Vol. 7 No. 1 January-2022

ISSN: 2319-4227

Ranchi University Journal of SCIENCE and TECHNOLOGY

RUJOST

Ranchi University
Ranchi – 834001, Jharkhand
India

Ranchi University Journal of Science & Technology

(RUJOST : ISSN 2319-4227)

A biannual, peer reviewed, referred **Ranchi University Journal of Science & Technology** published by Ranchi University, Ranchi, Jharkhand, India, every January & July

RUJOST

ISSN : 2319-4227

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Vol. 7 No. 1 January, 2022

Chief Editor

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Ranchi- 834008, Jharkhand, India

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From Editor's Desk:

It is a matter of great pleasure that our prestigious Ranchi University Science Journal**, RUJOST (Ranchi University Journal of Science and Technology, ISSN: 2319-4227), Volume 7, January, 2022 issue** is being brought to you as a result of continuous exercise of the editorial team & office of the Dean, Faculty of Science, Ranchi University, Ranchi. As a matter of fact this Journal is a biannual in nature published every January & July but due to some constraints the issue is being released a little delayed for which we sincerely regret and apologise.

The Journal mainly covers the research articles of all the six Science University Departments- Botany, Chemistry, Geology, Mathematics, Physics & Zoology with the acronym **BCGMPZ**. The journal is on a verge to upload online to enhance the domain of its readers as well as in the process of enlistment in the UGC.

The layout of the cover page having the hexagons symbolizes six different University Science Departments- **BCGMPZ**, which gets established as the copyright front page design of RUJOST which should be maintained in future as the first look identity of the Journal.

This journal has already made a big headway by entering into its seventh year of continuous publication and is successful in propelling many research initiatives for the socio-economic uplift of the state of Jharkhand. It is intended to make this journal a guiding resource to all the research activities for the development of our Nation and State.

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The Ranchi University Journal of Science and Technology (RUJOST) is a medium to provide upto date information on all scientific investigations together with their applications. The journal includes full length and mini reviews, original articles and scientific reports submitted to the Chief Editor. The journal hopes to attract the most important and highly innovative papers from the current research from academic and research communities, most importantly from young investigators and students, RUJOST features scholarly articles in rapidly moving science and technology related research areas like Botany, Zoology, Agriculture, Ethnobotany, Microbiology, Genetics, Molecular Biology, Biotechnology, Biochemistry, Geology, Physics, Mathematics, Bioinformatics, Pharmacology, Chemistry, Physiology and other sciences, which will be published subject to acceptability after referee and editorial assessment.

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RUJOST

ISSN : 2319-4227

A biannual, peer reviewed, referred **Ranchi University Journal of Science & Technology** published by Ranchi University, Ranchi, Jharkhand, India, every January & July

Volume 7 **No. 1** **January, 2022**

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Botany

Study of some ethnomedicinal plants used to cure kidney problems in Ranchi District, Jharkhand

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Abstract: Over the years, knowledge about the basic uses of indigenous medicinal plants has been accumulated. In order to promote their continued use in society, indigenous medicinal knowledge may have practical consequences for the creation of new medicines and provide more insights. Traditionally, for their sustenance, health and wellbeing, native societies worldwide are largely dependent on plants. Today, kidney disorders are widespread and cause people to suffer. An ethnobotanical survey was carried out in a specific area in India to collect information on plants used for kidney disease among the inhabitants of the Ranchi District of Jharkhand. This organ plays a very important role in filtering blood and removing waste from our body. Active substances that have a beneficial effect on the kidney tract system are found in medicinal herbs. The study found that 6 plant species belonging to 6 different families were reported to treat the problem in the area (e.g. Nyctaginaceae, Phyllanthaceae, Capparaceae etc.). In this research, a formal questionnaire was used to survey 78 households to learn what plants were used for this medicinal purpose. The medicinal preparations include powder, decoction, juice, etc. Future work with modern assays should confirm such methods, as then the importance of their conventional herbal practices in treating or appearing to cure kidney diseases in some cases will offer major benefits to society.

Keywords: Kidney disorders, indigenous medicinal plant, cure kidney disease.

INTRODUCTION

Plants are the backbone of life on earth and are central to the sustenance of humans. After fulfilling the primary needs like food and shelter, man has sought for a suitable remedy among plants for curing various diseases. For medicinal purposes, plants were used even before prehistoric times. As a rich resource of ingredients medicinal plants can be used to treat so many diseases. Medicinal plant care is known to be very effective as there are no or limited side effects. Plants from ancient times are man's oldest relations. The first science originating with the evolution or life of man on this planet is ethno botany. The study of the information structure relating to the multidimensional perspective of life, history, customs and the relationship of human societies with their local flora or fauna refers to ethnobotany.[1] There is a glance into the Vedic text of our knowledge of ethnomedicine, and there is a very strong connection between indigenous culture and biodiversity. This relation is an ethnobotanical one. Ethnobotany is the study of human-plant relationships and the use of plants by humans. Ethno refers to the religion, knowledge, language and aesthetics of individuals and their society. Botany is the biological branch that deals with plant science, and ethnobotany is the relationship between people and plants.[2]

Now a days, kidney problems are very common. If this is not taken care of, it is really concerning. After that to maintain life, the kidney can fail and require dialysis or kidney transplantation. By filtering the blood and removing excess water, contaminants and waste materials from the body in the form of urine, the kidneys are an amazing organ to keep us healthy. Acute renal failure or acute kidney injury (AKI) is one of the kidney problems. This is a causal

or sudden failure of the kidney or kidney damage that occurs within a few hours or a few days and is usually diagnosed with accumulation of end products of creatinine & urea or reduced production of urine.[3] Despite advances in supportive care, the death rate for AKI has remained high since the advent of hemodialysis 25 years ago. Acute renal failure is a very morbid and expensive condition, with a large percentage of patients advancing to end-stage renal disease and requiring dialysis.[4]

It remains a global public health issue that affects around 13.3 million patients each year. In addition, while direct causality between AKI and death has been controversial. AKI correlates about 1.7 million deaths per year with high morbidity, increased costs, and mortality.[5] People with renal failure can now live considerably longer than they could in the past because to the invention of dialysis, resulting in a huge pool of patients with severe chronic sickness. Although prolonging life is advantageous to patients suffering from renal failure, it comes at a significant price, both financially and in terms of quality of life. Medicinal plants, on the other hand, are another option to avoid this issue. Which is incredibly natural, inexpensive, and readily available.

Natural plants have been used for kidney disorders and their complications as alternative therapies. For the preparation of traditional medicines, different plant components are used.[6] Pretreatment with some herbs in a decoction may have distinct therapeutic effects and lower the toxicity of other medications. Several herbal components of the decoction are routinely changed by Chinese traditional physicians in response to changes in the patient's symptoms and indicators. The therapeutic effects of a prescription can be altered by changing a specific herbal component. Traditional Chinese medicine's science and pharmacology presents an ideal option for intervention in a chronic disease from a modern medical standpoint because it emphasizes the treatment of the variable and different targets related to disorders in the whole body and allows for individual medication modification whenever the symptom and sign change.[7] A prospective open label trial in 151 individuals with a serum creatinine of roughly 3.7 mg/dL found that rhubarb treatment reduced the progression of CKD.[8] Finally, we'll talk about future plans to assess the safety and efficacy of traditional herbal therapies for renal disorders.

MATERIALS & METHODS

The ethnobotanical survey was carried out in Ranchi's six separate blocks: Nagri, Ratu, Kanke, Angara, Namkum, and Ormanjhi. On the basis of their traditional knowledge and folklore formulation, which they prescribed to their patients, the required ethnomedicinal qualities of several plants were recorded through direct conversation with local people and practitioners. Plant species were identified by their local names.

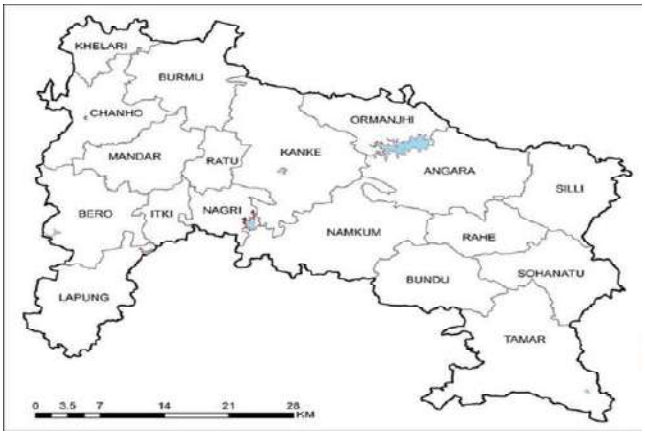

Fig. 1: Block map of Ranchi District of Jharkhand

Ranchi, in the state of Jharkhand, has an abundance of biodiversity and ethnomedicinal knowledge. Ranchi is located between 22° 30 N and 23° 30 N latitude and 85° E and 86° E longitude. It is situated at a height of 654 metres above sea level. Ranchi receives roughly 1530 mm of rain on an annual basis. Red sandy soil covers the entire region. With a forest area of 23,605 sq km, or 29 percent of the entire geographic area, Jharkhand is abundant in biological diversity and traditional wisdom. It is also rich in ethnomedicine, with around 80% of the population living in rural areas.[9] During field study, a formal questionnaire was used to survey 78 households to learn what plants were used to cure kidney problems. Following the documentation, the use of the formulation was double-checked and confirmed. The plants were identified with the herbaria of Ranchi University and Department of Forestry of Birsa Agriculture University, Ranchi, Jharkhand. The botanical names and families of the plants obtained were identified using literature and book of Botany of Bihar and Orissa (Haines, 1921-25).[10]

