes of *E. leucocephala* plant latex, the effect of different proteases activators have been evaluated. Table 4 shows the proteolytic activity of the protease enzyme of latex after its activation with the following class specific activators Cysteine- HCl (Activator of Cysteine protease) and β- mercaptoethanol (reducing agent) using casein substrate. Maximum activation of proteolytic activity of plant latex containing enzyme occurs in presence of Cysteine- HCl. Enzymes show the significant activation in the presence of β- mercaptoethanol on the activity of latex enzymes.

Table 4: Effect of Activators on proteolytic activity of E.L.CE

Sr. No	Activators (mM)	Cyst-HCl TU/ml/min	β-Mercaptoethanol TU/ml/min
1	*	1.33	4.67
2	2	3	8.33
3	4	4.17	9.67
4	6	5.67	14.33

5	8	9	16
6	10	6	11

***** = Control without activator.

We would like to suggest following major findings of present investigation.

1. Latex of CE (crude enzyme) has at least two proteases having caesinolytic activity.
2. Effect of various parameters like pH, temperature, surfactant, oxidizing agent, organic solvents, inhibitor, activator on protease of CE has been carried out.
3. Interestingly the study shows that latex do contain two enzymes as graphs has two peaks, peak I and peak II. This is also true for effect of temperature study. This clearly indicates peak I enzyme is chemically different from peak II enzyme.
4. The question arises what is peak I enzyme and peak II enzyme? To answer this, the results obtained with two types of inhibitors shows that either peak I would be serine/ Cysteine or peak II would be Cysteine/ serine. Study shows that there is complete inhibition of latex protein with PMSF and $HgCl_2$. More evidently the activity varies with concentration of inhibitor also in case $HgCl_2$.
5. Latex protease activity is enhanced by the reducing agent like Cysteine- HCl and β- mercaptoethanol.
6. No retardation of activity is seen with EDTA treatment.
7. Both enzymes are blocked by methanol, isopropanol and H_2O_2, however the activity is retain with ethanol.

Conclusion

Isolated protease enzymes have

i. Two temperature optima 30^o C and 50^oC and Two optima pH 3 and 8.
ii. Presence of two enzymes Cysteine and serine.
iii. Apparent Km is 3.25mg/ ml of caesin.
iv. Presence of isozymes is ruled out by use of specific inhibitor for given enzyme.
v. Future prospect: Maybe rennet substitute for cheese production.

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Preparation and characterization of Chitin Nano-Fertilizers and their applications

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Abstract

Nanotechnology can be fruitfully used for improving crop quantity as well as quality. Chitin is used as nano-carrier agent to pass essential macro as well as micronutrients for the optimum growth of plants. Chitin nanoparticles were prepared and characterized by means FTIR spectroscopy. Potassium nitrate, urea and ferrous sulphate were encapsulated with chitin nanoparticles by ionic gelation method to formulate nanofertilizers to be implemented in crop fields.

Keywords: *Chitin, Nanotechnology, Macronutrients, Micronutrients*

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Introduction

Henri Braconnot, a French professor of natural history, discovered chitin in 1811 in mushrooms. It is large-structural polysaccharide, made from chains of modified glucose. It exists as the main component of the exoskeleton of insects and cell walls of fungi. Structurally, it is long chain polymer of N-acetylglucosamine, an amide derivative of glucose. It is amorphous, whitish natural polysaccharide that is biodegradable, insoluble in water but soluble in strong acids such as sulphuric acid. It is a second most abundant biodegradable polymer (Gooday 1990; Gohel et al 2006; Gow & Gooday 1983).

The term "nano" is adapted from the Greek word meaning "dwarf." The word "nano" means one billionth part of a meter. Particles with at least one dimension less than 100 nm are considered as nanoparticles. Undetectable by human eye, nanoparticles usually distinguished from microparticles (1-1000 μm). They are classified into different types according to size, morphology, physical & chemical properties (Bari & Mishra 2016; Bari & Mishra 2017).

Nanofertilizer

Nanofertilizer is defined as material in the nanometer scale, usually in the form of nanoparticles, containing micro & macronutrients that are delivered to crops in controlled mode. It is important in agriculture, to increase crop yield and nutrient use efficiency, and to reduce excessive use of chemical fertilizers. Most important nutrients for almost all crops are macronutrients such as nitrogen, phosphorus and potassium. They have high surface area, sorption capacity and controlled release kinetics for targeted sites, attributing them as smart delivery system (Dimpka & Bindraban 2017).

Nanofertilizers are being prepared by encapsulating plant nutrients into nanomaterials, employing thin coating of nanomaterials on plant nutrients, and delivering in the form of nano-sized emulsions. They have high solubility and stability. They improve nutrient-use efficiency and acts as simulating agents for plant growth and activate metabolic process in plants. Macronutrient nanofertilizers providing nitrogen, phosphorus, sulphur, potassium, calcium & magnesium are needed for proper growth of plants (Zulfiqar et al 2019).

Urea

Urea is a source of nitrogen, an essential nutrient, crucial for crop growth and development. Urea is the most important nitrogenous fertilizer in the country because of its high N content (46 % N). It also has industrial applications such as the production of plastics and as a nutritional supplement for cattle. The standard nitrogen fertilizer application rate is 0.1 to 0.2 pounds per 100 square feet. This translates into 0.5 to 1 pound ammonium sulphate, 0.3 to 0.6 pounds of ammonium nitrate, or 0.2 to 0.4 pounds of urea. The most common urea fertilizer types are the crystalline and granular variants. Crystalline urea fertilizers are the finest products and feature the quickest release time of the dry urea applications. Granular urea is in nano-form, acting as an intravenous injection rather than a popping capsule. The ultra-small particles are better absorbed directly from the leaf than through the soil. More than 70 % of the conventional urea applied in the soil remains unabsorbed by plants and it gets wasted in the environment (Saharan & Pal 2016).

Primary nutrients, also known as macronutrients, are those usually required in the largest amounts. They are carbon (C), hydrogen (H), nitrogen (N), oxygen (O), phosphorus (P), calcium (Cl), sulphur (S), magnesium (Mg), and potassium (K). Most micronutrients are part of the enzyme systems of plants. Micronutrients play important roles in redox reactions and during photosynthesis. Micronutrients are important in reactions such as N_2 fixation, protein synthesis etc. Macronutrients are taken up in relatively large amounts (10 -100 kg or more per hectare), while the amount of micronutrients that is taken up by plants is mostly limited to several grams per hectare.

Potassium nitrate

Potassium nitrate (KNO_3) a crystalline salt, is also a strong oxidizer, used especially in making gunpowder, as a fertilizer, and in medicine. Potassium nitrate is the inorganic nitrate salt of potassium. It is a soluble source of two major essential plant nutrients. It is commonly used as a fertilizer for high-value crops, benefited from nitrate (NO_3-) nutrition and a source of potassium (K^+) free of chloride (Cl^-). Further, it is easy to handle and apply, and is compatible with many other fertilizers, including specialty fertilizers for many high-value specialty crops, as well as those used on grain and fibre crops.

Ferrous sulphate

Ferrous sulphate, also known as iron sulphate represents a spectrum of salts having the formula of $FeSO_4$. Ferrous sulphate, Green vitriol, Iron vitriol, Copperas, Melanterite, and Szomolnokite are some of the other names for iron sulphate. Iron sulphate created when iron filings are added to a copper sulphate solution and thereby iron pushes the copper out since it is more reactive. Iron Sulphate is a bluish-green chemical, used in variety of application such as inks, dye and medicine. Melanterite $FeSO_4H_2O$ and Rozenite $FeSO_4H_2O$ are two well-known mineral forms of ferrous sulphate. Ferrous sulphate fertilizer is used to correct Chlorosis (yellowing of the leaves). In the plant, iron plays a role in photosynthetic electron transport, respiration, chlorophyll formation, and numerous enzymatic reactions.

In the present study, we have prepared and characterize chitin nanoparticles, followed by its encapsulation with few essential macro as well as micronutrients, in the form of urea, ferrous sulphate and potassium nitrate by ionic gelation method.

Experimental

- **For nano-chitin synthesis**

Material

Chitin, 3M HCL, 96% Ethanol, Distilled Water

Method

For Chitin hydrolysis, 3M HCl with 3 different chitin and acid ratio i.e 1:10 (M1), 1:12 (M2), 1:14 (M3) were stirred at 100^{o}C for 90 min. at one atmosphere pressure. After hydrolysis, the acid solution was removed and then replaced with 96% ethanol. Chitin suspension in ethanol was crushed mechanically with mortar and pestle for 10 min. Product obtained was boiled at 100^{o}C for 90 minutes, in water bath to mix the particles, followed by centrifugation at 6000 rpm for 30 min. The supernant was removed & ppt obtained was rewashed in ethanol and again centrifuged at 6000 rpm for 10 min. Final product was dried at 80^{o}C for 4 h (Jayakumar et al 2010; Saharan & Pal 2006)**.**

- **Synthesis of Chitin nanofertilizers, using ionic gelation Method**

A. Chitin-KNO_3 nanofertilizer

Material

Chitin nanoparticles, Distilled Water, 0.3% KNO_3 (0.3 gm KNO_3 in 100 ml distilled water)

Method

The nanofertilizer was prepared by adding 0.3 % KNO_3 into chitin nanoparticles emulsion.

B. Chitin-Urea nanofertilizer

Material

Chitin, Acetic acid (1%), Twin 80, Urea (10 gm), Sodium tri-polyphosphate (STPP), Distilled water

Method:

1.5 g chitin was dissolved in1% acetic acid. Then twin 80 was added, and stirred overnight at 350 rpm. 10 gm of urea was dissolved in chitin-acetic acid solution. STPP solution was added drop by drop (16 drops/minute) using filtration funnel at 450 rpm under stirring. Then ultra-sonication treatment was provided by using sonicator with 35% amplitude for 15 minutes under ice bath condition. Finally nanochitin-urea composite was freeze-dried in powdered form.

C. Chitin-$FeSO_4$ nanofertilizer

Material

Chitin nanoparticles, $FeCl_2$ (0.15 M), $FeCl_3$ (0.30 M), Distilled Water, NH_4OH (60 M)

Method

Initially two different solutions namely 0.15M $FeCl_2$ and 0.30 M $FeCl_3$ were prepared using distilled water and allowed them to mix homogeneously via passing ultrasonic waves. Then, NH_4OH was rapidly added to mixture to obtain black particles of Fe_3O_4 at room temperature. These particles were washed with water and dried in oven. Thus prepared 0.5 g Ferrofluid was added in chitin nanoparticles solution and subjected to ultrasonic irradiation for 10 min on sonicator. Finally dried the product, that was obtained after multiple washing and centrifugation at 3000 rpm for 20 min.

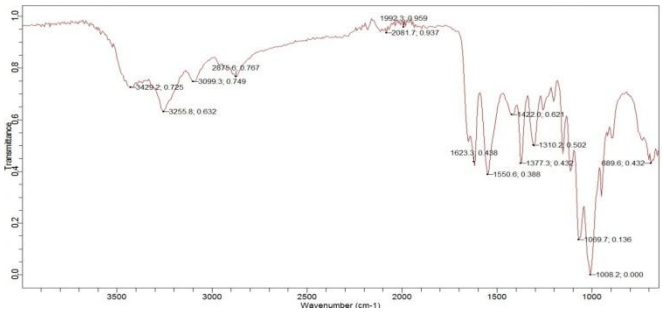
Characterization

1. **Fourier-Transform Infrared Spectroscopy (FTIR)**

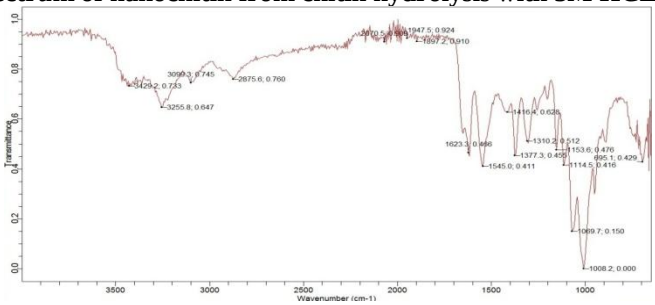
This is a rapid method that can detect a range of functional groups and is sensitive to changes in molecular structure. Samples were scanned over the frequency range of 400-4000 cm^{-1} with resolution of 4 cm^{-1} in absorbance mode for 8 – 128 scans at room temperature by using FTIR, Agilent Technologies, and Santa Clara, CA, USA. The sample of FTIR analysis is prepared by grinding the dry blended powders.

Results & Discussion

FTIR spectroscopy of two nanochitin samples, namely M2 and M3 are shown in graph 1 and graph 2 respectively. In both spectra, two bands at 1659 cm^{-1} and 1624 cm^{-1} correspond to the stretching of amide-I, band at 1659 cm^{-1} correspond to stretching of the C = O group, hydrogen bonded to N–H of the neighbouring intra-sheet chain, and the 1624 cm^{-1} band may indicate a specific hydrogen bond of C = O with the hydroxyl-methyl group of the next chitin residue of the same chain.