RESULTS & DISCUSSION

In the ethnobotanical surveys, a total of 78 informants were chosen from six blocks in Ranchi district (thirteen

respondents from each block) have been presented in fig-1 and table-1 shows a list of plants in alphabetical order, followed by their Botanical name, local name, family, plant parts used, and therapeutic purposes. The documentation was created using information gathered from knowledgeable local practitioners, patients who had been treated, and local plant collectors of the area. The average number of plant species cited by all of the informants was 6, which belongs to 6 different families such as Apiaceae, Nyctaginaceae, Pinaceae, Poaceae, Phyllanthaceae, Capparaceae. According to the findings, traditional herbal medicine is an important aspect of the healthcare of both tribal and non-tribal groups in Ranchi, Jharkhand. The ailments include several cysts in the kidneys (Polycystic disease), diabetes, kidney Infection, high Blood Pressure, longstanding blockage (Such as Kidney stones and tumor), Inflammation (Glomerulonephritis), that leads to renal failure.[11,12]

Table-1: List of important medicinal plants in Ranchi district and their uses to cure kidney problems.

Sl. No.	Botanical name	Local name	Family	Plant parts used	Therapeutic purposes
1.	*Coriandrum sativum*	Dhaniya	Apiaceae	Leaves, Seeds	improving the filtration rate, kidney infection.
2.	*Boerhavia diffusa*	Punarnava	Nyctaginaceae	Roots, Leaves	Kidney inflammation, kidney stone.
3.	*Pinus longifolia*	Chir	Pinaceae	Leaves, Twig	kidney and urinary problem.
4.	*Cynodon dactylon*	Doob grass	Poaceae	Whole parts	polycystic kidney disease, kidney pain
5.	*Phyllanthus niruri*	Bhumi amla	Phyllanthaceae	Whorl plant	kidney inflammation, improving the filtration rate of kidney.
6.	*Crateva religiosa*	Varun	Capparaceae	Bark, Leaves	polycystic kidney disease, kidney inflammation, renal failure.

The photographs of these ethnomedicinal plants have given in figure 2

Fig. 2: Ethnomedicinal plants to cure Kidney problems in Ranchi, Jharkhand.
(a) *Coriandrum sativum*, (b) *Boerhavia diffusa*, (c) *Pinus longifolia*

(d) *Cynodon dactylon*, (e) *Phyllanthus niruri*, (f) *Crateva religiosa*

CONCLUSION

Traditional indigenous medicines may be integrated into the national health-care system. Plants have been used as a medication for ages, first as a traditional preparation and then as a pure active component. The tribals of Ranchi have a strong belief in the efficacy and success of traditional medicine, and the findings of this study show that medicinal plants continue to play an important part in the renal function of Ranchi's tribal and non-tribal communities. So that the kidney remains heathy and it would not have to face dialysis stage. It will benefit mankind and future generations as well.

ACKNOWLEDGEMENT

Author is thankful to Prof.(Dr.) Kunul Kandir, Ranchi University, Ranchi for providing all necessary facilities.

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RUJOST, ISSN: 2319-4227, Vol.7, No.1, January, 2022, pp. 5- 8

A Biaanual Peer Reviewed Referred Ranchi University Journal of Science & Technology

Botany

Traversing mycorrhizal fungi and other microbes as an alternative to chemical fertilizers for a successful carrot production

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Abstract: Carrot (*Daucus carota* L.) is an important vegetable crop of family Apiaceae. Mycorrhiza inoculum resulted in better morphological and biochemical traits in vegetables. Whereas carrot production is still dependent on the use of chemical fertilizers. Thus, here the outcome of Arbuscular mycorrhizal fungi (AMF) and other microbes' inoculation was studied. Largely, there seems to be a promising opportunity of AMF in carrot because of enhanced morphological parameters, root weight, as well as disparities in nutrients and metabolite. The AMF appears to be in an option to boost plant growth, quality and yield of carrot. The treatment with *Pseudomonas fluorescens* determined the maximum values for the biochemical and the morphological traits, and *Gigaspora gigantea*. Surprisingly, the consortium treatment with the three different microbes was not at par for any of the trait measured. Overall, our study highlights the efficacy of mycorrhizal fungi and other microbes in achieving a successful carrot production.

Keywords: Carrot, *Gigaspora gigantea*, micorbes, mycorrhizal fungi, *Pseudomonas fluorescens*, soil

INTRODUCTION

Carrot (*Daucus carota* L.) is an economically important root crop.[1] The carrot is believed to be a native of the Western Asia (Afghanistan). Botanically, the carrot is more a biennial herb that is cultivated as an annual vegetable for its taproots. Carrot varieties are classified as European/Temperate types and Asiatic/tropical types based on their temperature requirements at the time for bolting. The foliage of western carrots is much dissected, and the ancestors are unbranched. The carrot storage root is an excellent supply of dietary fibre, vitamins, and carotenoids, and it is also full of antioxidants and minerals.[2] With rising wellness awareness, carrots are starting to be very popular because of their plentiful benefits and nutrients for human health. The bulk of research on carrots has centred on growth, nutrient content, tissue culture, breeding, and then carotenoid synthesis regulation. Mychorhizza inoculum resulted in better morphological and biochemical traits in vegetables, including carrots.[3] Moreover, in some cases, enhanced tolerances for stresses have been reported as the result of mycorrhizal inoculum. Arbuscular mycorrhizal fungi (AMF) are identified to have a significant amount of impact on host plant physiology and biochemistry, along with secondary metabolites. *Pseudomonas fluorescens*, is essential microbe for optimum plant growth and development. The AMF and the PSB combination might ameliorate plant development and maybe a substitute for synthetic fertilizers.[4]

Soil microbes regularly improve soil biodiversity by changing the unfavourable environment. AMF are ubiquitous obligate symbionts where fungal associates assist the host by improving the water and nutrients absorption.[5] Recently, it was recognized that the combined effects of bio inoculants boost soil development that ultimately results in terrific progress of plants. Preceding work on other root crops confirmed the progressive impact of AMF on growth as well as basic yield. Furthermore, it identifies change in the sucrose, and sugar and also fructose and greater starch at maximum advancement due to AMF.[6]

It is helpful to recommend that AMF in the rhizosphere can has an additive effect on development details in the development of carrot.

Therefore, the present study was carried out with a popular carrot variety to determine the effect of AMF and PSB on morphological and biochemical traits of the carrot.

MATERIALS & METHODS

Experimental setup

A pot experiment was conducted under the net house (24 ± 5 °C and 49–66% relative humidity) at the University Department of Botany, Ranchi University, Ranchi Jharkhand, India using the cultivar Hisar Gairic in the first week of October in 2019. The treatments were planted in a randomized complete block design in three replications with 15 plants in each replication. All plant production practices were followed based on package and practices define elsewhere. Using a mixture of ground soil as well as sand (3:1) was used for the experiment, with 70.8% sand, 24.5% silt, and 4.0% clay. The chemical composition of the soil was 0.042% N, 0.017% P, 0.06% organic carbon and a pH of 7.4.[7] Furthermore, the soil was autoclaved at 121°C.

Microbial inoculum preparation

Inoculum of *Glomus mosseae* containing 80-86% colonization (root pieces) and 780-800 AM spores (w/w) was procured from Department of Botany, Kurukshetra University Kurukshetra and inoculum of *Gigaspora gigantea* containing 75-79% colonization (root pieces) and 870-890 AM spores (w/w) was procured from Forest Pathology Discipline, Forest Protection Division, FRI, Dehradun. Both inoculums were then mass multiplied using Maize as host for 3 months, to get the starter inoculum for the experiment. After mass production, the inoculum containing 77-82% colonization/infection (maize root pieces) and 820-860 *Glomus mosseae* spores (w/w) and 74-78% colonization/infection (maize root pieces) and 840-880 *Gigaspora gigantea* spores (w/w) were taken. For single treatment, 10g of each inoculum was added per pot and 5+5g (*Glomus mosseae* + *Gigaspora gigantea*) for dual and consortium treatments. *Pseudomonas fluorescens* was purchased from the Institute of Microbial Technology (Imtech), Chandigarh, India. It was then multiplied in a nutrient broth medium containing beef extract, peptone and NaCl, 3g/L, 5 g/L, and 5g/L, respectively, and incubated at 32°C for 48 hours for proper growth of bacteria. For *Pseudomonas fluorescens* treatment, all the seeds of carrot except control were dipped in the nutrient broth medium for 10 minutes.[8] Microbial inoculums used in the present study are shown in Table 1. The following eight treatments (Tt) were studied in the present study to inoculate the carrots.