Graph 1: FTIR spectrum of nanochitin from chitin hydrolysis with 3M HCL at 1:12 ratio (M2)



Graph 2: FTIR spectrum of nanochitin from chitin hydrolysis with 3M HCL at 1:14 ratio (M3)

The absorption bands at 1556 and 1315 cm^{-1} correspond to amide II (N-H bending) and amide III (C-N stretching) respectively, confirming the presence of nanochitin in sample (Dahmane et at 2014). Further characterization for estimating the nano-size of products by electron microscope is under process.

Conclusion

Nanotechnology is very useful in improving the growth, yield and health of crops. Its most important advantage is in the form of nanofertilizers, which account for most of the research in this field. Present study confirms the formation of nanofertilizers by means of FTIR spectroscopy. When sprayed on crops at very low concentration, these compounds may have a direct effect by increasing crop growth and final product quality.

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Preparation and characterization of Chitosan Nano-Fertilizers and their applications

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Abstract

In agricultural sector, nanofertilizers have a significant impact on productivity and resistance to a biotic stress. Advantage of nanofertilizers lie in increased production of crops, that are capable to withstand the atmospheric changes. Comparatively with conventional fertilizer, nanofertilizers reduce leaching and volatilization. Through utilization of nanofertilizer, product quality & quantity increases which is beneficial in profit margin for plant breeders/growers. For the preparation of chitosan nanofertilizers and their application for encapsulation bioactive ingredients, the nano-structure chitosan can be used as a carrier. This is attributed to favorable biological properties of chitosan including non-toxicity, bio-capability, bio-degradability, and antibacterial ability, along with high surface area as well as aspect ratio of their nanoparticles. Chitosan nanofertilizer was prepared by ionic gelation method & characterized by various spectro/microscopic techniques.

Keywords: *Chitosan, Nano-fertilizer, Crop-improvement*

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Introduction

Different approaches can be used to synthesize nanoparticles for production of nanofertilizers, such as top-down or bottom-up approaches (Singh & Rattanpal, 2014; Pradhan & Mailapalli, 2017). With these innovative fertilizers, nutrients are released at a very slow rate that corresponds to crop growth. Using these nanofertilizers, it is possible to increase the efficiency of nutrient use, resulting in precision agriculture. These fertilizers can be a great alternative to conventional fertilizers, which are expensive and require large quantities, and can also protect the soil and water from nutrient pollution (Dimkpa & Bindraban, 2017). A variety of nano fertilizers, including macro & micronutrients like N, P, K, Cu, Fe, Mn, Mo, Zn etc., have been shown to achieve an excellent control release for targeted delivery efficiency (Liu & Lal, 2015).

There are numerous applications for chitosan-based nanoparticles due to their biodegradability, high permeability, non-toxicity to humans, and cost-effectiveness (Shukla et al., 2013). Functional bioactive ingredients have received much attention from the scientific community, consumers, and food manufacturers. As scientific evidence for health-promoting ingredients emerges steadily (Elliott & Ong, 2002), it is challenging to retain the health benefits of functional ingredients in conditions encountered during processing or in the gut (pH, enzymes, and other nutrients). To protect these molecules from harsh conditions, they must be encapsulated or immobilized. Alkaline de-acetylation of chitin produces chitosan, which is a partially de-acetylated polymer of N-acetyl glucosamine. It consists of a β- (1, 4)-linked D-glucosamine residue with acetylated amine groups randomly distributed across the residue (Sevda & McClure, 2004). Chitosan's amine and hydroxyl groups end it with many unique properties, enabling it to be used in a wide range of applications

and to be easily reacted with chemicals. As well as being safe and non-toxic, chitosan can interact with poly-anions to form complexes and gels (Sunil, Nadagouda & Tejraj 2004).

In an acidic medium, Chitosan nanoparticles are soluble and are prepared by the ionic gelation method with sodium tri-polyphosphate ion. In the ionic gelation, method nanoparticles are synthesized by electrostatic interaction between opposite charge types in the presence of a polymer under stirring mechanical conditions. In many agricultural fields Nanoparticles, made from chitosan (CS), have been used as nano pesticides, nano fertilizers, and nano herbicides (Choudhary et al., 2019). A wide range of CS-NP or CS-NPs-loaded active ingredients (Cu, Zn, salicylic acid, NPK, urea, and ferrous) have been studied recently for their potential to control plant diseases and enhance plant growth, depending on concentration and application method, and have attracted much attention. CS-NPs are synthesized using the ionic gelation method and a present forward-looking perspective on the use of CS-NPs in plant disease management (Sharma et al. 2020). Encapsulation of two or more compounds in a single carrier is a packaging process used to increase shelf life. It will Increase the functionality of nutrients and bioactive compounds.

Advantages of Nano-Fertilizer

Nano-scale additives enhance plant uptake or inhibit pathogen growth in conventional fertilizers. It is possible to encapsulate or carry fertilizers using nanomaterials, including chitosan. By reducing overall chemical usage, reducing toxic residues, and enhancing management, they contribute to an environmentally friendly and sustainable agriculture system (Zulfiqar et al., 2019).

Experimental

Materials:

Chitosan, Sodium tri-poly-phosphate, Acetic acid, Tween 80, Deionized water, NaOH, Copper sulfate, Salicylic acid, Ferric chloride, Ferrous chloride, Ammonium hydroxide, Urea

Methods:

1) Synthesis of Chitosan nanoparticles using ionic gelation Method

0.5 gm chitosan was dissolved in 1% acetic acid premixed with Tween 80, by overnight stirring at 360 rpm. 0.25 gm Sodium tri-polyphosphate (STPP) was dissolved in deionized water and stirred at 360 rpm. Using Whatman filter paper no.1, chitosan and STPP solution was filtered out separately and then cross linked with each other. This cross linking was carefully achieved by keeping the dropping speed of 80-100/min for chitosan solution via separating funnel, under constant stirring of STPP anions at 450 rpm. pH was maintained at 4.5-5.5 by adding NaOH.

2) Co-encapsulation with Copper

0.04 gm $CuSO_4$ was added to the chitosan nanoparticles solution with 0.2 gm salicylic acid. The solution was centrifuged at 10,000 rpm for 10 minutes. Sonication for 10 minutes was performed in the bath sonicator, followed by multiple washing with distilled water. Finally, semi-solid material was obtained and dried.

3) Co-encapsulation with Ferrous

2.44 gm $FeCl_2$ was added to the 100 ml deionized water and 3.08 gm $FeCl_3$ was added to the 100 ml deionized water. The mixture was irradiated with ultrasonic waves for 15 minutes. NH_4OH solution was rapidly added to the mixture to obtain black particles of Fe_3O_4. Wet particles were dried in the oven. These Ferro-nanoparticles

were added to the chitosan solution and subjected to ultrasonic irradiation for 10 minutes on a bath sonicator. This is followed by their centrifugation for 20 minutes and finally vacuum-dried particles were obtained.

4) Co-encapsulation with Urea

1.5 gm chitosan was dissolved in 1% acetic acid containing Tween 80 with 450 rpm while stirring. Similarly 10 gm urea was dissolved in chitosan-acetic acid suspension, under stirring at 300 rpm. 1 gm STTP was dissolved in deionized water under stirring at 620 rpm and then added to urea-chitosan-acetic acid suspension at 16 drops per minute using a filtration funnel under continuous stirring at 450 rpm. The suspension was further subjected for sonication using an ultrasonicator with 35 % amplitude for 15 minutes under ice bath conditions.

Characterizations

1. **UV–Visible Spectroscopy**

With a UV–Vis spectrophotometer (UV-1601, Shimadzu, Japan), the absorption spectra of synthesized chitosan nanoparticles/nano fertilizer were measured in the range of 200–800 nm.

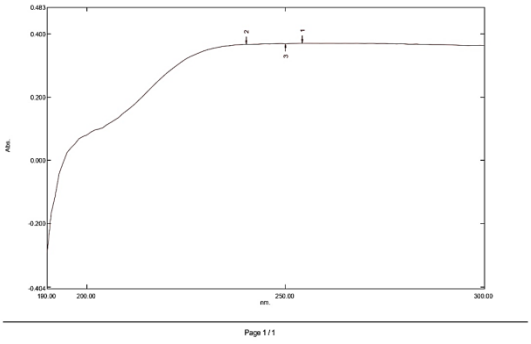
2. Fourier Transform Infrared Spectroscopy (FTIR)

To identify the possible functional groups present in synthesized chitosan nanoparticles/nano fertilizer, Fourier Transform Infrared Spectroscopy (FTIR, Agilent Technologies, and Santa Clara, CA, USA) was carried out at 450 to 4000 cm^{-1}.

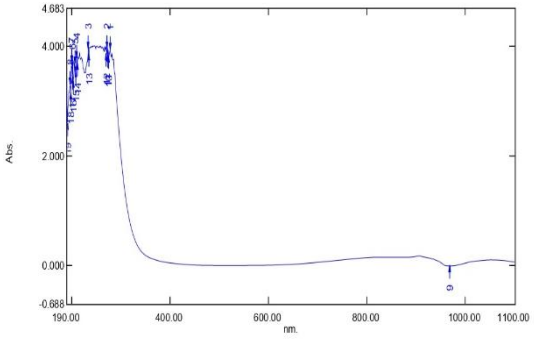
Results & Discussion

1. **UV–VIS Spectroscopy**

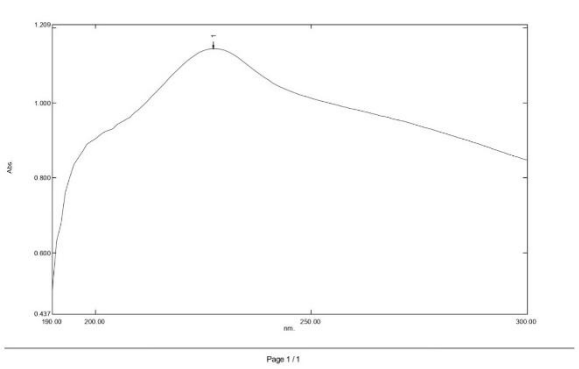
The optical properties of biopolymer materials were analyzed by UV- Vis spectroscopy and are shown in graph no. 1 to 4. The absorption wavelength can be noticed at 260 nm in graph 1; 340 nm in graph 2; 227 nm in graph 3 and 198 nm in graph 4. The intensity obtained of peaks is due to formation of nanoparticles (Sharma et al 2020).



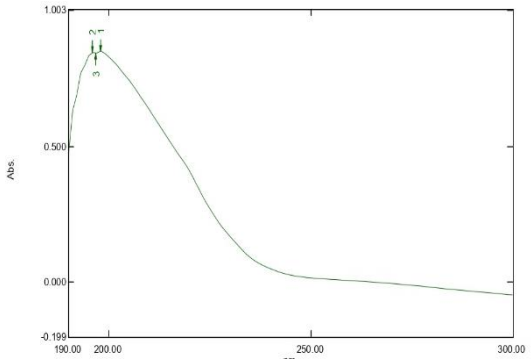
Graph 1: Chitosan Nano Fertilizer



Graph 2: Chitosan Nano fertilizer Encapsulated with Copper



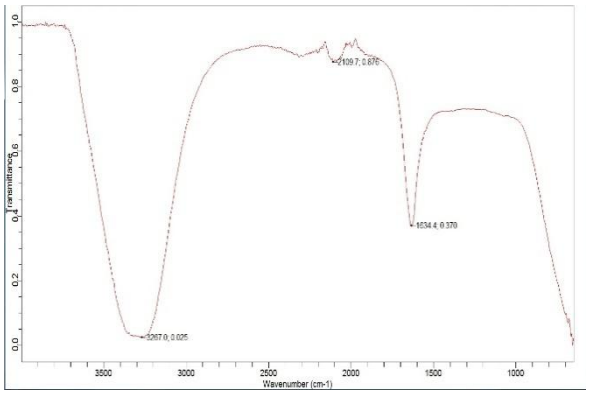
Graph 3: Chitosan Nano fertilizer Encapsulated with Ferrous



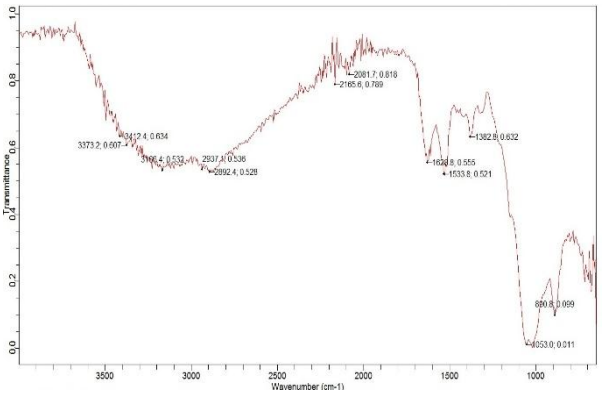
Graph 4: Chitosan Nano fertilizer Encapsulated with Urea

2. Fourier Transform Infrared Spectroscopy (FTIR)

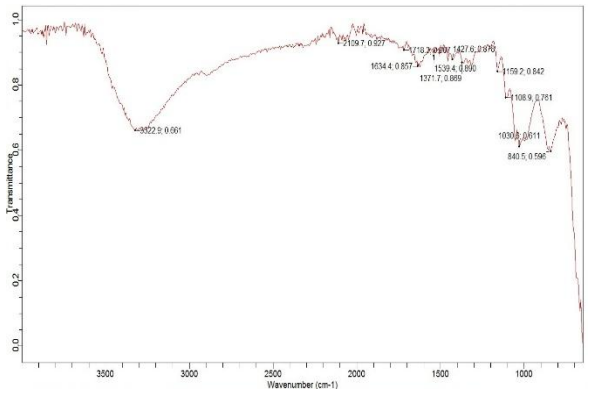
Functional groups of the samples were studied by FTIR spectroscopy and respective spectra are shown in graphs 5 to 8. A peak at 3500-3300 cm^{-1} was observed for the main functional group of chitosan and is due to O-H group stretching vibrations. The presence of absorption peak at 3200-3300 cm^{-1} is due to N-H stretching. The peak at 3093-2870 cm^{-1} is due to C-H stretching, 3166-2937 cm^{-1} is due to O-H stretching, 2892 cm^{-1} is due to N-H stretching, 2165 cm^{-1} is due to S-C≡N stretching, 2109 cm^{-1} is due to C≡C group stretching, 2081 cm^{-1} is due to N=C=S group stretching, 1628-1634 cm^{-1} is due to C=C stretching, 1718 cm^{-1} is due to C=O group stretching, 1500-1550 cm^{-1} is due to N-O stretching, 1382 cm^{-1} is due to C-H stretching, 1377-1421 cm^{-1} is due to O-H stretching, 1371cm^{-1} is due to S=O stretching, 1108,1159-1215 cm^{-1} is due to C-O group stretching, 1153 cm^{-1} is due to C-N group stretching, 1030-1058 cm^{-1} is due to S=O stretching, 1053 cm^{-1} is due to C-O group stretching, 840-890 cm^{-1} is due to C=C group stretching (Chandrasekaran et at 2019).



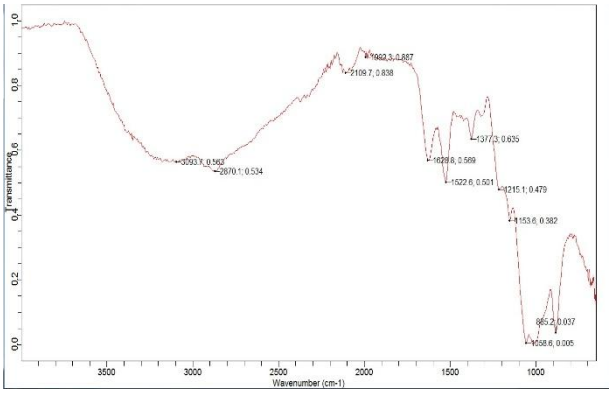
Graph 5: Chitosan Nano Fertilizer



Graph 6: Chitosan Nano fertilizer Encapsulated with Copper



Graph 7: Chitosan Nano fertilizer Encapsulated with Ferrous



Graph 8: Chitosan Nano fertilizer Encapsulated with Urea

Conclusion

From the present study, we concluded that nano-structured chitosan can be used as a carrier molecule for the transport of fruitful macronutrients (N, P, K) for plant growth. These nanofertilizers are potentially developed by encapsulation via ionic gelation method. Spectroscopic analysis confirmed the presence of various macromolecules in nanofertilizer. However, most of this study is still in progress, including microscopic analysis of nanoparticles/nanofertilizers via SEM/TEM, along with in depth evaluation of the effect of nanofertilizer for different physio-chemical properties. We expect that co-encapsulation approach used in present study could further be expanded for synthesis of nanofertilizers, having more than one micronutrient or macronutrient deliver to the crops.

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Isolation, identification and characterization of lipolytic bacteria from oil spilled garage soil

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Abstract

Lipases are a hydrolases class of enzymes and multifaceted with the ability to perform a specific range of biotransformation. In present investigation different sources like waste water of sweetmart, garden soil, garage soil and vegetable spilled soil of Jalgaon (Maharashtra) were used for isolation of lipase containing microorganism. The colony obtained from oil spilled garage soil sample showed zone of clearance on Tributryin –agar media and further subjected to gram staining and biochemical assay. The olive oil was used as substrate for determining lipase activity of crude enzyme by calculating EU/ml using titrimetric assay. Enzyme produced by potential isolates showed optimum enzyme activity at pH (7), temperature (37^0c) and 48 hours of incubation time.

Key words: Lipase, biotransformation, isolates, enzymes, media etc

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1. Introduction

Lipases were discovered in 1856 by Claude Bernard when he was studying the role of pancreas in digestion of fat (Petersen, 1994). Lipase are the enzyme that catalyzes hydrolysis of the ester bonds at the interface between insoluble substrate phase and the aqueous phase in which the enzyme is dissolved under a natural condition, such as in the absence of water, they are capable of reversing the reaction (KE *et al.,* 2002). Lipases catalyze various reactions since they have ability to act on wide range of substrates that may be artificial or natural and possess stability over a wide range from pH 4 to 11 and temperature optima in the range from 10^0C to 96^0C. Compared to plants and animals, microorganisms have been found to produce high amount of lipase (Mehta et al., 2017).Lipase producing microorganisms including bacteria, yeast and fungi are found in various habitats for example coal tips, compost heaps, decaying food, dairies, industrial wastes, oil-proc essing factories, oil seeds, soil contaminated with oil and waste water. Among all these microorganisms several species of bacteria including mainly *Achromobacter, Alcaligenes, Arthrobacter, Pseudomonas*, *Staphylococcus* and *Chromobacterium species* are very efficient in producing extracellular lipases (Veerapagu et al., 2013) while *Bacillus* and *Pseudomonas*,spp. are the most efficient.

Bacterial enzymes are preferable over fungal enzymes due to its high activity and alkaline pH optima. The bacterial cells also have shorter generation times, common nutritional requirement and easy screening procedure compare to fungal enzymes. Some gram positive lipase producing species includes *Staphylococcus aureus* and *Staphylococcus hyicus, Streptomyces* and *Bacillus*. Among all the gram positive bacteria's, the bacillus species is the most useful lipase producing genus at industrial level. Moreover, microbial lipases as multipurpose biological enzymes are of significant interest for various applications in biological and industrial processes

including food and drink, leather, detergents, cosmetic, textile, agrochemicals, the pharmaceutical industries, and waste treatment.

The oily environment (oil mill outlet) may provide a good environment for isolation of lipase producing microorganisms. It was also observed that concocting an artificial mixture of metal ions and plastic-supplemented nutrient molecules from soil extract under shaking conditions, a number of lipolytic bacteria were explored, and their adaptability under harsh conditions was improved (Van Hong Thi Pham et al., 2021**).** It has been demonstrated that lipases can be utilized in a wide spectrum of substrates and is highly stable under extreme conditions of temperature, pH, and organic solvents (Andualema and Gessesse, 2012). Sadatullaha, 2018 isolated lipase producing species from service digs waste water collected from Dir lower, Peshawar and Kohat districts of KPK by using tributyrene agar plate assay and identified as *Pseudomonas* species biochemically. They characterized partially purified lipases for lipolytic activity at different pH and incubation time. During their study they found that molecular mass of purified lipase was approximately 50 kDa using SDS-PAGE and maximum lipolytic activity was observed at pH 7 after 48 hours of incubation and 37°C. Among all the isolates, isolate HSWPC showed highest activity of 110.11U /ml at pH 7 after 48 hours of incubation and 37°C. However, other studies have demonstrated that bacteria may secrete the highest lipase enzyme at either low or high temperatures, such as at 10^0C or 40^0c (Hassan et al., 2018).

Lipases occupy a place of prominence among biocatalysts according to their ability to catalyze a wide variety of reactions & are an important group of biotechnologically relevant enzymes; they find massive application. Many attempts have been made to isolate lipase producing microorganisms since this enzyme is used in numerous biotechnological processes including food, leather, cosmetics, detergents, and pharmaceuticals industries and in industrial waste management. Considering the lipase enzyme as an important enzyme, the purpose of the present study was to isolate and identify a novel lipase producing bacteria from different sources of oil contaminated soils and from the root nodules of some of the plants and study the activity of these isolates.

2. Materials and Methods

For the present study soil samples were collected from different sources of different places such as garage soils, soil exposed to oils for longer period, wastewater of sweet mart, waste from kitchen and restaurants of Jalgaon, Maharashtra. The soils were collected from trench of about 10-15 centimeters deep dug using sterile spatula to avoid contamination. Then samples were homogenized using mortar pestle and sieved through a sieve with pore size 2mm and stored in sterile glass beakers.

2.1 Isolation of lipase containing microorganism

The isolation process was performed by serially diluting the soil sample upto 10^{-7} and inoculating it on to the Nutrient Agar plates. The presence of lipase containing microorganism was carried out on Tributryin-agar media. An isolated colony was transferred into a 50 ml Erlenmeyer flask containing nutrient broth from preserved culture slant. The Flask was then incubated for 24 hours at 120 rpm at 37^oC. The 24 hours freshly grown culture was used as inoculum for inoculation of production media.

2.2 Screening of lipase containing isolates

The pure isolates were screened for lipase production on tributyrene agar plates. The bacterial isolates were

streaked, on tributyrin agar plates and incubated for 48 hour at 25°C, 30°C and 37°C. Formation of clear zones around colonies at 37°C indicates lipase production (Parsad and Manjunath , 2012). Due to their stability in organic solvents, lipases are listed as the third largest group of commercialized enzymes after protease and carbohydrase (Casas-Godoy et al., 2012).

2.3 Biochemical characterization

The biochemical and sugar fermentation tests were carried out using standard protocol given by Tembhurkar et al., 2012

2.4 Optimization of media for lipase activity

The different parameters like incubation time, temperature and pH were used for maximum enzyme activity as follows**,**

2.4.1 Effect of incubation time on lipase production

Studies were done to evaluate the effect of incubation time on lipase activity. Optimal enzyme activity was determined by incubating the reaction mixture at different time interval like 24 hr, 48 hr and 72 hr keeping other parameters same.

2.4.2 Effect of temperature on lipase production

Studies were done to evaluate the effect of temperature on lipase activity. Optimal temperature for enzyme activity was determined by incubating the reaction mixture at varying temperature in range 25 to 55°C keeping other parameters same (Tembhurkar et al., 2012).

2.4.3 Effect of pH on enzyme production

Studies were done to evaluate the effect of pH on lipase activity. The crude enzyme used for assay was the culture broth after separation of cells and particles. The enzyme was normally stored at 4°C until used. Optimal pH for enzyme activity was determined by incubating the reaction mixture at 37°C in buffer solutions of pH values ranging from 6 to 9, keeping other parameters same. Effect of pH on lipase action was analyzed by substituting the buffer in reaction mixture with the different buffers for different pH (Phosphate Buffer (0.1M) for pH 6, 6.5, 7, 7.5, 8, 8.5; Ammonia Buffer (0.1M) for pH 9) (Tembhurkar et al., 2012).

2.4 Determination of lipase activity

Lipase activity was measured titrimetrically using olive oil as a substrate. One ml of the culture was added to reaction mixture containing 2 ml of phosphate buffer with pH-7.0 and 1 ml of olive oil and incubated at 37^{o}C for 60 minutes. The reaction was stopped and fatty acids were extracted by addition 1.0 ml of acetone: ethanol solution (1:1). The amount of fatty acids liberated was estimated by titrating with 0.1 N NaOH until pH 10.5 using phenolphthaline as indicator. One unit of enzyme activity required to liberate 1µmol of equivalent fatty acid under the standard assay conditions.

3. **Result and Discussion**

3.1 Isolation and screening of lipase containing microorganism

In present study bacterial strain were selected and subjected to the further characterization from different sources shown in table 3.1 and purified by four flame method and spreaded on tributyrin agar plate.

Table 3.1 Sources used for isolation of lipase containing microorganism

Sr. No	Soil sample	Result
1	Waste water of sweet mart	No Zone of clearance
2	Garden soil	No Zone of clearance
3	Vegetable soil spillage	No Zone of clearance
4	Garage soil	Zone of clearance

The clear area surrounding the bacterial growth was observed on TBA media shown in fig 3.1 inoculated with garage soil sample which showed presence of lipase producing bacteria in garage soil sample.

Fig 3.1Colonies showing zone of clearance on TBA media

3.2 Biochemical and sugar fermentation test

The result obtained of biochemical and sugar fermentation test were given as follow;

Biochemical test	Result
Indole	Negative
MR	Positive
VP	Positive
Citrate	Positive
Urease	Positive
Casein Hydrolysis	Negative
Sugar fermentation tests	
Glucose	Positive
Lactose	Positive
Sucrose	Positive

From the above result it is concluded that the isolated lipase containing microorganism may be strains of *Staphylococcus aureus.*

3.2 Effect of pH on media

The initial pH of the growth medium influences the rate of lipase production. It was inferred from the results that the bacteria is capable of producing lipase from the initial pH of medium from pH 4.0 to pH 10.0. The enzyme production varied considerably from 12.0Uml-1 to 114Uml-1. This result is correlated with lipase enzyme activity ranges between 6 to 9 isolated from *bacillus sp*. using kitchen grease as source of lipase containing microorganism Jaiganesh and Jaganathan, 2018).