Table 1- Microbial inoculums

Treatments	Code
Control	T1
G_m†	T2
P_f	T3
G_G	T4
G_m+P_f	T5
G_m+G_G	T6
P_f+G_G	T7
G_m+P_f+G_G	T8

Plant characterization and data analysis

Root length and shoot length of all of the plants in replication were measured at maturity, i.e., after 80 days of sowing. The same sample was used for the measurement of root and shoot length. Where for the chlorophyll and carotenoids content were estimated 0.1 g of fresh leaf samples and tap root sample, respectively. The supernatant was carefully gathered, and also absorbance was recorded utilizing a UV Vis. Spectrophotometer (Specord 205 Analytik Jena AG, Jena, Germany) at 645 nm as well as 663 nm for chlorophyll a plus chlorophyll b, respectively, and also at 520 nm for carotenoids using 80 % acetone as being a blank.[8] Arbuscular mycorrhizal fungi (AMF) spores and AMF root colonization (%) was determined based on the procedures defined elsewhere.[9] The data analysis was performed using the SPSS software (11.5 version).

RESULTS & DISCUSSION

There were significant differences among the means of seven treatment groups and the control (Table. 2). Effect of Bioinoculants on Biochemical and Physiological attributes of *Daucus carota*.

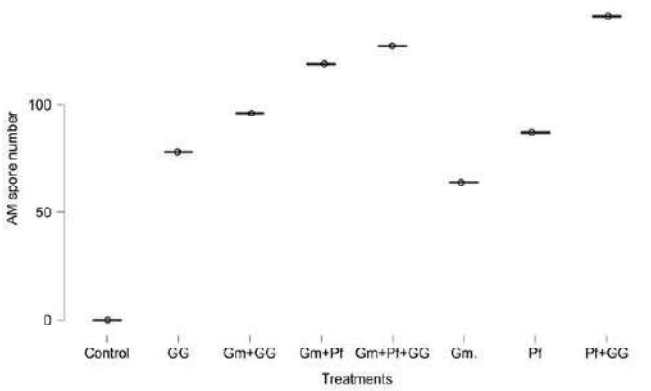
The maximum shoot length of treated *Beta vulgaris* was found in *P. fluorescens* + *G. gigantea* (P_f+G_G) inoculants which also corresponded with shoot weight (Table. 2). Similarly, the root length and root weight were maximum in the same treatment (P_f+G_G) (Table 2). The shelf-life of carrot was found to be highest in P_f+G_G treatment (Table 2). The maximum chlorophyll content and carotenoids were determined by the P_f+G_G treatment

Table 2- Means of seven treatment groups and the control

Treatments	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)	AM spore number	AM root colonization (%)	Chlorophyll a (mg FW^{-g})	Chlorophyll b (mg FW^{-g})	Total chlorophyll (mg FW^{-g})	Total carotenoids (mg FW^{-g})
Control	9.86±1.11^{g}	3.95±0.84^{h}	13.38±1.27^{h}	13.24±1.53^{g}	0±0^{h}	0±0^{f}	11.52±1.67^{g}	5.29±1.12^{g}	16.81±2.27^{g}	10.21±0.86^{g}
G_m†	15.28±0.61^{f}	8.11±0.82^{g}	23.56±1.02^{g}	31.82±2.61^{f}	64±6.6^{g}	41.2±5.7^{e}	18.51±1.49^{f}	8.61±1.25^{f}	27.12±1.32^{f}	15.67±0.74^{f}
P_f	20.37±0.98^{d}	18.14±0.71^{e}	34.46±1.57^{e}	64.78±1.08^{d}	87±5.1^{e}	58.4±3.7^{c}	25.49±1.74^{d}	12.85±1.05^{d}	38.34±2.03^{d}	21.15±0.79^{d}
G_G	18.16±0.77^{e}	11.15±0.68^{f}	27.54±1.46^{f}	48.14±1.73^{e}	78±6.3^{f}	50.2±3.9^{d}	22.29±2.08^{e}	10.54±1.84^{e}	32.84±3.21^{e}	18.66±0.81^{e}
G_m+P_f	23.32±0.81^{c}	72.08±0.96^{d}	48.74±1.18^{c}	115.46±2.21^{b}	119±6.8^{c}	62.6±7.5^{c}	28.43±1.31^{c}	13.23±1.25^{cd}	41.67±2.44^{c}	24.67±0.72^{c}
G_m+G_G	21.52±1.06^{d}	79.91±0.71^{c}	40.78±2.55^{d}	82.88±1.91^{e}	96±6.5^{d}	61.8±3.1^{c}	26.14±1.82^{d}	14.86±1.06^{bc}	41.01±2.48^{cd}	24.56±0.91^{c}
P_f+G_G	34.39±1.53^{a}	115.47±1.63^{a}	68.02±1.88^{a}	190.22±1.81^{a}	141±3.5^{a}	78.6±5.4^{a}	35.12±1.17^{a}	17.81±1.48^{a}	52.93±2.25^{a}	28.34±1.61^{a}
G_m+P_f+G_G	25.23±1.27^{b}	92.59±1.09^{b}	55.44±1.05^{b}	116.04±1.29^{b}	127±5.2^{b}	69.8±3.9^{b}	32.43±1.39^{b}	15.34±1.96^{b}	47.78±2.92^{b}	26.28±1.29^{b}
LSD ($P \leq 0.05$)	1.353	1.261	2.031	2.358	7.048	6.021	2.076	1.831	3.128	1.302
ANOVA (7, 32)	238.071	1.030.677	648.283	4.847.789	328.494	133.852	110.562	40.062	112.786	180.085

†Gm- *Glomus mosseae*, Pf- *Pseudomonas fluorescens*, GG- *Gigaspora gigantea* ±- Standard deviation; ‡values in a column followed by the same letter are not significantly different; pd"0.05- LSD (least significant difference test); FW- Fresh Weight

followed by the consortium treatment (Table 2). The total chlorophyll and carotenoid content were too highest in P_f+G_G treatment because of the greatest acidic and alkaline phosphatase activity. This agrees with the highest AM number and colonization (Table 2). The AM spore numbers were significantly higher in the treatments as compared to control. The maximum AM spore number was determined for the P_f+G_G treatment which was much as 141.35 (Fig. 1.) AM spore number identified from the beetroots among the different treatments studied in the present study.



Successful carrot production requires high nutrient supply.[10] Due to its root structure, and that is why the absorption of nutrition is rather difficult, especially P, that is essential for carrots roots developments.[11] As we understand, in soil that is natural, there are countless microbial communities. These nutrients cycling are guided by microorganisms residing in soil and useful in the development and plant growth.[12] This confirms the study of ours that the shoot development, as well as its water absorption capacity, was improved. Likewise, the weights of carrots have been much higher in inoculated vegetation because of a lot more water content. The blend of nitrifying bacteria, AMF and PSB for carrot might facilitate efficient uptake of P and N without also working with synthetic fertilizers.[13] AMF is advantageous for photosynthesis, and that it's complete growth and development.[14] Nevertheless, during experimentation, it's additionally been observed that because of microbial inoculation quantity of biochemical parameters has improved significantly.[15] This is in agreement with the results of ours that indicate precisely how AMF affect secondary metabolites generation, as demonstrated by various other plants additionally. Whereas, they besides, discovered that raising fertilizer quantity had a comparable impact on grow yield as well as NPK uptake.[16]

CONCLUSIONS

AMF treatment, along with the *Pseudomonas* appears to be promising for the carrot. In this direction, the AMF seems to be in a place to increase plant development, yield and quality of carrot. The optimum values for the biochemical and also the morphological traits have been based on the treatment with *P. fluorescens*, and *G. gigantea*. Astonishingly, the consortium therapy with the 3 distinct microbes wasn't at par for the characteristic measured. Overall, our analysis highlights the efficacy of other microbes and mycorrhizal fungi in attaining a profitable carrot production.

CONFLICT OF INTEREST

Authors declare there is no conflict of interest.

ACKNOWLEDGEMENT

The corresponding authors extend their appreciation to the Head of University Department of Botany, Ranchi University, Ranchi, Jharkhand, India.

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RUJOST, ISSN: 2319-4227, Vol.7, No.1, January, 2022, pp. 9-10

A Biaanual Peer Reviewed Referred Ranchi University Journal of Science & Technology

Botany

Comparative determination of extractive value of some potential anxiolytic plants

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Abstract: Present study was carried out to determine the extractive values of some potential anxiolytic plants which include *Atrocarpus hetrophyllus*, *Azadirachta indica*, *Ficus religiosa, Mangifera indica*, *Murraya koenigii* and *Neolamarckia cadamba*. Different solvents (methanol, benzene, chloroform, water and ethanol) were used for the extraction of plant material. Maximum extractive value was observed in *N. cadamba* with 12.7% whereas lowest was observed in *A. hetrophyllus* with 4.1%.

Keywords: Extractive value, anxiolytic, mental disorder, solvents

INTRODUCTION

According to WHO more than billion people suffers from neurological and mental disorders worldwide.[1] Anxiety disorder is a term which includes various mental disorders like panic disorders, post-traumatic stress disorder, obsessive compulsive disorder, specific phobia etc.[2] At present time, Benzodiazepines (BDZs) which acts on γ- aminobutyric acid (GABA) and selective serotonin reuptake inhibitors (SSRIs) are the most common medications available for the treatment of anxiety disorders but are limited due to their side effects hence there is high requirement of alternative medicine with minimal or nil side effects.[3,4]

Some of the most commonly found plants were observed having anxiolytic activities. From previous works methanolic extract of *Atrocarpus hetrophyllus*[5], aqueous extract of *Azadirachta indica*[6], aqueous extract of *Ficus religiosa*[7], *Mangifera indica*[8], ethanolic extracts of *Murraya koenigii*[9] and aqueous & ethanolic extracts of *Neolamarckia cadamba*[10] showed anxiolytic effects.

MATERIALS & METHODS

Fresh leaves of plants were collected from the Morabadi area of Ranchi. Leaves were cleaned and dried in shade till week. Dried leaves were grinded into powder using mortar- pestle and kept in air tight container.

Determination of extractive value

Powdered plant material of each plant material was extracted with different solvent (methanol, benzene, chloroform, water and ethanol). 2 gm of powdered plant material was weighed and transferred into dry and clean 250 ml conical flask and then filled with different solvents separately. Flask then kept at room temperature while shaking frequently for 24 hrs. Mixtures were filtered using whatman filter paper into 50 ml measuring cylinder. Filtrates were then poured into clean and dry weighed petri plates and then kept till concentrated extracts were obtained. To determine extractive value following formula was applied:

$$\text{Extractive value (\%)} = \frac{\text{weight of dried extract}}{\text{weight of plant extract}} \times 100$$

RESULT & DISCUSSION

Extractive value determination is useful in finding effective solvent for the extraction of drugs. The maximum extractive value observed in *N. cadamba* (12.7%, Table 6) followed by *A. indica* (12.3%, Table 2), *M. koenigii* (10.2%, Table 5), *F. religiosa* (9.9%, Table 3), *M. indica* (8.4%, Table 4) and *A. hetrophyllus* (4.1%, Table 1). Methanolic extract followed by water extract was found to be having highest extractive value whereas benzene was lowest. Most of the extracts were of light green colour in different solvents but water extracts were of brown colour except in *N. cadamba* which was light green in colour.

Table 1: Extractive value of *A. hetrophyllus*

S. No.	Type of Extract	Plant material (g)	Colour of Extract	Extractive value (%)
1.	Methanol	2	Light green	2.9
2.	Benzene	2	Light brown	0.6
3.	Chloroform	2	Dark green	1.5
4.	Water	2	Light brown	4.1
5.	Ethanol	2	Light green	2.9

Table 2: Extractive value of *A. indica*

S. No.	Type of Extract	Plant material (g)	Colour of Extract	Extractive value (%)
1.	Methanol	2	Light green	9.3
2.	Benzene	2	Brownish green	1.4
3.	Chloroform	2	Dark green	3.1
4.	Water	2	Light brown	12.3
5.	Ethanol	2	Light green	3.9

Table 3: Extractive value of *F. religiosa*

S. No.	Type of Extract	Plant material (g)	Colour of Extract	Extractive value (%)
1.	Methanol	2	Light green	9.9
2.	Benzene	2	Light brown	2.3
3.	Chloroform	2	Dark green	2.7
4.	Water	2	Brown	7.4
5.	Ethanol	2	Light green	3.6

Table 4: Extractive value of *M. indica*

S. No.	Type of Extract	Plant material (g)	Colour of Extract	Extractive value (%)
1.	Methanol	2	Dark green	8.4
2.	Benzene	2	Light brown	2.1
3.	Chloroform	2	Dark green	3.7
4.	Water	2	Dark brown	6.4
5.	Ethanol	2	Dark green	6.5

Table 5: Extractive value of *M. koenigii*

S. No.	Type of Extract	Plant material (g)	Colour of Extract	Extractive value (%)
1.	Methanol	2	Light green	10.2
2.	Benzene	2	Light green	1.3
3.	Chloroform	2	Light green	2.3
4.	Water	2	Brown	7.6
5.	Ethanol	2	Light green	8.3

Table 6: Extractive value of *N. cadamba*

S. No.	Type of Extract	Plant material (g)	Colour of Extract	Extractive value (%)
1.	Methanol	2	Light green	12.7
2.	Benzene	2	Dark green	2.4
3.	Chloroform	2	Brownish green	4.6
4.	Water	2	Light green	12.2
5.	Ethanol	2	Light green	6.5

CONCLUSION

Extractive value provides the parameter of chemical constituents present in plant material which also helps in detection of adulteration of drugs which will be useful in medicine field. Methanol was observed as a suitable solvent for the extraction process.

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RUJOST, ISSN: 2319-4227, Vol.7, No.1, January, 2022, pp. 11-16
A Biaanual Peer Reviewed Referred Ranchi University Journal of Science & Technology

Documentation on some dye-yielding plants in Ranchi District of Jharkhand

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Abstract: Extensive field trips were carried out to different village/ agriculture fields of Ranchi district and location near Ranchi University campus. Plant biodiversity of Ranchi District is rich and abundant wealth of functional germplasm resources and plant kingdom is a treasure-house of various natural products. Dyes are one of the natural products obtained from plants. The worldwide demand for natural dyes is nowadays of great interest due to the increased awareness on therapeutic properties of natural dyes among the public. Natural dyes are derived from naturally occurring sources such as plants, insects, animals and minerals. The structure and functional properties of natural dyes have been identified in the recent past. Some dyes extracted from plants have important medicinal properties like; antimicrobial activities, antifungal activities. Main objective of this paper to write the literature which is related with survey and documentation of dye-yielding plants found in Ranchi district, Jharkhand. These efforts can be helpfuli the development of pharmaceutical formulations.

Keywords: Dyes, Medicinal values, Natural dyes.

INTRODUCTION

The plants are used not only for maintaining the basic life sustaining needs like food, fuel, shelter, but also for making clothes and natural dye for colouring the clothes.[1] The worldwide demand for natural dyes is nowadays of great interest due to the increased awareness on therapeutic properties of natural dyes among the public. Natural dyes are derived from naturally occurring sources such as plants, insects, animals and minerals. The use of natural products for therapeutic use is as ancient as human civilization and for a long time, minerals, plants and animal products were the main sources of drugs.[2]

Among all the natural dyes, plant-based pigments have a wide range of medicinal values. Many of the plants used for dye extraction are classified as medicinal and some of these have recently been shown to possess remarkable antimicrobial activity.[3-7]

Natural dyes are not only used to impart colour to an infinite variety of materials such as textile, paper, wood, etc. but they are also widely used in the cosmetics, food, and pharmaceutical industry. Many plant and animal/ insect sources have been identified for the extraction of colour and their diversified use in textile dyeing[8-10] and functional finishing[11-13], food colouration[11], cosmetics[14]. They have a wide range of medicinal importance in the pharmaceutical industry.[15] Apart from dye-yielding properties, some plants are also used traditionally for medicinal purposes.[16]

Natural dyes are environment-friendly. For example, turmeric, the brightest of naturally occurring yellow dyes is a powerful antiseptic, which revitalizes the skin. Another natural dye, wild indigo gives a cooling sensation.[17]

However, researches have shown that synthetic dyes are suspected to release harmful chemicals that are allergic, carcinogenic and detrimental to human health. In 1996, Germany became the first country to ban certain azo dyes.[18]

Natural dye-yielding plants are found in many places of Jharkhand but research on their medicinal potential is still lacking. Unfortunately, no serious attempt has been made to document and preserve this eminent treasure of traditional knowledge of natural dye making associated with the indigenous people. The main objective of this paper is to write the literature which is related with survey and documentation of dye-yielding plants found in the Ranchi district, Jharkhand. These efforts can be helpful in the development of pharmaceutical formulations.

Study Area

The study was conducted in the Ranchi District of Jharkhand and its surrounding native areas (Fig. 1). Ranchi, the beautiful green city of waterfall, lakes and dense forest, is the capital of the Indian state of Jharkhand and is located on latitude 23.23° North and longitude 85.23° East at a mean elevation of 2100 feet above the sea level.

Fig. 1- Ranchi District in Jharkhand

METHODOLOGY

Extensive field trips were carried out to different village/ agriculture fields of the Ranchi district location near Ranchi University campus. The plants specimen was photographed and identified with the help of different flora. The herbarium of collected specimen were also made by proper methods. Chemical constituent of investigated plants was studied by reference books and eminent authors.

The tribals of Jharkhand use a variety of plants in their daily life for food, medicine, shelter, clothes, etc. However, little is documented on medicinal uses of a wide variety of plants used by the tribals. This provides sufficient scope of research in this area.

RESULTS & DISCUSSIONS

During the survey, there is documentation of 16 dye-yielding plants with their medicinal value which is mentioned in table number 2. Today, dyeing is a complex and specialized discipline. Nearly all dyestuff is now produced from synthetic compounds. Some of the synthetic dyes are found to be associated with health hazards affecting human life causing skin diseases and pulmonary problems.[19]

Information regarding the different plants used for dye-yielding purpose, their properties, uses and effectiveness are collected through personal interview with the farmers and villagers. Plants were collected, made into herbarium, identified using local floras.

Natural dyes are less toxic, less polluting, less health hazardous, non-carcinogenic and non-poisonous.[20] Natural dyes do not pose a threat to the health of users, which is not the case with synthetic dyes. Moreover, natural dyes are commonly available and because of their availability at cheaper cost these are within the reach of common man.

Many of the plants used for dye extraction are classified for medicinal use. They have a wide range of medicinal importance in the pharmaceutical industry. So, the present study would be beneficial for society by throwing light on several unexplored potentialities of dye-yielding plants grown in Ranchi district.