3.3 Effect of temperature on media

Studies conducted for the optimization of temperature shows that the bacteria produces lipase in wide range of temperature from 20 °C to 50°C.The lipase enzyme produced at different range of temperature was from 34.2 U

ml-1 to 108.0U ml-1. The optimum temperature for lipase enzyme production was at 37°C (108.0U ml-1) and the enzyme production was affected and decreased after increase of temperature above 37°C to 50°C. It was also noted that the lipase enzyme production was ceased at temperature 50°C. . Similar result was reported that the maximum lipase production was at 37°C by *bacillus sp.* (Jaiganesh and Jaganathan, 2018).

3.4 Optimization of incubation time

The incubation time for enzyme production is governed by the characteristics of the culture and is based on growth rate. In the present study the production of lipase starts only after 24 hours of incubation .maximum lipase produced in 48 hours of incubation.

4. Conclusion

The present research work was aimed to isolate, identify and characterize lipolytic bacteria from oil spilled garage soil sample. From result it was observed that among all other sources garage soil sample found to be best source for lipase producing microorganisms. On the basis of highest zone of clearance around them, colonies were taken from plates of garage soil samples. The result showed that the pH (7), temperature (37^0c) and incubation time (48hr) found to best for maximum enzyme activity. It was also concluded that as temperature increases beyond the 37 ^{0}c the enzyme activity decreases. Their importance is increasing by the day in several industries, such as food, detergents, chemicals, pharmaceuticals, *etc*. On account of above facts, it could be concluded that the lipase production can be achieved by the isolated bacterial species from the natural sources. These isolates can be used for further studies and the gene which is responsible for lipase production can be identified, isolated and cloned in expression vector and can get increased production of lipase.

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Isolation, screening, characterization and production of pectinase containing microorganism from rotten fruits

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Abstract

Pectinase are one of the most widely disseminated enzymes of the bacteria, fungi and plants. Agro industrial residues are primarily composed of complex polysaccharides that strengthen microbial growth for the production of industrially important enzymes. In present study Czapek media was used for isolation of pectinase containing microorganism from rotten fruits. The isolated microorganism was confirmed as Aspergillus Niger using slide culture technique and microscopic examination using Lactophenol blue stain. The characterization of Pectinase activity was carried out using 1% pectin as substrate with varying pH (2-10) and temperature (20-70^{0}c). The enzyme activity was found to be the maximum on pH (5) and temperature 50 ^{0}c at 1% pectin concentration. The study also reveals that enzyme activity decreases beyond 50^{0}c. The maximum production of pectinase in the presence of 1% substrate concentration makes the enzyme useful in industrial sectors and clarification of fruit juice industry.

***Keywords**: Aspergillus Niger, pectinase, Czapek media*

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1. Introduction

Enzymes are bio-active compounds or catalysts that regulate many chemical reactions in living tissues and cells (Prathyusha and Suneetha, 2011). It also catalyses various reactions involved in the preparation of different food products. Enzymes are one of the important tools in modern food industry because they simplify many intermediate processes during food processing. Pectinases are one of the important and imminent enzymes of the commercial sector, especially, in the fruit juice industry as a pre-requisite for obtaining well clarified and stable juice with higher yields (Sandri et al., 2011). Pectinases, commonly referred to as pectic enzymes , are important class of enzyes for their uses in industries like wine ,paper, and food for processing of fruits, vegetables ,tea, coffee (Bhardwaj and Udupa , 2019). These enzymes have useful applications in paper, fruit and textile industries. Almost 75% of the estimated sale value among industrial enzymes in 1995 has been contributed by pectinase. As a result, pectinase is considered as one of the futuristically useful enzymes in commercial sector (Kashyap et al., 2001). The two major sources of the enzyme pectinase are plants and microorganisms. In nature, microorganisms have been endowed with vast potentials which can be exploited for the utilization of waste material. The main source of the microorganisms that produce pectinolytic enzymes are yeast, bacteria and large varieties of fungi, insects, nematodes and protozoas (Jayani et al., 2010). Almost all the commercial preparations of pectinase are produced from fungal sources. Aspergillus *niger* is the most commonly used fungal species for industrial production of pectinolytic enzymes (Oumer and Abate, 2018). In

recent years, there has been a great increase in industrial applications of enzymes owing to their significant biotechnological potential. The present investigation therefore, aims to produce industrially valuable pectinase from *Aspergillus Niger* isolated from rotten fruits.

2. Materials and methods

2.1 Collection of fruits

The fruits were collected from Jalgaon fruit market for isolation of pectinase containing microorganism. The fruits were brought to the biotechnology laboratory in sterile polythene bags and rinsed with sterile distilled water for isolation of pectinase containing microorganism.

2.2. Isolation of pectinase containing microorganism from fruits

For isolation of pectinase containing microorganism fruits were ground and made into slurry using sterile distilled water using sterile pestle and mortar. An aliquot of this sample were serially diluted with dilution blanks and plated on Czapek agar medium for growth of fungal microorganism .The Czapek media supplemented as carbon source with citrus peel used for the development of *Aspergillus niger*.

2.3. Morphological identification of fungal isolate

The fungal isolate were identified based on their morphology, mycelia structure and spore formation. The identified fungal strains were stained by lacto phenol cotton blue stain.

2.4. Estimation of total sugar

In order to measure reducing total sugar 3, 5 dinitrosalicylic acid (DNS) colometric method was followed to measure the reducing sugar of sample with glucose as standard (Miller, 1959) .The crude enzyme was centrifuged at 10,000 rpm for 15 min at 4^0C. The mixture of 1 ml DNS and 1 ml of water was boiled for 5 min and colour intensity was measured spectrophotometrically at 550 nm.

2.5. Estimation of protein

The determination of protein content was carried using Lowry *et al.*, (1951), method and bovine serum albumin was used as standard.

2.6. Determination of pectinase activity

Enzyme activity was measured by dinitrosalicylic acid reagent (DNS) method .The crude enzyme obtained from rotten fruits was centrifuged at 10,000 rpm for 15 min at 4^0C. The reaction mixture containing 1 ml sodium citrate buffer (pH 5), 0.1 ml crude enzyme extract and 0.1 ml of purified pectin (1%) was incubated for 25 min at 50 ^{0}C then after 3 ml DNS reagent in each test tube was added. The tubes were now shaken for 10 min and cooled down at room temperature. At last the enzyme activity was measured spectrometrically by taking absorbance at 570 nm and compared with standard pectinase.

2.6.1. Effect of pH on enzyme activity

The buffers with different pH like (pH 2 to 3) using glycine –HCl buffer,(pH 3 , 4 to 6) using citrate buffer , (pH 5) using sodium acetate buffer , (pH7) using of phosphate buffer ,(pH 8 to 9) using tris-HCl buffer and (pH 10) glycine NaOH buffer were used to study the effect of pH on pectinase activity.

2.6.2. Effect of temperature on enzyme activity

The effect of temperature on enzyme was studied by using 1% 0.1ml pectin. The 0.1 crude enzymes were incubated at the desired temperature, in the range of 20^0C– 70^0c, for 15 min in sodium citrate buffer (pH 6) and DNSA was carried out.

3. Results & Discussion

3.1. Morphological characterization of fungal isolate

The native mycoflora isolated in large numbers from rotten fruits samples were identified as *Aspergillus Niger* based on the basis of black color of fungus produced by slide culture method shown in fig 3.1.1 as primary screening and from microscopic observation (fig 3.1.2) of fungal hyphae it was observed that isolated fungus was may be an Aspergillus *niger*.

Fig 3.1.1 Slide culture technique

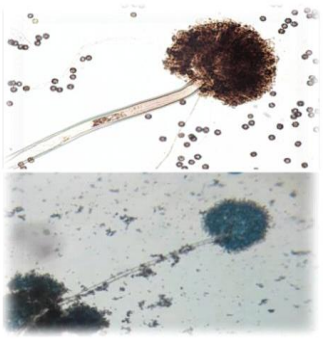

Fig 3.1.2 Microscopic examination of fungus

3.2. Characterization pectinase activity

3.2.1 Effect of pH on enzyme activity

The obtained results demonstrated that among different pH (2-10) used the activity of pectinase enzyme produced from *A. niger* was foun to be maximum at pH (5) and from result it was also observed that the specific activity of enzyme obtained from isolate was similar to standard pectinase. The optimum pH of our findings is comparable to the pectinase of *Aspergillus niger* (Zhang et al., 2009). Freitas et al., *(*2006) reported that pH (5.5) showed optimum pectinase activity from thermo-tolerant *Aspergillus sp*. The pH was compared with standard pectinase ranges from 2 to 6 respectively.

3.2.2 Effect of temperature on enzyme activity

To find out the thermo stability of pectinase, enzyme was incubated at various temperatures ranging from 20 to 70 °C. The results revealed that 50 °C was optimum temperature of purified pectinase whereas, enzyme activity suppressed at temperature higher than 50 °C. Similar results were reported by Esquivel and Voget, 2004 for polygalacturonase from *Aspergillus species.*

4. Conclusion

The present research work was aimed to isolate, screen, characterize pectinase containing microorganism from rotten fruits. On the basis of microscopic examination it was found that the isolate may be of Aspergillus Niger. This reveals that Aspergillus niger can produce pectinase enzyme at different pH and temperature range. The pH 5 and temperature 50^0c found to be best for maximum pectinase enzyme activity. The uses of the pectinase enzyme in commercial application can be seen everyday life and can be seen in the production lines including fruit juices and wine brewing. To conclude, it must be noted that our study and such studies involving culture optimization and enzyme standardization can help to find enzymes that have a more useful temperature

withstanding abilities to the one in hand, either for this application or for other use.

5. Acknowledgement

The present work was carried out at Department of Biotechnology, KCEPG College Jalgaon. Authors are very much thankful to Krishna Pectins Pvt. Ltd. Jalgaon for providing material for the present study.

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Statistics

A Statistical Analysis of Impact of COVID–19 on Human Beings

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Abstract

The outbreak of COVID-19 affected the lives of all sections of society as people were asked to self-quarantine in their homes to prevent the spread of the virus. The lockdown seriously affected mental health, resulting in psychological problems including frustration, stress, and depression. The explore to explore the impacts of this pandemic on the lives of human beings and students, we surveyed duals of different age groups from various Jalgaon cities. Moreover, our research found that participants adopted different coping mechanisms to deal with stress and anxiety and r near ones to deal with stress and anxiety.

Keywords: *COVID-19, Chi-Square, Factor Analysis, Minitab 20, IBM SPSS 25*

Introduction

This practical training is very much important as theoretical knowledge supported by practical experiences makes better understanding; it gives pleasure to submit this Research report. The purpose of the research paper was to understand the statistical techniques used or identify the problems and for forecasting for next year. It gives pleasure to us to submit this project report on "A Statistical Analysis of Impact of COVID-19 on Human Beings".

Objective

1. The objective of the project is to study the effects of the changes that occurred on human beings in daily activities like
    - Exercise
    - learning platform
    - official work
    - difficulties after vaccination
    - academic difficulties, etc

    of the various section of society during the COVID-19 pandemic period.
2. The main focus/ highlights of our project are to study the problems faced by the students after the introduction of the new digital learning platform.

What is Coronavirus (COVID–19) and How does it spread?

The Coronavirus (COVID-19) is a new respiratory illness first identified during an investigation into an outbreak in Wuhan, China, but has now spread to other parts of the world, including India. The virus that causes COVID-19 is called SARS-CoV-2 and is spread between people who are in close contact with each other (within about six feet), mostly through respiratory droplets produced when an infected person coughs or sneezes. People are thought to be most contagious when they are the sickest (symptomatic); however, growing evidence suggests that COVID-19 may be spread by people before they start showing symptoms (pre-symptomatic) or by people who have the virus but never show symptoms (asymptomatic). The virus can also live on certain surfaces for a period which can make it possible for people to get COVID-19 if they touch a contaminated object and then touch their mouth, nose, or eyes. That is why it is important to take preventive

steps to avoid getting sick.

Impact of COVID–19 on human beings:

COVID-19 (Coronavirus) has affected day-to-day life and is slowing down the global economy. This pandemic has affected thousands of people, who are either sick or are being killed due to the spread of this disease. The most common symptoms of this viral infection are fever, cold, cough, bone pain, and breathing problems, ultimately leading to pneumonia. This, being a new viral disease affecting humans for the first time, vaccines are not yet available. Thus, the emphasis is on taking extensive precautions such as extensive hygiene protocol (e.g., regularly washing hands, avoidance of face-to-face interaction, etc.,), and social distancing. This virus is spreading exponentially region-wise. Countries are banning gatherings of people to spread and break the exponential curve.

COVID-19 has rapidly affected our day-to-day life, and businesses, and disrupted world trade and movements. Identification of the disease at an early stage is vital to control the spread of the virus because it very rapidly spreads from person to person. This virus creates significant knock-on effects on the daily life of citizens, as well as on the global economy. Presently the impacts of COVID-19 in daily life are extensive and have far-reaching consequences. These can be divided into various categories:

Survey Details:

For the research paper "**A Statistical Analysis of Impact of Covid-19 on Human Beings,"** we collect the data on the COVID-19 pandemic situation of the people and students by Google Form. We take a sample of respondents. We have decided to take the samples using simple random sampling without replacement. Then we take 517 samples from the overall respondents and get start the analysis.

Data Representation:

Table No. 01: Basic Distribution of responses by 517 (Students/Persons) Respondents according to their personal attributes.