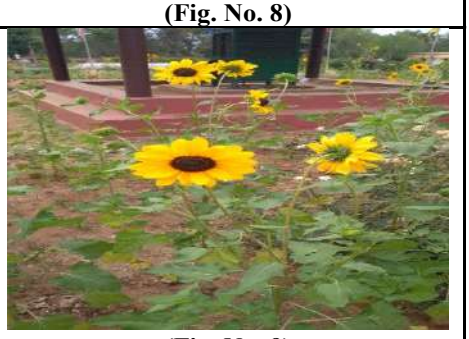
Existing documented works suggest that natural dye-yielding plants have a wide range of medicinal importance to the pharmaceutical industry. Natural dyes find use in the colouration of textiles, food, drugs and cosmetics. These plants have also been reported to exhibit inhibitory activities against different fungi and bacteria. Antioxidant activity of natural dye has also been reported. Medicinal uses of various types of natural dye-yielding plants include treating skin disorder, diarrhoea dysentery, cancer, cough, tumour, etc.

Table 1- Plants taxa collected from different areas of Ranchi District

S.No.	Plants Species	Places of Collection	Dates of Collection
1	*Lawsonia inermis* L.	Vikas Vidyalaya Ranchi	05.09.2021
2	*Bougainvillea glabra*	Vikas Vidyalaya Ranchi	05.09.2021
3	*Nyctanthes arbour tristis*	Vikas Vidyalaya Ranchi	25.10.2021
4	*Hibiscus rosa-sinensis* L.	Vikas Vidyalaya Ranchi	05.09.2021
5	*Hibiscus sabdariffa*	Vikas Vidyalaya Ranchi	06.01.2022
6	*Tagetes erecta* L.	Vikas Vidyalaya Ranchi	10.09.2021
7	*Mallotus philippensis* Muell.	Vikas Vidyalaya Ranchi / Ranchi University Ranchi Campus	18.02.2022
8	*Helianthus annuus* L.	Vikas Vidyalaya Ranchi	25.10.2021
9	*Spathodea companulata*	Nucleus Mall Ranchi	02.03.2022
10	*Allium cepa* L.	Vikas Vidyalaya Ranchi	06.03.2022
11	*Bixa orellena* L.	Vikas Vidyalaya Ranchi / Ranchi University Ranchi Campus	18.02.2022
12	*Butea monosperma* Lam. Taubert.	BIT Mesra, Bundu	20.03.2022
13	*Solanum lycopersicum* L.	Vikas Vidyalaya Ranchi	05.03.2022
14	*Artocarpus heterophyllous* Lam.	Vikas Vidyalaya Ranchi	02.03.2022
15	*Curcuma longa* L.	Vikas Vidyalaya Ranchi	22.02.2022
16	*Punica granatum* L.	Vikas Vidyalaya Ranchi	25.10.2021

Table 2- Some important dye-yielding plants with its medicinal values and pictures

Details	Pictures
Botanical Name: *Lawsonia inermis* L. **(Fig. No. 2)** Family Name: Lythraceae Common Name: Henna Habit: Shrub Parts used: Leaves Colour Obtained: Red-orange Medicinal Uses: Antibacterial, antifungal, anti-parasitic, antiviral, anticancer, antidiabetic, anti-inflammatory, antifertility and wound healing properties.	 **(Fig. No. 2)**
Botanical Name: *Bougainvillea glabra* **(Fig. No. 3)** Family Name: Nyctaginaceae Common Name: Bougainvillea Habit: Evergreen climber Parts used: Flowers Colour Obtained: Grey Medicinal Uses: To treat diarrhoea, reduces acidity, cough and sore decoction of dried flowers for the blood vessels and leucorrhea and decoction of the stem in hepatitis.	 **(Fig. No. 3)**
Botanical Name: *Nyctanthes arbour tristis Linn.* **(Fig. No. 4)** Family Name: Oleaceae Common Name: Harsingar (Night Jasmine) Habit: Tree Parts used: Flowers Colour Obtained: Yellow Medicinal Uses: It provides treatments for Dengue, Chikungunya, Malaria and Arthritis. It prevents gas, radical damage, treats cough, fights breathing problems, etc. Additionally, it has anti-bacterial, anti-viral and anti-fungal properties which make it fight various infections in the body.	 **(Fig. No. 4)**
Botanical Name: *Hibiscus rosa-sinensis* L. **(Fig. No. 5)** Family Name: Malvaceae Common Name: Chinese hibiscus Habit: Evergreen shrub Parts used: Flowers Colour Obtained: Deep red Medicinal Uses: Treatment in swelling, pain, mumps, fever.	 **(Fig. No. 5)**
Botanical Name: *Hibiscus sabdariffa* **(Fig. No. 6)** Family Name: Malvaccae Common Name: Kudrum / Roselle Habit: Herb or Woody-based subshrub Parts used: Fruits / Calyxes Colour Obtained: Red Medicinal Uses: Used to lower blood pressure, relieve dry coughs, and topically treat skin afflictions.	 **(Fig. No. 6)**

Botanical Name: *Tagetes erecta* L. **(Fig. No. 7)** Family Name: Asteraceae Common Name: Marigold Habit: Herb Parts used: Flowers Colour Obtained: Dark yellow Medicinal Uses: Internally to treat indigestion, colic, severe constipation, dysentery, cough, and fever and externally to treat sores, ulcers, eczema, sore eyes and rheumatism.	 **(Fig. No. 7)**
Botanical Name: *Mallotus philippensis* Muell. **(Fig. No. 8)** Family Name: Euphorbiaceae Common Name: Kamala tree Habit: Evergreen tree Parts used: Fruits Colour Obtained: Red Medicinal Uses: Treatment of bronchitis, abdominal diseases, spleen enlargement, etc.	 **(Fig. No. 8)**
Botanical Name: *Helianthus annuus* L. **(Fig. No. 9)** Family Name: Asteraceae Common Name: Wild Sunflower Habit: Herbs Parts used: Flower, leaves and seeds Colour Obtained: Red, Deep Purple and White Medicinal Uses: Flower tea is used for lung ailments and malaria. Leaf tea reduces high fevers and has astringent properties. Leaf poultice may be used on snakebites and insect bites. Leaves are also diuretic and expectorant, as are seeds.	 **(Fig. No. 9)**
Botanical Name: *Spathodea companulata* **(Fig. No. 10)** Family Name: Bignoniaceae Common Name: African Tulip, Rugtoora Habit: Tree Parts used: Flower Colour Obtained: Brown Medicinal Uses: Used for treatment of malaria, diabetes, stomach, ulcers, wounds, skin infections and viral diseases.	 **(Fig. No. 10)**
Botanical Name: *Bixa orellena* L. **(Fig. No. 11)** Family Name: Bixaceae Common Name: Sindoor Plant / Lipstick tree Habit: Tree Parts used: Seeds Colour Obtained: Orange, Red Medicinal Uses: Used for diabetes, diarrhoea, fevers, fluids retention, heartburn, malaria and hepatitis.	 **(Fig. No. 11)**
Botanical Name: *Butea monosperma* Lam. Taubert. **(Fig. No. 12)** Family Name: Fabaceae Common Name: Flame of the forest / Palash Habit: Small Tree Parts used: Flowers Colour Obtained: Yellow, Orange Medicinal Uses: Used for piles, tumour and menstrual disorders, Gum is astringent and used in diarrhoea.	 **(Fig. No. 12)**

Botanical Name: *Allium cepa* L. **(Fig. No. 13)** Family Name: Amaryllidaceae /Liliaceae Common Name: Onion Habit: Herbs Parts Used: Skins Colour Obtained: Yellow, Orange Medicinal Uses: Onion is used for treating digestion problems including loss of appetite, upset stomach, and gallbladder disorders; for treating heart and blood vessels problems including chest pain(angina) and high blood pressure; and for preventing atherosclerosis.	 **(Fig. No. 13)**
Botanical Name: *Solanum lycopersicum* L. **(Fig. No. 14)** Family Name: Solanaceae Common Name: Tomato Habit: Herbs Parts Used: Fruits Colour Obtained: Red Medicinal Uses: Antibacterial, antifungal, anti-mutagenic, used in prostate cancer.	 **(Fig. No. 14)**
Botanical Name: *Artocarpus heterophyllous* Lam. **(Fig. No. 15)** Family Name: Moraceae Common Name: Jackfruit Habit: Tree Parts Used: Leaves and Wood Colour Obtained: Yellowish Brown & Bark Brown Medicinal Uses: Anti-aging, Diabetics, Detoxification, Anti-oxidants, Control blood pressure and weight losses.	 **(Fig. No. 15)**
Botanical Name: *Curcuma longa* L. **(Fig. No. 16)** Family Name: Zingiberaceae Common Name: Turmeric Habit: Herbs Parts Used: Roots/ Rhizomes Colour Obtained: Yellow Medicinal Uses: Anti-oxidant, anti-inflammatory, anti-cancer, anti-fungal, anti-bacterial effects and anti-septic agent.	 **(Fig. No. 16)**
Botanical Name: *Punica granatum* L. **(Fig. No. 17)** Family Name: Punicaceae / Lythraceae Common Name: Pomegranate Habit: Shrubs Parts Used: Fruits Colour Obtained: Yellow Medicinal Uses: Fruits contains anti-carcinogenic, anti-microbial and anti-viral compounds.	 **(Fig. No. 17)**

CONCLUSION

From this research work, it can be concluded that due to their non-toxic properties, low pollution and less side effects, natural dyes are used in day-to-day food products. Unfortunately, no serious attempts have been made to document and preserve this immense treasure of traditional knowledge of natural dye-making associated with the indigenous people.