Sr. No.	Questions	Option	Frequency	Percentage
1	Gender	Male	310	59.97%
		Female	207	40.03%
2	Age	0-15	18	3.49%
		15-30	314	60.73%
		30-45	161	31.14%
		45 & above	24	4.64%
3	Locality	Rural	340	65.77%
		Urban	177	34.23%
4	Education Qualification	10^{th}	31	5.99%
		12^{th}	111	21.47%
		Graduation	160	30.95%
		Post-Graduation	198	38.30%
		Other	17	3.29%
5	Occupation	Student	247	47.78%
		Government Employee	109	21.083%

		Private Sector Employee	103	19.92%
		Farmer	55	10.64%
		Other	3	0.580%

From **Table No. 01**, we observe that there are a total of 310 Male and 207 Female responses. i.e., the proportion of male and female responses is nearly (3:2) and there are a total of 314 responses having aged 15 years to 30 years, a total of 161 responses having aged 30 to 45 years, and so on.

Table No. 02: Distribution of responses to the pandemic in COVID – 19 by 517 Respondents according to their personal attributes.

Sr. No	Question	Option	Male	Female	Total	% Male	% Female
1	COVID-19 has affected families financially or economically	Yes	255	158	413	61.75%	38.25%
		No	55	49	104	52.88%	47.12%
2	Any changes in your behaviour	Yes	231	79	310	74.52%	25.48%
		No	156	51	207	75.36%	24.64%
3	Doing exercise regularly	Yes	241	69	310	77.74%	22.26%
		No	152	55	207	73.43%	26.57%
4	Are you Vaccinated	Yes	268	42	310	86.45%	13.55%
		No	174	33	207	84.06%	15.94%
5	How many Doses taken	One	64	36	100	64%	36%
		Two	204	138	342	59.65%	40.35%
6	Learning/working do you like the	Online	205	104	309	66.34%	33.66%
		Offline	105	103	208	50.48%	49.52%
7	liked attending offline classwork	Yes	255	182	437	58.35%	41.65%
		No	55	25	80	68.75%	31.25%
8	Vaccine are you taken	Covid Shield	116	112	228	50.88%	49.12%
		Co-Vaccine	84	52	136	61.76%	38.24%
		Moderna	13	6	19	68.42%	31.58%
		Sputnik V	5	4	9	55.56%	44.44%
9	Academic Difficulties	Completing Work	33	25	58	56.90%	43.10%
		Online Learning	91	56	147	61.90%	38.10%
		Concentration	100	73	173	57.80%	42.20%
		Reduced Motivation	54	29	83	65.06%	34.94%
		No Difficulties	32	24	56	57.14%	42.86%

From Table No. 02, the Percentage of Male and Female respondents is nearly equal for each question

STATISTICAL ANALYSIS OF COVID-19

Test 1 : Chi-Square for Association:

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

The null hypothesis H_0 is given by,

H_0: There is no association between the two variables.

Test Statistics are given by,

$$\chi^2 = \sum_{i=1}^{m}\sum_{i=1}^{n}\frac{(O_{ij} - E_{ij})^2}{E_{ij}}$$

Where;

m is the number of levels for one categorical variable and

n is the number of levels for another categorical variable.

So, the chi-square test for the variables in **Table 1** is as follows.

Aim: To test the association between any two pairs of categorical variables in **Table 2**

Table No. 03: Chi-Square test with Conclusions

Sr. No.	Null hypothesis	χ^2	DF	p-value	Decision	Conclusion
1	H_{01}: Pandemic of COVID-19 doing exercise regularly is Independent of Gender.	1.266	1	0.261	p-value>α i.e., accept H_{01}	In a pandemic of COVID-19 doing exercise regularly is not associated with Gender. i.e., Independent of their Gender.
2	H_{02}: Pandemic of COVID-19 doing exercise regularly is Independent of Age groups.	21.431	3	0.0001	p-value< α i.e., reject H_{02}	In a pandemic of COVID-19 doing exercise regularly is associated with Age groups. i.e., Depends on their Age groups.
3	H_{03}: Pandemic of COVID-19 doing exercise regularly is Independent of Locality.	0.283	1	0.109	p-value> α i.e., accept H_{03}	In a pandemic of COVID-19 doing exercise regularly is not associated with Locality. i.e., Independent of their Locality.
4	H_{04}: Pandemic of COVID-19 doing exercise regularly is Independent of Edu. Qualifications.	13.412	4	0.009	p-value< α i.e., reject H_{04}	In a pandemic of COVID-19 doing exercise regularly is associated with Edu. Qualifications. i.e., Depends on their Edu. Qualifications.

5	H_{05}: Pandemic of COVID-19 doing exercise regularly is Independent of Occupation.	24.699	4	0.0001	p-value< α i.e., reject H_{05}	In a pandemic of COVID-19 doing exercise regularly is associated with Occupation. i.e., Depends on their Occupation.

Test 02: Exploratory Factor Analysis

For factor analysis, we take some questions from our questionnaire which has 4 or more answers they are

14. What bothered you after taking the vaccination dose?

	Never	Almost Never	Some Times	Fairly Often	Very Often
Headache					
Fever					
Body Pain					
Long Time Hand Pain					
Cold					

17. Which physical problems, did you face in online education or working? (Fill Rank wise)

	1st	2nd	3rd	4th
Eyesight problem				
Lack of physical activity				
Neck problem				
Headache				

18. Which Mental Problems, did you face in online classes and education or work

	1st	2nd	3rd	4th
Stress & Anxiety				
Lack of Confidence				
Lack of interest				
Depression				

The collected data of 517 respondents correspond the 20 questions. Responses corresponding to the questions that are useful for extracting Bothered after taking Vaccination Dose are collected on 5 points. Scale as follows.

1 = Never, 2 = Almost Never, 3 = Some Times, 4 = Fairly Often, 5 = very Often.

As well as Physical Problems and Mental Problems face in online education or online working (Q.17 & Q.18) are collected on 4 points in COVID-19. The scale is as Follows (Rank Wise)

1 = 1st rank, 2 = 2st rank, 3 = 3st rank, 4 = 4st rank.

Hence, for our data m = 13, p = 3, n = 517 and X = (X_1, X_2, X_3, …, X_{13}).

L = Factor Loading Matrix of order 13 × 3

F = Vector of extracted factors of order 3 × 1

Hence the model for the above data becomes,

$$(\boldsymbol{X} - \boldsymbol{\mu})_{\boldsymbol{13\times 1}} = \boldsymbol{L}_{\boldsymbol{13\times 3}} * \boldsymbol{F}_{\boldsymbol{3\times 1}} + \in_{\boldsymbol{13\times 1}}$$

I. The adequacy of data in Table No.6.1 for the factor analysis had checked using KMO Test in the KMO test following hypothesis is tested,

H_0: The data is adequate for structure detection.

i.e., Factor Analysis is suitable for the data.

KMO test statistics = 0.809

It leads to rejecting H_0 i.e., Data is Suitable to Factor Analysis.

II. The independency of variables under study is tested using Bartlett's Test of Sphericity.

H_0: The population correlation matrix of variables under study is an identity matrix.

Bartlett's Test Statistics, $\boldsymbol{\chi^2_{78}}$ **= 1788.572** with d.f. 78 and p-value = 0.0001

Conclusion:

Bartlett's Test of Sphericity is significant (0.0001). i.e., a p-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. i.e., The variables under consideration are correlated. So, we can perform the factor analysis using these variables.

The following is the output of Factor Analysis performed on the 517 responses using SPSS software of version 25.

- **Communalities:**

  Following is a table of communalities which shows how much of the Variance (i.e., the communality value which should be more than 0.5 to consider for further Analysis. Else these Variables are to be removed from further steps of factor analysis) on the variable has been accounted for by the extracted factors.

Table 05 : **Communities**

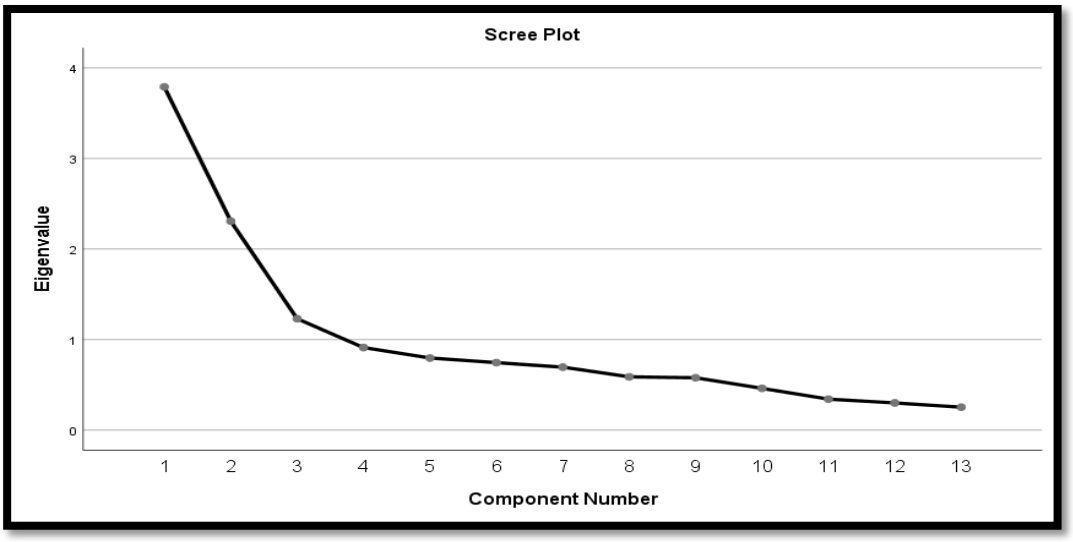
	Q14 .1	**Q14 .2**	**Q14 .3**	**Q14 .4**	**Q14 .5**	**Q17 .1**	**Q17 .2**	**Q17 .3**	**Q17 .1**	**Q18 .1**	**Q18 .2**	**Q18 .3**	**Q18 .4**
Initial	1	1	1	1	1	1	1	1	1	1	1	1	1
Extraction	0.70	0.77	0.75	0.74	0.62	0.71	0.40	0.52	0.54	0.43	0.48	0.36	0.33
Extraction Method: Principal Component Analysis.													

From Table 05, For instance over 77% of the variance in "Q14 (2)" is accounted for, while 33% of the variance in "Q18 (4)" is accounted for.

- **Scree Plot:**

  The Screes plot is a graph of the Eigenvalues against all the factors. The graph is useful for determining how many factors to retain. The point of interest is where the curve starts to flatten

Figure 01: Scree Plot



Conclusion:

In Figure 01 It can be seen that the curve beings to flatten between factors 3 and 4. After the 4 factors, the Eigenvalues are not so significant.

- **Component Matrix:**

  The idea of rotation is to reduce the number of factors on which variables under investigation have high loadings. Rotation does not change anything but makes the interpretation of the analysis easier. Looking at Table 6.5 all the variables are substantially loaded on Factors. These Factors can be used as variables for further analysis.

Table 06: Component Transformation Matrix

Component	1	2	3
1	0.836	0.414	0.361
2	-0.548	0.667	0.505
3	-0.032	-0.62	0.784
Extraction Method: Principal Component Analysis.			
Rotation Method: Varimax with Kaiser Normalization.			

Table 07: Component Score Coefficient Matrix

	Component		
	1	2	3
Q14.1	0.23	-0.192	0.185
Q14.2	0.261	-0.089	0.06
Q14.3	0.26	-0.009	-0.014
Q14.4	0.258	0.092	-0.167
Q14.5	0.231	0.097	-0.147

Q17.1	-0.028	-0.196	0.542
Q17.2	-0.005	0.127	0.198
Q17.3	-0.022	0.382	-0.126
Q17.1	-0.016	0.401	-0.162
Q18.1	-0.04	-0.002	0.342
Q18.2	-0.049	0.138	0.241
Q18.3	-0.032	0.259	0.022
Q18.4	-0.013	0.201	0.081
Extraction Method: Principal Component Analysis.			
Rotation Method: Varimax with Kaiser Normalization Component Scores			

- **Component Plot in Rotated Space:**

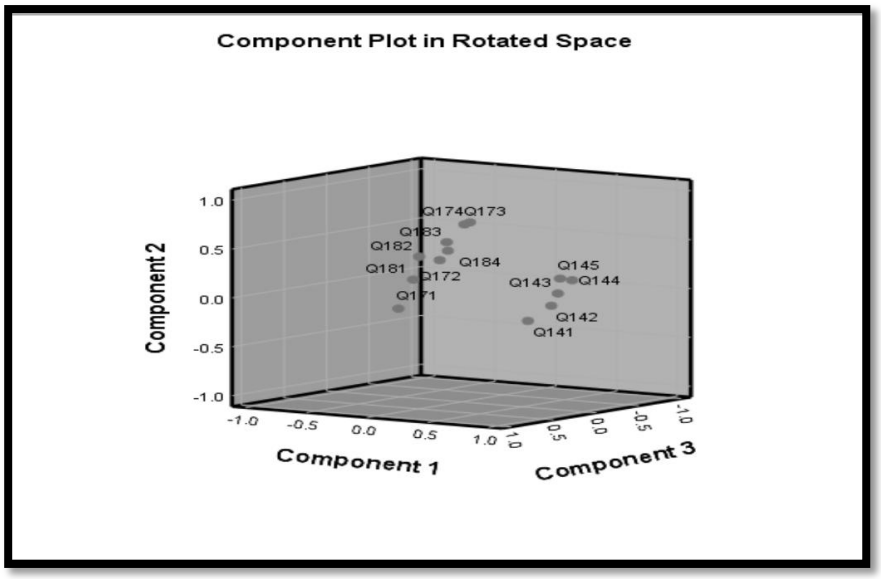


Figure 02: Component Plot in Rotated Space

Overall Conclusion:-

i) In COVID -19 there are 61.75% of males and 38.25% of females were affected by family financially and economically.

ii) There are 74.52% of males and 25.48% of females have changed their behaviours.

iii) In our study we conclude that most people have taken Covid Shield Vaccine.

iv) In our project we can conclude that; In the pandemic of COVID-19 doing exercise regularly depends on the age group of respondents.

v) In our project shows that respondents with both online learning and concentration difficulties have seen mostly in rural areas.

Limitations while filling questionnaire:

1. Most of the student are not fill out forms well even if we explain them.

2. Responses of questionnaire are filled up by Students and other people
3. All responses are filled up by educated persons.

References:-

1. https://www.unidosus.org/issues/health/covid19/?gclid=EAIaIQobChMIs8eNps7m9gIV1auWCh3uXwlEEAAYAiAAEgIdYvD_BwE
2. Effects of COVID-19 pandemic in daily life, Abid Haleem1, Mohd Javaid, New Delhi, India
3. Google Form Link : https://forms.gle/eowRuRuGbHix7hSV8

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A Statistical Analysis of Agricultural Production

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Abstract

Agriculture is an important sector in India. On an average, about 70% of the households and 10% of the urban population is dependent on agriculture as their source of livelihood. Today, India is a major suppliers of several agricultural commodities like tea, coffee, rice, spices, oil meals, fresh fruits, fresh vegetables and marine products to the international market. The finding of present analysis revealed that there has been a clear transition from food grain consumption to non-food grain consumption. The objective of the project is to examine the production and consumption pattern among Indian people.

Keywords: *Agriculture, Crops, India, Experiments etc.*

Introduction

We got interested in knowing how to handle real life data sets by using statistical analysis. India is the 2nd largest agricultural producer and 7th largest exporter of agricultural goods. Agriculture plays a vital role in the Indian Economy. Agriculture shown the practical implementation of statistical theories and techniques related to designing and recording of observations at the field. Here we have taken our best efforts to implement statistical planning, designing and analysis for the actual work performed at the fields. On completion of this project work will be confident for analyzing data sets. This project will be also helpful for those who are handling, planning, designing and analysis of statistical experiments in the agriculture, private sector / Industry.

Objectives:

i. To study the production of major crops, exports and imports of agricultural crops in India.
ii. To see how the various types of statistical designs are used in Agricultural fields.
iii. To study various parameters related to agricultural experiments.
iv. To study which type of production is mostly preferred in our India.

- **About Indian Agriculture system:**

Agriculture is an important sector of Indian Economy as it contributes about 17% to the total GDP and provides employment to over 60% of the population. Study of soil and climate, plant, nutrients, manures and fertilizers, field crops, horticulture crops, different diseases of crops plants, pests of crops plants, weeds and weed control, plant propagation and nursery, advance techniques in agriculture, green house and poly house. The agricultural crop year in India is from July to June. The Indian cropping season is classified into two main seasons-

1)Kharif and 2) Rabi based on the monsoon. The Kharif cropping season Is from July-October during the south-west monsoon and Rabi cropping season is from October-March (Winter).

Data Collection:

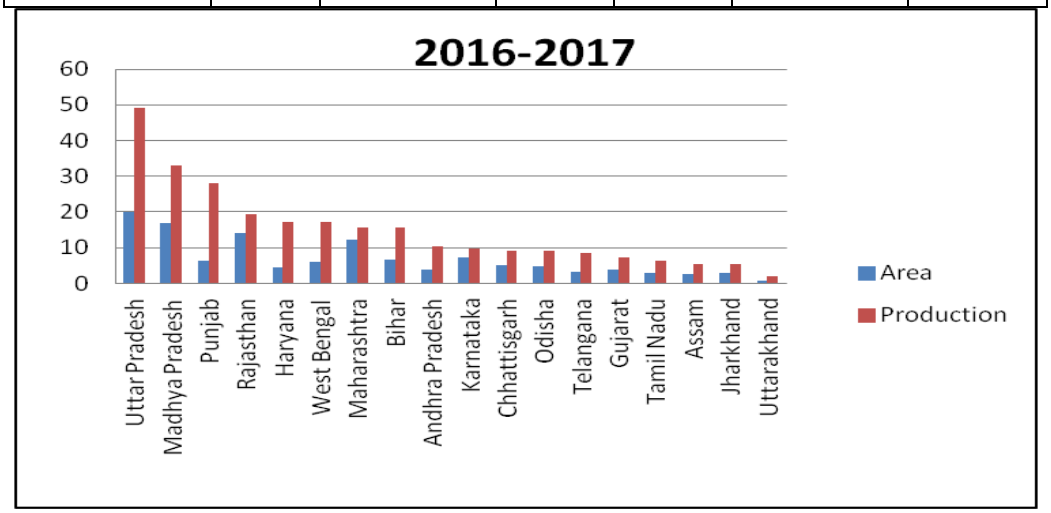
India has a unique culture and is one of the oldest and greatest civilization in the world. Thecountry can be divided into six zones mainly north, south, east, west, and central and northeast zone. It has state and eight union territories.

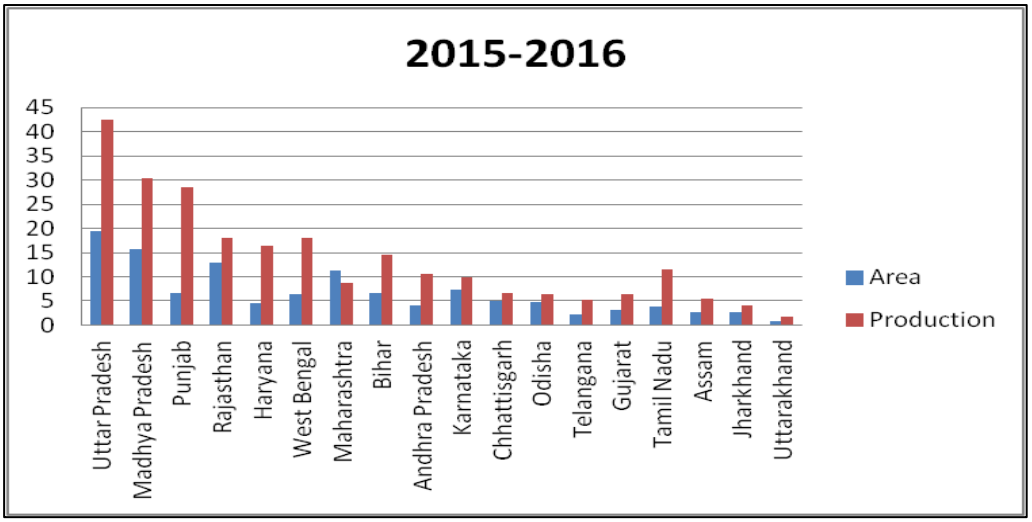
India is geographically a vast country so it has various food and non-food crops which Rabi, Kharif and Zaid. Food crops – Rice, Wheat, Milts, Maize and Pulses. Cash crops sugarcane, Oilseeds. Horticulture crops Tea, Coffee, Rubber, Cotton and Jute.

We have secondary data of agricultural production and also collected some data fromdifferent sites. “The book of ministry of agriculture and farmers welfare” this book was so helpful in our work.

State-wise Area, Production and Yield:

State	2016-17			2015-16		
	Area	Production	Yield	Area	Production	Yield
Uttar Pradesh	19.92	49.14	2467	19.36	42.55	2198
Madhya Pradesh	17.03	32.98	1937	15.66	30.39	1941
Punjab	6.42	27.99	4360	6.65	28.4	4269
Rajasthan	14.11	19.28	1367	12.98	18.04	1390
Haryana	4.59	17.16	3735	4.48	16.36	3648
West Bengal	5.98	17.06	2853	6.38	18.01	2823
Maharashtra	12.16	15.79	1298	11.21	8.75	781
Bihar	6.61	15.58	2355	6.57	14.51	2208
Andhra Pradesh	3.97	10.37	2610	4.14	10.63	2571
Karnataka	7.29	9.64	1323	7.33	9.92	1354
Chhattisgarh	5.05	9.23	1827	4.99	6.65	1334
Odisha	4.8	9.06	1887	4.82	6.41	1330
Telangana	3.29	8.37	2545	2.18	5.13	2353
Gujarat	3.8	7.42	1953	3.14	6.28	2000
Tamil Nadu	2.99	6.22	2084	3.75	11.48	3063
Assam	2.67	5.47	2049	2.68	5.36	1997
Jharkhand	2.89	5.37	1860	2.65	4.09	1546
Uttarakhand	0.88	1.87	2131	0.88	1.75	1989

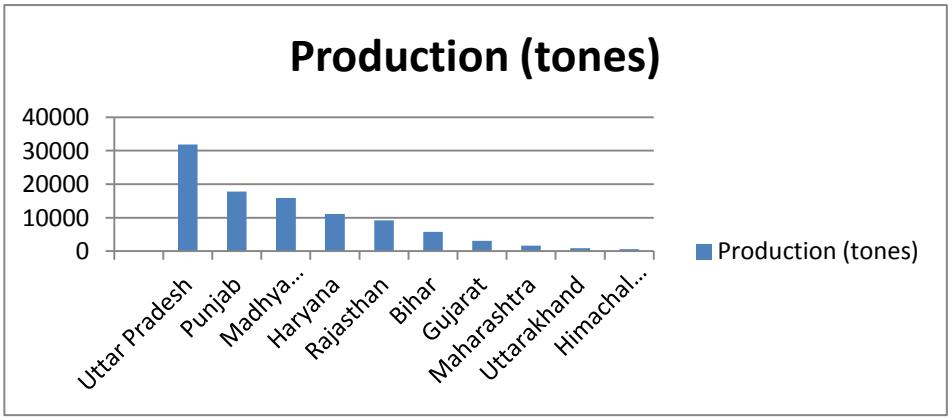




Interpretation From above graph, we observed that Uttar Pradesh and Madhya Pradesh has maximum production in both the years. From all over the observation it is clear that this two states have maximum production in our India.

State-wise production of major crop wheat:

Sr. no.	**State**	**Production (tones)**
1.	Uttar Pradesh	31,880.00
2.	Punjab	17,850.00
3.	Madhya Pradesh	15,910.00
4.	Haryana	11,160.00
5.	Rajasthan	9,190.00
6.	Bihar	5,740.00
7.	Gujarat	3,100.00
8.	Maharashtra	1,620.00
9.	Uttarakhand	910.00
10.	Himachal Pradesh	590.00

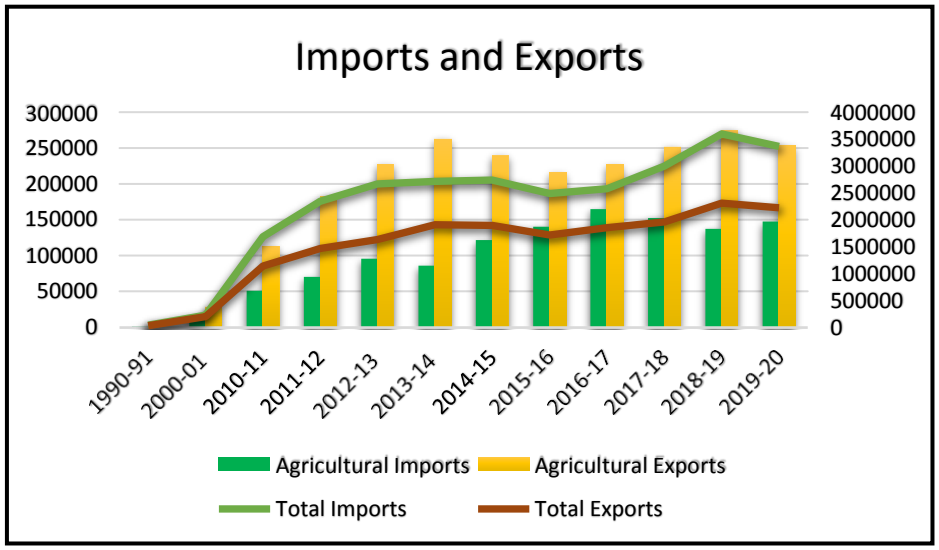


Interpretation : We concludethat Uttar Pradesh is highly producing state of Wheat and Punjab is second highest producing state of Wheat in India.

Agricultural Imports and Exports:

Year	Agricultural Imports	Agricultural Exports	Total Imports	Total Exports
1990-91	1205.86	6012.76	43170.82	32527.28
2000-01	12086.23	28657.37	228306.64	201356.45
2010-11	51073.97	113046.58	1683466.96	1136964.22
2011-12	70164.51	182801	2345463.24	1465959.31
2012-13	95718.89	227192.61	2669161.96	1634318.29
2013-14	85727.3	262778.54	2715433.91	1905011
2014-15	121319.02	239681.04	2737086.58	1896445.47
2015-16	140289.22	215396.55	2490298.08	1716378.05
2016-17	164726.83	226651.94	2577665.59	1849428.76
2017-18	152095.2	251563.94	3001028.71	1956514.52
2018-19	137019.46	274571.28	3594674.22	2307726.19
2019-20	147445.81	252976.06	3360954.45	2219854.17

Interpretation: From above graph, we can see that Agricultural Exports are greater than Agricultural



Imports but both are not increase or decrease constantly it means that there isvariation in every year. Total Imports are greater than Total Exports. Recently from total imports 4% of share is of agricultural imports and from total exports 11% share is of agricultural exports.