To conclude, there is an urgent need for proper collection, documentation, assessment and characterization of dye-yielding plants and their dyes, as well as research to overcome the limitation of natural dyes.

ACKNOWLEDGEMENT

I express my sincere gratitude to the Dean, Faculty of Science and Head, Department of Botany, Ranchi

University, Prof. (Dr.) Kunul Kandir for providing necessary facilities and encouragement.

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RUJOST, ISSN: 2319-4227, Vol.7, No.1, January, 2022, pp. 17-22

A Biaanual Peer Reviewed Referred Ranchi University Journal of Science & Technology

Botany

The most reliable and exotic ethnomedicinal *Kalanchoe* species used against Urolithiasis and Cholelithiasis in Jharkhand

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Abstract: Genus *Kalanchoe* (syn. *Bryophyllum*) of Crassulaceae family has 125 species worldwide of tropical-subtropical, succulent and flowering plants, mostly originated in Madagascar and South-East Africa. The subtropical climate of India also promotes its wide distribution. The ethnomedicinal importance of several of these globally accepted species are successfully used against various ailments of human, especially urolithiasis and cholelithiasis, in district Ranchi of Jharkhand also. In Ayurveda, Kalanchoe plants are described as PATHARCHATTA (stone dissolving plants). As the miracle leaves of these plants help in the management of kidney stones as well as gall bladder stones. They are juicy, succulent and have great medicinal values, rich in phytochemicals, especially polyphenols, like - flavanoids, citric acid, and malic acid. These secondary metabolites involved in the protection of kidney and gall bladder from stone formation. Various research confirms that they are antipyretics, anti-inflammatory, diuretic, antilithiatic, antiviral, antibacterial, antioxidant, hepatoprotective, nephroprotective, antitumor, etc. The presence of cytotoxin Bufadienolide Cardiac Glycosides make some *Kalanchoe* spp. a grazing hazard for animals, with documented issues in Brazil, South Africa and Australia; not in India. *Kalanchoe* plants propagate through epiphyllus buds i.e., new buds with roots are produced in the serrated leaf-margin, each at the end of a vein which detaches and develops into new plant. In this article a brief description of 12 most reliable edible spp. of *Kalanchoe* has been taken, viz. *K. pinnata, K. lacianiata, K. blossfeldiana, K. mortagei*, etc. *K. pinnata* is the most popular and widely used species and best-known representative of this genus. The study has an aim to explore the valuable ethnomedicinal potential of *Kalanchoe* spp. in Jharkhand, their availability, popularity and preservation.

Keywords: *Kalanchoe* spp., Patharchatta, epiphyllus buds, flavanoids, antilithiatic, cardiac glycosides.

INTRODUCTION

Family – Crassulaceae J. St.-Hil.

Genus – *Kalanchoe* Adans.

Common Name[1]- Patharchatta, Stone crop, Air plant, Cathedral bells, Life plant, Magic leaf, Mother of thousands, Mother of millions, Sprout leaf plant, Miracle plant, etc.

Genus *Kalanchoe* (Crassulaceae) encompasses succulent, shrubs or herbs usually perennial plants, albeit, some are annual or biennial, native to both Old and New World; they are especially abundant in Madagascar. They are cosmopolitan in warm climate. Subtropical and warm climate of India also naturalized them and are used by many ethnicities to treat a variety of human ailments, thus being called "miracle leaf".[2] Despite their exotic and invasive presence, *Kalanchoe* spp. have ethnobotanical uses wherever they are found.

According to Ayurveda, all kinds of medicinal properties are found in Patharchatta (as name used in Ayurveda for *Kalanchoe* Plants) and it is used to make medicines for many diseases.

District Ranchi[3] of Jharkhand state is a great hub of medicinal plants and its ethnicity. 56.86% of rural population along with some part of urban have great credence in the role and use of ethnomedicinal plants. The climate of Ranchi is warm and temperate. The average annual temperature is 23.1°C and precipitation here is about 1430 mm per year.[4] The surrounding dense tropical forests make its biodiversity rich. It is a plateau region (average

elevation- 651m, above the sea level) and the urolithiasis (kidney stone) and cholelithiasis (gall-bladder stone) are common occurring stone problems in hilly region due to geographical/ genetic variation/ industrialization and modern life-style. The frequent reoccurrence is associated with these two diseases. The surgery and modern drug therapy are not sufficient and satisfactory.

Role of *Kalanchoe* spp. in the management of urolithiasis and cholelithiasis[5] is miraculous. Sufferer's first choice is the intake of *Kalanchoe* leaves juice and other preparations. According to Gehrig *et al.* (2001)[6] 125 species of genus *Kalanchoe* can be distinguished, divided into 15 taxonomic groups; they possess very high levels of bioactive compounds[7]; such as, flavonols, phenolic acid glucoside, flavonoid- kaempferol, quercetin and quercitrin, citric acid, malic acid, etc. Some sps. are edible, too. Those exhibit remarkable antioxidant activities and have been employed as natural antioxidants. The therapeutic use of *Kalanchoe* is considerably limited by lack of clinical evidence.

Flavonoids[7,8] are the main phytochemicals, which involve in the protection of kidney and gall-bladder from the stone formation.

The leaves of *Kalanchoe* plants are the main source of all its medicinal properties, used internally as well as externally, prepared as decoction, infusion, juice, syrup, poultice, paste, etc.[9]

Morphologically, the leaves and inflorescences are different in different *Kalanchoe* spp. The plant heights either do not exceed one meter or can reach 6 meters. Stem may be woody in bigger spp. Leaves are succulent, fleshy, greenish in colour and edges are serrated, crenate or tooth-shaped. These are highly variable in shape and size among varieties, viz. elliptical, round, oval, cylindrical, etc. Flowers appear in panicles, corymbs or cymes, which are erect or pendulous and are of various beautiful colours- red, yellow, orange, etc.; thus, the plants are ornamentally important, too. Flowers bloom in winter, which may remain fresh for 6-8 weeks.

A unique feature of *Kalanchoe* plants is the method of reproduction[10] i.e., through epiphyllous buds, which are adventitious or foliar buds; produced on the crenate margin of the leaves, each at the end of a vein. These buds may drop from the leaf and grow up into new plants, or they may drop together with the leaf and then grow up. Thus, the plant is termed as "sprout leaf plant". An evolutionary advantage to survive even in the adverse environmental conditions attributed towards its ability to propagate through epiphyllous budding.

Anatomically, *Kalanchoe* representing Crassulacean Acid Plants[11] (CAMp), which are recognized as a photosynthetic pathway distinct from C_3 and C_4. So, these plants are nocturnal oxygenators and also have air purifying property. These plants are termed succulent plants, characterized by thickened stems and leaves modified for water and acid storage.

MATERIAL & METHOD

Field Survey and Plant Collection-

The study was conducted in Ranchi district, the capital city of Jharkhand, lies between 23°15 N Latitude and 85°15 to 85°24 Longitude, near to the Tropic of Cancer. The physical factors, like, average annual temperature and precipitation of Ranchi create suitable climate for *Kalanchoe* propagation. Twelve species of genus *Kalanchoe* were collected during the study period January, 2021 to January, 2022 from 8 different administrative blocks of Ranchi district, Jharkhand - Ormanjhi, Angara, Namkum, Kanke, Ratu, Bero, Bundu and Burmu. The plant specimens were photograghed and identified with the help of different flora.[12]

Identification-

The tradition knowledge was collected from informants using open interviews and semi-structured questionnaires. All local plant names collected during this study were authenticated by Taxonomic Department, University Department of Botany, Ranchi University, Ranchi, which were further identified and correlated their medicinal properties by the following Botanical Databases (update-2018) -

I. Royal Botanical Gardens, Kew/ Plants of the World Online.
II. Medicinal Plant Names Services (MPNS) v.10 (2010)
III. Integrated Taxonomic Information System (ITIS)

Chemical constituents of investigated plants were also studied by the recent open access authentic research articles.

RESULT

This research is on finding the 12 spp. of genus *Kalanchoe* of crassulaceae family in different location of Ranchi

district of Jharkhand, which are the most reliable and exotic ethnomedicinal spp. used against urolithiasis and cholelithiasis by the endemic- ethnic people, albeit, various other ailments are also cured by these wonderful plants. The following pictures are of those findings: -

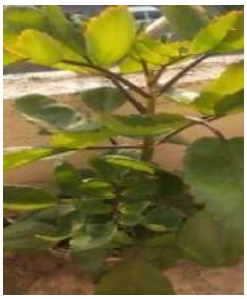

Fig.1- *Kalanchoe pinnata* (Lam.) Pers.

Fig.2- *Kalanchoe mortagei* Raym.-Hamet & H. Perrier

Fig.3- *Kalanchoe fedtschenkoi* Raym.-Hamet & H. Perrier

Fig.4- *Kalanchoe laciniata* (L.) DC.

Fig.5- *Kalanchoe prolifera* (Bow.) R.-Hamet

Fig.6- *Kalanchoe delagoensis* Eckl. & Zeyh.

Fig.7- *Kalanchoe laxiflora* Baker

Fig.8- *Kalanchoe spathulata* DC.

Fig.9- *Kalanchoe blossfeldiana* Poelln.

Fig.10- *Kalanchoe gastonis-bonnieri* Raym.-Hamet & H. Perrier

Fig.11- *Kalanchoe daigremontiana* Raym.-Hamet & H. Perrier

Fig.12- *Kalanchoe brasiliensis* Camb.

DISCUSSION

The omnipresence of various species of genus *Kalanchoe* are often referenced in folklore, and commonly used in traditional medicine worldwide for the treatment of urolithiasis and cholelithiasis, beside these also used for fever, pain, abscesses, bruises, contused wounds, coughs, skin diseases, microbial infections, hypertension, respiratory diseases, gastritis, ulcers, diabetes, cancer tumors, rheumatism and inflammation.

The presence of important bioactive phytochemicals in the 12 selected *Kalanchoe* spp. are responsible for their great medicinal values; flavonoids are the common secondary metabolite among them, they are polyphenols, responsible for all antioxidant, anti-inflammatory, antiurolithiatic, anticholelithiatic and immunomodulatory activities in human health.

1. *Kalanchoe pinnata* (Lam.) Pers.[13,14] [Fig.1] "Leaf of life, Tree of life, Air plant" is the most popular, easily available and reliable succulent in folklores and is widely naturalized throughout Jharkhand. Its leaves are astringent, antibacterial, antiseptic, diuretics, and many more; thus, used in the treatment of acute nephritis and lithiasis.

   It contains polyphenolic compounds such as flavonoids and phenolic acids (e.g., *p*-hydroxycinnamic, caffeic, *p*-coumaric, ferulic, *p*-hydroxybenzoic, protocatechuic acids, quercetin, kaempferol, luteolin, astragalin, rutin, and patuletin).
2. *Kalanchoe mortagei* Raym. - Hamet & H. Perrie[15] [Fig.2]: The plant contains caffeic acid, quercetin, flavonoids, kaempferol, phenolic compounds, *p*-coumeric acid, ferulic acid, lupeol, lupeol acetate, B-sitosterol, etc. It has antimicrobial, anticancer, etc. property and used to treat digestive disorder, roots are used for treating parasitic worm related diseases.
3. *Kalanchoe fedtschenkoi* Raym. - Hamet & H. Perrier[15] [Fig.3]: It has caffeic acid, quercetin, kaempferol, *p*-coumaric acid, ferulic acid. It is a modal organism for research into Crassulacean acid metabolism (CAM). This spp. is used as an analgesic, antimicrobial.
4. *Kalanchoe laciniata* (L.) DC.[14] [Fig.4]: "Christmas tree plant", "Cathedral bells", "Hemasagar plant"- ornamental and medicinal herb. Same medicinal values, as the leaves possess diuretic, astringent, and hemostatic properties
5. *Kalanchoe prolifera* (Bow.) R.-Hamet[16] [Fig.5]: "Blooming boxes, Flaming Katie, Jurassic kale, etc." The fresh leaves may well be used in a similar way as with *K. pinnata*
6. *Kalanchoe delagoensis* Eckl. & Zeyh. (Syn. *Kalanchoe tubiflora* Harv.) [Fig.6]: often called 'mother of millions' or 'chandelier plant'; ornamental- fleshy, cylindrical green with grayish spots on leaves, each leaf apex may have 4-6 teeth from which arise plantlets and orange, red, or purple bell-shaped tubular flowers on an unbranched stem appear gorgeous look. It has the same medicinal values, but, doses greater than 5 grams should not consumed due to toxicity.
7. *Kalanchoe laxiflora* Baker[17] [Fig.7]: "Milky widow's thrill"- ornamental and medicinal both. It has beautiful elliptically shaped leaves with red tips.
8. *Kalanchoe integra* (Medik.) Kuntze[18] (Syn. *Kalnchoe spathulata* DC.) [Fig.8]: "Never die, Parnabija"- hepatoprotective, diuretic, anti-inflammatory, antidepressant and central nervous system depressing

activities. Its folklore medicine is used for hypertension, cardiovascular disorders like stroke, earache, asthma, leaf water is sedative, root-decoction is tonic for pregnant women (in Kerala), leaf-sap is for intercostal and intestinal pain. This species is very similar to *K. laciniata*, although, *K. integra* leaves are entire, irregularly crenate, and the cymes are glandular-pubscent, while *K. laciniata* leaves are entire to lobed and the cymes are not glandular.

9. *Kalanchoe blossfeldiana* Poelln.[19] [Fig.9]: "Flaming Katy", "Florist Kalanchoe"- ornamental and medicinal herb. Its leaves are used in medicinal purposes to treat inflammation, rashes, burns, ulcer, skin infections, pain, etc.
10. *Kalanchoe gastonis-bonnieri* Raym.- Hamet & H. Perrier [Fig.10]: "Life Plant", "Donkeys ear", "Mal madre"- possesses large leaves that can measure up to 50cm in length. Leaves have been used in tradition system of medicine against genitor-urinary troubles and vaginal infections. Its leaf juice is employed vaginally as a contraceptive in Mexican traditional medicine.
11. *Kalanchoe daigremontiana* Raym.- Hamet & H. Perrier[20,21] [Fig.11]: "Devil's backbone" is used to treat lithiasis, rhinitis, ulcers, cancer, back sprain, disease of ear, nose & throat, protects the female reproductive system and are good analgesic and muscle relaxant. It contains α- and β-amyryne, stigmasterol, phenolic compounds, organic acids, alkaloids, vitamin E & C, and tannins.
12. *Kalanchoe brasiliensis* Camb. [Fig.12]: It is the only native *Kalanchoe* spp. in Brazil, widely used to treat urinary infection, injury, allergy and inflammatory processes. It seems very similar to *Kalanchoe pinnata* both in medicinal property and morphology. The phytochemicals present are flavonoids quercitrin and isoquercitrin, patuletin rhamnosides, kalambroside A, B and C.

Kalanchoe gracilis (L.) DC- native to Assam, Taiwan. Possess same medicinal property to treat lithiasis. It is used for the treatment of tissue inflammation.

Most *Kalanchoe* species are cytotoxic[22] due to the presence of some cardiotoxins in high level, there are others that, if used correctly, can help us improve health. *Kalanchoe* plants contain Bufadienolide Cardiac Glycosides, that can cause cardiac poisoning, particularly in grazing animals and pets. WHO placed cardiac glycosides in class 1a; means highly poisonous. Several toxicological studies have been conducted that dealt with isolation and elucidation of its major compounds. The aerial part of the plant contain more toxins; the leaves contain Bufadienolides, like, Bryotoxin A, B, C which are very similar in structure and activity as two other cardiac glycosides, Digoxin and Digitoxin and possess antibacterial, antitumor, cancer preventive and insecticidal actions.

CONCLUSION

From this research work it can be concluded that the selected global ethnomedicinal *Kalanchoe* spp. are the most reliable in having Cardiac Glycosides in low quantity, natural antioxidants and substantial in using against urolithiasis and cholelithiasis diseases. The Baidyas or herbal practitioners of Jharkhand are successful in using these plants.

The Flavonoids- rich these reliable species ensure their antilithiatic activities and show promising effect in the management of kidney stones and gall-stones. *Kalanchoe* leaf is an astringent, sour in taste and has hot potency. The high and diverse bioactivity of *Kalanchoe* is important but from the medical point of view, it needs to be excluded from broad uncontrolled use, particularly as a nutrient, because of the possible toxicity. Plant should not be used in pregnancy, since it has traditionally been used during labour and can stimulate the uterus. It is immune-modulating and should not be used chronically for long periods of time. Its use is restricted to cardiac patients. It is stated that, in too high doses, *Kalanchoe* is dangerous to human, also not used by animals.

Kalanchoe pinnata is the most popular and widely used species, now, most of these *Kalanchoe* spp. become endangered, which needs to be conserved as well as explored for its significant biological properties. Their potential medicinal values may be used as crude material in the pharmaceutical industry.

The information reported in this work might contribution to the recognition of the importance of edible and reliable *Kalanchoe* spp. as well as to direct further studies.

ACKNOWLEDGEMENT

The authors wish to thank Prof. (Dr.) Kunul Kandir, Dean, Faculty of Science and HOD, University Department of Botany, Ranchi University, Ranchi for valuable

suggestions and technical support. Also thankful to the local eminent for sharing their valuable knowledge and experience in completing he research.

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RUJOST, ISSN: 2319-4227, Vol.7, No.1, January, 2022, pp. 23-26

A Biaanual Peer Reviewed Referred Ranchi University Journal of Science & Technology

Botany

Ethnomedicinal and ethnobotanical studies of *Spinacia oleracea*: An important leafy vegetable used in Indian diet

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Abstract: *Spinacia oleracea* commonly known as palak is an edible flowering plant belongs to *Amaranthacea* family. These leafy vegetables are natural antibacterial agent with numerous nutritional and medicinal benefits to human being. It exhibits the presence of proteins, glycosides, carbohydrates, quinones, terpenoids etc. Spinach having large quantity of vitamin A which moderate the oil in skin pores and hair follicles to produce moisturizing activity in the skin and hair. This paper includes ethnobotanical and ethnomedicinal properties of *Spinacia oleracea* plant species found in Ranchi district of Jharkhand and widely used by people in curing various diseases like anaemia, sore throat, lung and liver inflammation etc.

Keywords: *Spinacia oleracea,* disease, nutrition, ethnomedicinal properties, health.