Testing of Hypothesis:

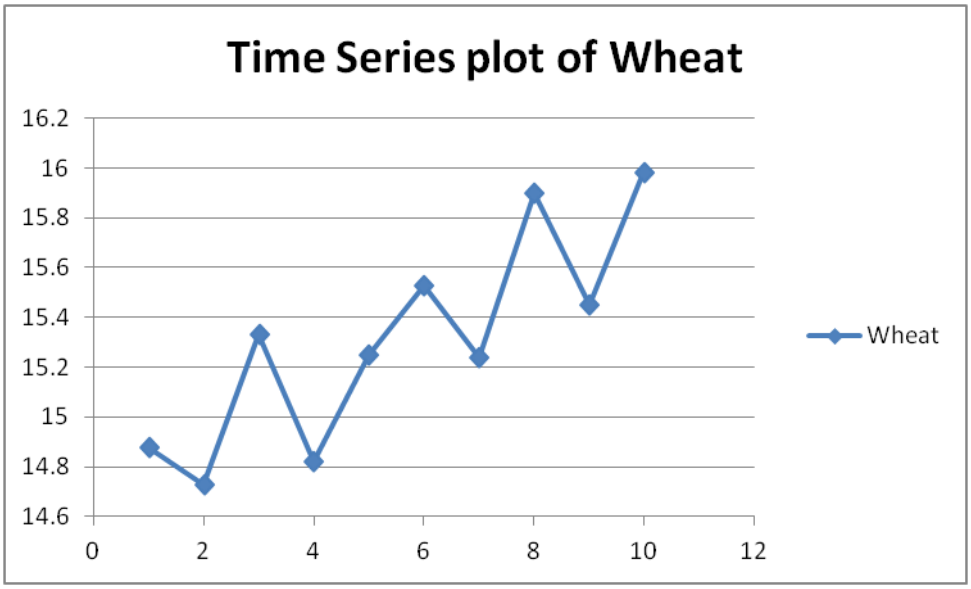
- Paired-t test for comparing food grain and horticulture production

H_0: There is significant difference between food grain and horticulture production. H_1: There is no significant difference between food grain and horticulture production.P-value: 0.887 then accept H_0.

- Forecasting: Area under major crop wheat

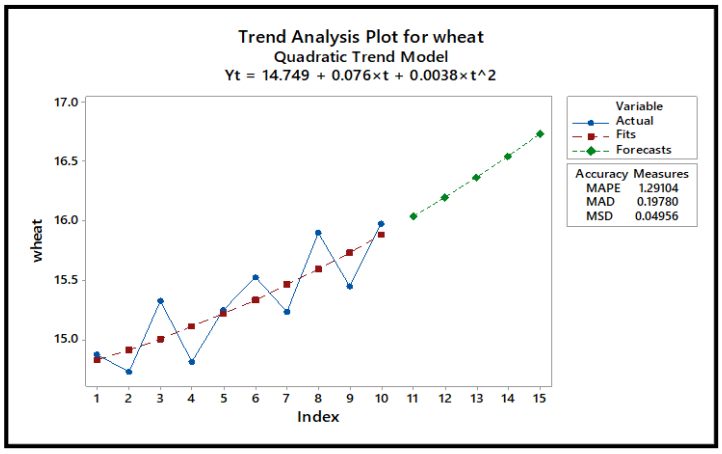
Year	Wheat
2007-08	14.88
2008-09	14.73
2009-10	15.33

2010-11	14.82
2011-12	15.25
2012-13	15.53
2013-14	15.24
2014-15	15.9
2015-16	15.45
2016-17	15.98



In this model, represents the average change from one period to the next. The ***quadratic trendmodel*** which can account for simple curvature in **t**he data. Fitted trend equation:

$$Y_t = 14.749 + 0.076X_t + 0.0038X_{t^2}$$



Forecast value:

Period	Forecast
18	16.03
19	16.20
20	16.36
21	16.54
22	16.73

MAPE	1.29104
MAD	0.19780
MSD	0.04956

Measures of accuracy:

Three measures of accuracy of the fitted model: MAPE, MAD and MSD for each of the simple forecasting and smoothing methods. For all three measures, the smaller the value, thebetter the fit of the model. Use these statistics to compare the fit of the different methods.

- One-way ANOVA: Nitorgen, Phosphorous, PotassiumNull hypothesis: All means are equal
  Alternative hypothesis: At least one mean is differentSignificance level:
  $\alpha = 0.05$

Factor	N	Mean	St-Dev	95% CI
Nitorgen	7	169.26	3.04	(164.44, 174.08)
Phosphorous	7	68.62	8.85	(63.79, 73.44)
Potassium	7	25.28	4.82	(20.45, 30.10)

P-value: 0.001 then reject H_0

- Regression Analysis: Yield(Y) versus temperature(X_1), rainfall(X_2), PH(X_3), and nitrogen(X_4)

S	R-sq	R-sq(adj)	R-sq(pred)
1.79679	56.26%	54.74%	53.40%

$$Yield\_(Y) = 61.6 - 1.828\ Temp\_(X_1) + 0.0468\ Rainfall\_(X_2) + 0.822\ pH\_(X_3) - 0.01648\ Nitrogen\_(X_4)$$

➢ **Overall conclusion:**

- We conclude that, the foodgrain and horticulture production have difference. It means that foodgrain production is mostly preferred in India and also foodgrain production is exported in other countries.
- Our major crops are wheat, cotton and rice. Mostly these crop production is profitable for us. (Our soil is familiar with these crops so it gave better profit than others).

Area under major crops are also different in different year.

- Chemical nutrients are helpful in production but there quantities are also different asper requirement.
- We observe that, many factors affecting yield like rainfall, PH, temperature and nitrogen. we have shown the relation between dependent and independent factors.

➢ **Reference:**

- R. RANGASWAMY "A text book of AGRICULTURE STATISTICS".
- https://www.kaggle.com/datasets?search=Agriculture+production+in+india
- The Book Ministry of Agriculture and Farmers Welfare (Directorate of Economicsand Statistics

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Analysis of Heart Disease

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Abstract

Heart is the most essential or crucial portion of our body. There are a lot of cases in the world related to heart disease. In this project statistical survey is carried out to study various factors which impact to heart disease of a person. We have taken various independent factors like age, gender, diabetes, habit of smoking, history of heart disease, etc. to study their behaviour. We have 319795 records. If we want to study one or more categorical or continuous variables such as age, habit of smoking, and others with the objective of saving time, money, man power and giving proper medical treatment, a proper medical strategy is created with the help of statistical tools which will contribute to overall medical growth and prosperity in corporate and society.

Keywords: *Chi-Square test, Proportion test, Binary Logistics Regression, Minitab17, IBM-SPSS25, MS-Excel.*

Introduction:

It gives pleasure to submit this project research paper on **"Analysis of Heart Disease".** This practical knowledge is very important for us. In this dataset we have taken various types of 13 independent factors which depend on heart disease. According to the CDC, heart disease is one of the leading causes of death for people of most races in the US (African Americans, American Indians and Alaska Natives). The dataset come from the CDC and is a major part of the Behavioural Risk Factor Surveillance System (BRFSS), which conducts annual telephone surveys to gather data on the health status of U.S. residents. BRFSS completes more than 319795 adult interviews each year, making it the largest continuously conducted health survey system in the world. By this project we will be able to know the reason behind the heart disease between human beings.

Objective:

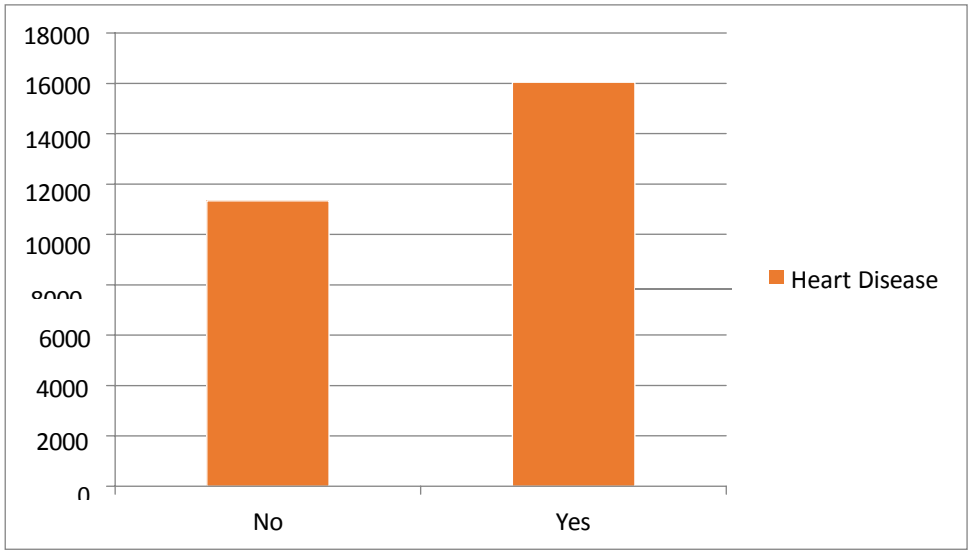
1. The objective of project is to determine risk of heart disease in day to day life based on gender, age, smoking, high blood pressure, diabetic, BMI, asthma, alcohol drinking, stroke, mental health, physical activity, physical health, difficulty in walking, age-category, sleep time, kidney disease.
2. To check whether the dependency / independency of different pair of clinical factors.
3. To study the risk factors are the most significant causes of heart disease.

- **Causes of Heart Disease:**
  1. Smoking
  2. Alcohol Drinking
  3. High Blood Pressure
  4. Kidney Disease

Data Representation:

For the project **"Analysis of Heart Disease".** We collect the data from kaggle. We take a sample of 319795 for an analysis. The main idea of this survey is to detect the risk of heart disease between human beings.

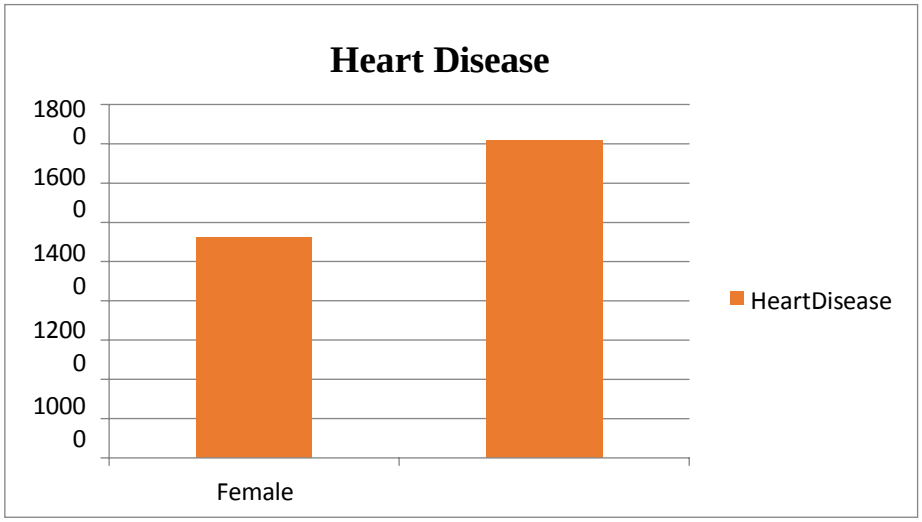
FigureNo.1: Smoking among people having Heart Disease:



Conclusion: From this graph we can conclude that smoking is bad for health.

Figure No.: 2 Heart Disease between Male and Female:

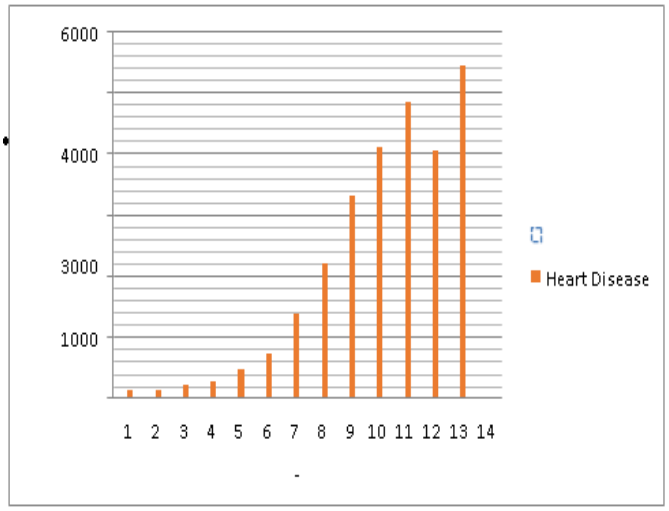
	Heart Disease	
Female	**11234**	
Male	**16139**	



Conclusion: From this graph we can conclude that male suffer more than female in heart disease.

Age-wise people having Heart Disease:

Age	Heart disease
18-24	130
25-29	133
30-34	226
35-39	296
40-44	486
45-49	744
50-54	1383
55-59	2202
60-64	3327
65-69	4101
70-74	4847
75-79	4049
80 or older	5449



Conclusion: As age increases the risk of heart disease is also increases.

- **Statistical Analysis and Interpretation:**
- **Chi-square test for association:**

Aim: To test association between heart disease and other parameters.

Chi-Square Test for Association: Heart Disease, Smoking:

H0: There is no evidence between heart disease and smoking.

H1: There is evidence between heart disease and smoking.

Rows: Heart Disease Columns: Smoking

	0	1	All
	176551	115871	292422
0	171805	120617	
1	11336	16037	27373
	16082	11291	
All	187887	131908	319795

Cell Contents: Count

Expected count

Pearson Chi-Square=3713.816**, DF**=1, **p-Value**=0.000

Likelihood Ratio Chi-Square=3645.329, **DF**=1, **p-Value**=0.000

Conclusion: By using Chi-square association test, p-value is 0.000 are < 0.05. So, we reject null hypothesis. Thus, there is evidence of an association between heart disease and smoking.

- **Binary Logistics Regression:**

- Logistic regression is an extension of simple linear regression. Where the dependent variable is dichotomous or binary in nature, we cannot use simple linear regression. Logistic regression is the statistical technique used to predict the relationship between predictors(our independent variables)and a predicted variable (the dependent variable) where the dependent variable is binary (e.g., sex [male vs. female], smoker [yes vs. no] etc……

The basic equation of Univariate binary logistic regression is

$$\pi(x) = \frac{e^{\beta 0+\beta 1x}}{1+e^{\beta 0+\beta 1x}}$$

Predicted Probability of logistic regression is

$$\frac{1}{1+e^{-(b0+b1x1+b2x2+\cdots+bpxp)}}$$

Hosmer and LemeshowTest			
Step	Chi-square	Df	Sig.
1	2.517	5	.774

H0: The fitted model is good for the data.

H1: The fitted model is not good for the data.

The p-value is 0.774(>0.05), therefore accept H_0.

Conclusion: The fitted model is good for the data.

Overall Conclusion:

1) Male suffers more from heart disease than female.
2) Smoking is bad for health.
3) As age gets bigger, the risk of heart-disease increases.

- From given results physical activity, diff walking, alcohol drinking, kidney disease, asthma, diabetics and stroke seems to have an inverse relationship with heart disease.

- By using Chi-square association test, there is evidence of an association between heart disease and alcohol drinking, smoking, stroke, diabetics, diff-walking, asthma, physical activity.

- By using SPSS software binary logistic regression model is fit for our data.

References:

- www.kaggle.com
- Wikipedia.org
- Reference book : Logistics Regression Model
- D.C.Montgomery, E. A. Peck, G. Geoffrey Vinning (2014) , "Introduction of linear Regression Analysis" (Third Edition), Reprint by Wiley India Pvt. Ltd.

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A Statistical Analysis of Stress level Among Human Beings

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Abstract

In modern life, stress is a common problem. The negative effects of stressaffect individual health and performance. As a result, individuals have their own stress perceptions and they develop different kinds of strategies in order to manage stressful situations.

Keywords: *Human Stress, Chi-Square test, Proportion test, Factor Analysis, Logistics regression, Minitab20, SPSS25, MS-Excel.*

Introduction:

This practical training is very much important as theoretical knowledge supported by practical experiences makes better understanding; it gives pleasure to submit this project report. Stress & individuals' adaptive response to situation i.e., perceived as a challenging threatening to the person well-being. Human stress analysis has received special attention inthe recent decade due to its impact on human life performance. By this project we will be able to know the reason behind the stress of human beings. it gives pleasure to us to submit this project report on "A statistical Analysis of stress level Among Human beings,"

Objective

1. The objective of the project is to study the impact of stress on human beings in day-to-daylife based on like gender, family type, area, occupation, nature of job, expenditure, marital status, qualification, income, sleeping time and habits .

2. The main purpose is to study on stress level on human beings.

Causes of Stress: As stated, earlier stress is caused by our reaction to external events and brings about changes in our response and our general behavior. It is important tolearn how to know when our stress levels are out of control Stress affects the mind, body, and behavior in many ways, and everyone experiences stress differently.

Data Representation: For the project **"Statistical Analysis of Stress Level among Human Beings".** We collect the data on the response on stress in the daily life of the people & students by google form. We take a sample of 608 respondents & conduct ananalysis. The main idea of this survey is to detect the causes that lead to stress & couldworsen the physical activity of the body.

Statistical Analysis of Stress data: Chi-Square for Association

Aim: To test the association between any two pairs of categorical variables.

Satisfactions of family income of individuals are associated with Age. i.e., depends of their Age.
Covers family monthly expenditure of individuals is not associated with Gender. i.e., independent of their Gender.
Trouble with sleep of individuals is not associated with Residential area. i.e., independent of their Residential area.
Addiction of individuals is associated with Residential area. i.e., depends of their Residential area.
Addiction after stress of individuals is associated with Occupation. i.e., depends of their Occupation.
Suffering from any disease of individuals is associated with Marital Status. i.e., depends of their Marital Status.

Exploratory Factor Analysis:

. Responses corresponding to the questions that are useful for extracting about Emotions ofrespondents are collected on 5 points. Scale as follows.

1 = Never, 2 = Almost Never, 3 = Some Times, 4 = Fairly Often, 5 = very Often for our data m = 9, p = 3, n = 608 and X = (X_1, X_2, X_3, …, X_{13}).

L = Factor Loading Matrix of order 9 × 3

F = Vector of extracted factors of order 3 × 1

Hence the model for the above data becomes,

$$\mathbf{(X - \mu)_{9\times 1}} = L_{9\times 3} * F_{3\times 1} + \in_{9\times 1}$$

I. The adequacy of data in Table No.6.1 for the factor analysis had checked using KMOTest in the KMO test following hypothesis is tested,

H_0: The data is adequate for structure detection.i.e., Factor Analysis is suitable for the data.

KMO test statistics = 0.892

It leads to rejecting H_0 i.e., Data is Suitable to Factor Analysis.

II. The independency of variables under study is tested using Bartlett's Test of Sphericity.H_0: The population correlation matrix of variables under study is an identity matrix.

Bartlett's Test Statistics, χ^2 = 2311.994 with d.f. 36 and p-value = 0.0001

Conclusion: Bartlett's Test of Sphericity is significant (0.0001). i.e., a p-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. i.e., The variables under consideration are correlated. So, we can perform the factor analysis using these variables.

Table 6.3: Communities

Communalities	FQ1	FQ2	FQ3	FQ4	FQ5	FQ6	FQ7	FQ8	FQ9
Initial	1	1	1	1	1	1	1	1	1
Extraction	0.8	0.73	0.929	0.61	0.655	0.698	0.702	0.642	0.7

Extraction Method: Principal Component Analysis.

From Table 6.2, For instance over 93% of the variance in "FQ3" is accounted for, while 61% of the variance in "FQ4" is accounted for on the basis of total variance explained one should note that the first factor accounts for 50% of the variance, the second 14%, and the third 8%. All the remaining factors are not significant.

- **Scree Plot:**

  The Screes plot is a graph of the Eigen values against all the factors. The graph is useful for determining how many factors to retain. The point of interest is where the curve starts to flatten.

Figure 6.1: Scree Plot

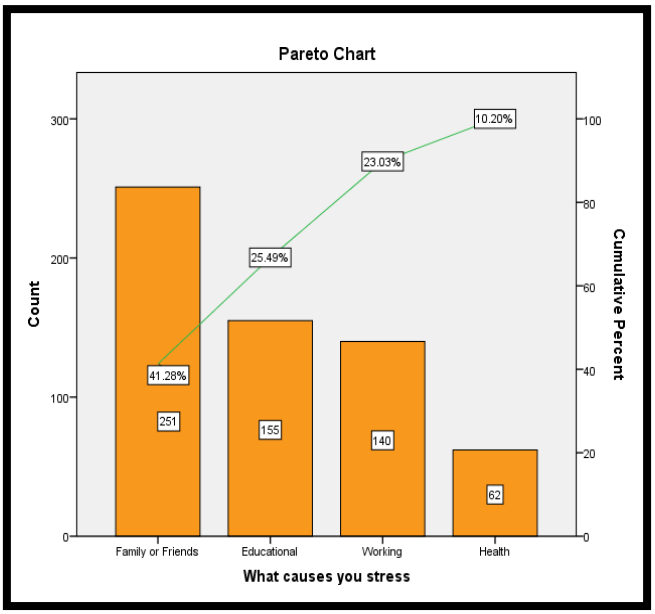
Conclusion:

It can be seen that the curve beings to flatten between factors 1 and 2. After the 4 factors, theEigen values are not so significant.

We have extracted three variables where the 9 items are divided into 3 variables according tothe most important items which similar responses in component 1 and simultaneously in component 2 and 3)

Pareto Chart

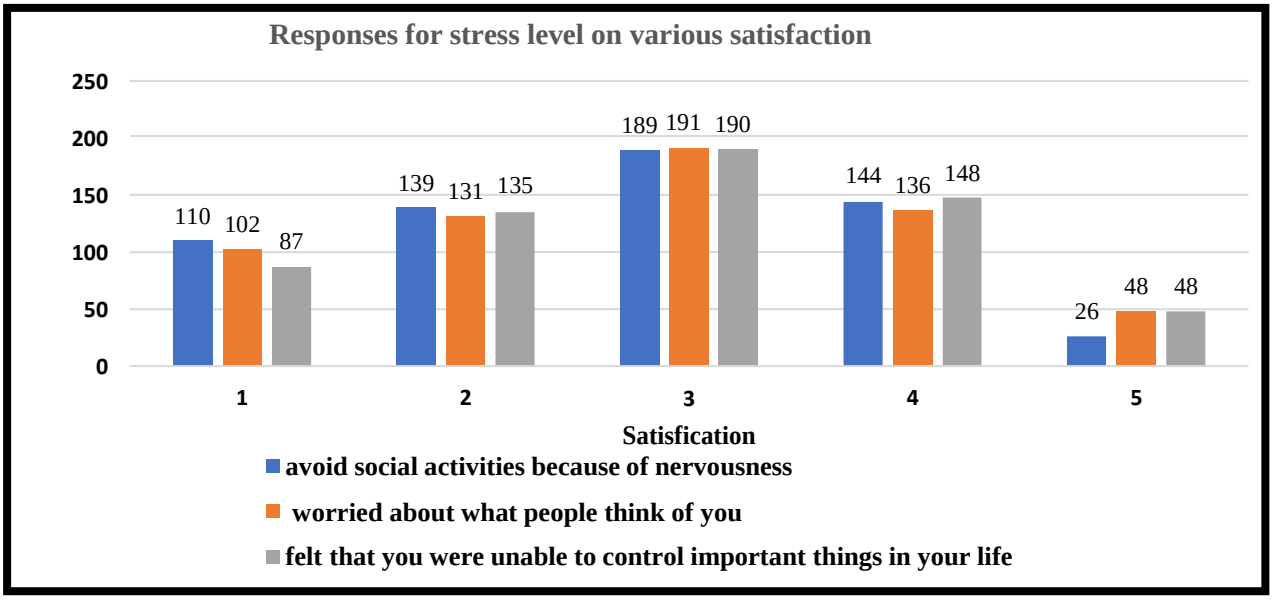
Causes you stress	Frequency	Percentage
Working (office/business/household/other)	140	23.02632
Family or Friends (relatives/colleague/officers)	251	41.28289
Educational (study)	155	25.49342
Health (disease)	62	10.19737



INTERPRETATION: In This case, the first two of four causes of stress together constitute 66.77%. So here, two most of friend and education causes will 66.77% of all causes.

Graph of responses for stress level on various satisfaction:

Satisfaction	Never	Almost Never	Some Time	Fairly Often	Very Often
You avoid social activities because of nervousness	110	139	189	144	26
You worried about what people think ofyou	102	131	190	136	48
You felt that you were unable to control important things in your life	87	135	191	148	48



Interpretation:

From above graph we can see those 189 human beings out of the608 population sometime avoid social activities because of nervousness. 190 human beings from the population are sometimes worried about what people think of you. 191 human beings from the population sometimes felt that they were unable to control important things in their life.

Logistic Regression

To Analyze the Relationship between Emotions and the family monthly income

Test of Significance of regression

H_0: There is insignificant Relationship between Emotions and the family of monthly income.H_1: At least one of the repressor variables are contributes significantly.

Conclusion: By Using logistic regression, here Likelihood Ratio Test P-value = 0.002<0.05Reject the null Hypothesis At least *one of* the repressors variable are contribute significantly.

Overall Conclusion:

- In our project, we can conclude that physical symptoms of stress mainly include troublewith sleep, memory problems, and blood pressure.
- The result of the project shows Factor that affects stress is worried about losing control,felting nervous and stress, taking rest in breaks and holidays.
- In this fast world today people having stress on health and depends on age, gender, area,family type.

Suggestions:

- Guided meditation is a great way to distract yourself from the stress of day-to-day life, practice deep breathing, maintain physical exercise and good nutrition, manage social media time and connect with others.
- Counseling, to help you recognize and release stress

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