INTRODUCTION

Spinach (*Spinacia oleracea*) is an edible flowering plant. It is green leafy vegetable grown in most part of the world. It is extensively cultivated in India for its nutritional leaves. Spinach leaves are best source of dietary magnesium. They help in energy metabolism, maintaining muscles, nerve's function, healthy immune system and blood pressure. They contain enormous number of micronutrients and chemical compounds that are having important medicinal uses, human well-being and healthy lifestyle.

Spinacia oleracea (spinach) is reported to be a good source of minerals, vitamin B complex, carotenoids, ascorbic acid, phenols and Omega 3 fatty acids. There are two types of spinach (*Amaranthus* sp.), the wild spinach and cultivated spinach. A cool climate is best for producing spinach. It is low in calories.

The phytonutrients present in green leafy vegetable gives many common health benefits such as protection from eye disorders, oxidative stress, iron deficiency etc.[1]

The Ranchi District of Jharkhand State is highly rich in biodiversity and ethnomedicinal properties. The district endowed with plant diversities due to its rich climatic conditions. The documentation and identification of medicinal plants, used by ethnic tribes of Chota Nagpur plateau has been worked out.[2]

Cultivation

Spinach is popular because of its high yield, wide adaptability to varying soil and climatic conditions and high nutritional value. It is a cold season crop. It can be grown pure or as a mixed crop with peas, cabbage and other comparatively longer duration vegetables. It is sown during September- November in the plains and during February- April in the hills.

Spinach naming

Hindi	–	Palak, Isfanaj
English	–	Spinach
Sanskrit	–	Chhurika
Kashmiri	–	Palakh
Bengali	–	Palang
Tamil	–	Pasalai, vasayleykiray
Marathi & Gujrati	–	Palak
Odiya	–	Palak sag, mitha palanga
Punjabi	–	Palak, valayati sag, Isfanaj
Assam	–	Malangas

Scientific classification

Kingdom	–	Plantae
Class	–	Angiosperm
Family	–	Amaranthacea
Genus	–	*Spinacia*
Species	–	*S. oleracea*

Ethnobotanical uses

Leaves – Leaves are digestible, wholesome, antipyretic, antihelminthic, useful in urinary concretion, sore throat pain in joints, inflammation of lungs and bowel, cold, sneezing and arrest vomiting.

Stem – Succulent, piped, smooth, sometimes reddish.

Plant – It is useful in diseases of blood and brain, asthma, leprosy. In experiments, it has been shown to have hypoglycemic properties.

Seeds – It is useful in fever, diseases of brain and heart, liver inflammation and jaundice etc.

Micronutrients

Spinach is a good source of minerals, vitamin B complex, ascorbic acid, carotene. It is also an important natural source of vitamin K. The total content of folic acid in spinach is 0.12 mg/ 100 hm. Phytochemicals are naturally occurring components in fruits, green vegetables, legumes, grains and plants food have been linked with reducing the risk of major degenerative disease.

Therefore, there is a need to evaluate the potential of vegetables in relation to the provision of basic micronutrients and phytochemicals which help in providing data for selection of proper green leafy vegetable.

Herbal medicine

According to WHO, at least 80% people in developing countries depend on herbal plants. The natural medications are widely formulated because they are better compatible with human body, easily available with less side effects.[3]

The interest in medicinal plants has grown possibly due to their availability, accessibility and the general belief that they demonstrate minimum side effects.

Ethnomedicinal survey and pharmacological studies shows that large number of plants have been found here which are utilized by local people.[4]

MATERIALS & METHODS

In this study, available literature on this plant was used. Ethnomedicinal study was carried out by extensive survey conducted in villages of Ranchi district of Jharkhand. The survey was conducted using questionnaire and discussion with knowledgeable persons including healers, vaidyas, local practitioners etc. Journals, book chapters, topic related information were also collected from internet.

RESULT & DISCUSSION

The information gathered from local healers, herbal practitioners were recorded. A number of research works have been performed on Ethnomedicinal plants. People have been using medicinal plants from time immemorial for the treatment of various types of disease. *Spinacia oleracea* is highly valuable for various therapeutic activities. Inorganic constituents present in spinach leaves are calcium, magnesium, sodium, potassium, iron.

Spinach is highly rich in fiber and water, both of which help to prevent constipation and promote healthy digestive tract and gives healthy skin.[5-8]

It is excellent source of antioxidants and vitamin A which is essential for normal vision and normal cell division, growth and development of bone and teeth and for health of skin and mucous membrane and tissues that line intestine, airways and other organs. This paper will add contribution to the existing knowledge regarding ethnobotanical and ethnomedicinal properties of *Spinacia oleracea.* This paper will also help in enriching the traditional knowledge of herbal medicines along with scientific touch.[9-14]

CONCLUSION

The commonly consumed green leafy vegetable in India selected for present study contain phytochemicals, vitamins, antioxidants etc. which are helpful in preventing some deadly disease. *Spinacia oleracea* is a good nutrient rich leafy vegetable with antioxidant property that can be used as therapeutic medicine.

This vegetable can be used as curative medicine for many oxidative stress induced diseases. Spinach having phytochemicals and other vitamins produce the moisturizing activity to skin and hair. This work shows that *Spinacia oleracea* is one of the most cherished vegetables in India. This study may be useful for future experimental and clinical studies and the development of future therapeutics.

ACKNOWLEDGEMENT

We would like to extend our sincere thanks to Prof. (Dr.) Kunul Kandir, Professor & Head, University Department of Botany, Ranchi University, Ranchi, Jharkhand, India for providing necessary laboratory facilities and continuous encouragement.

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RUJOST, ISSN: 2319-4227, Vol.7, No.1, January, 2022, pp. 27-30
A Biaanual Peer Reviewed Referred Ranchi University Journal of Science & Technology

Botany

Study of ethnomedicinal properties of aquatic plants grown in Basargadh Pond, Tupudana, Ranchi

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Abstract: Aquatic plants are important to maintain the aquatic ecosystem. These are the plants which spend at least a part of their life cycle in water. These plants are the source of number of chemicals which has immense medicinal values. An ethnobotanical survey was conducted in Basargadh Pond, Tupudana, Ranchi to collect information on plants used for various diseases among the inhabitants of Ranchi district. The present study is to analyse the medicinal uses of these aquatic plants and make aware the public about the importance of pond plants.

Keywords: Aquatic plants, ethnomedicinal, inhabitants, ethnobotany

INTRODUCTION

State capital of Jharkhand, Ranchi is blessed with rich biodiversity including number of water bodies like dams, waterfalls, ponds, rivers, ditches etc. These water bodies harbor a number of plant species. These plants spend their life in water and grow for at least a part of their life cycle in water. From the time immemorial, these plants are used as food, fodder, medicines etc. Several species of aquatic plants are the source of number of chemicals which has immense medicinal values.[1-3] Ethnobotany deals with the relation between man and plants which help to record the knowledge of traditional people about medicinal properties of diverse plants. Though in Jharkhand a large variety of locally available plant species are used by local people as herbal medicines in curing various diseases. Some of the herbal plants they use are aquatic plants. The present work reviews the medicinal use of aquatic plants with the help of authentic publications and by incorporating the traditional knowledge of tribal and kabirajs.

METHODOLOGY

A study of ethnomedicinal properties of aquatic and semi aquatic plants was carried out in Basargadh Pond, Tupudana, Ranchi. To make the accurate and intensive study of aquatic and semi aquatic species, the study area was visited at regular intervals,[4-6] twice or thrice in every season to encounter the plants at their flowering and fruiting conditions.[7]

During the field work several important characters like habit, habitat, height of plants, colour of flower, and association of plants with other plants, field numbers, local name and their uses were noted down. After collection, the plants were pressed in field herbarium press and news papers. Some plants were also kept in the dilute solution of formalin. After completion of specific identification of specimens, the plants were identified[8] with the help of local floras. Various use of the aquatic plants was gathered by the local people. In order to know the medicinal uses of the plants,[9] the local herbal practitioner, kabiraj vaidyas were also interviewed.

RESULT & DISCUSSION

Altogether 10 hydrophytes,[10] of medicinal importance were recorded from the study area. Followings are the plants utilized by the local people and vaidyas in curing various diseases.

***Oxalis corniculata* (Oxalidaceae)**

Annual or short-lived perennial plants growing only 5-10 cm tall but spreading at the roots to form a mat of growing 30 cm or more wide. This plant possesses anti-inflammatory, antioxidant, anticonvulsant, antifungal, antiulcer, anticancer, antidiabetic, hepatoprotective, antimicrobial and wound healing properties.

Leaves of the plant are used as antidote to poisoning by the seeds of dhatura species, arsenic and mercury. The leaf juice is applied to insect bite, burn and skin eruption. It is used in treatment of influenza, fever, diarrhoea, urinary tract infection, sprain and poisonous snake bites. The plant is used as an antiscorbutic in the treatment of scurvey.

***Nymphoides indica* (Menyantheceae)**

This plant is commonly known as kumudini. Fast growing perennial aquatic plants spread by rhizomes, forming cluster of leaves, with clustered white flower about 1cm across. A blooming colony indeed looks like snowflakes on water. It has flat round floating leaves and delicate white flower blooming in summer.

This plant is useful for treatment of brain and nervous system disorder like migraine, insomnia, anxiety and epilepsy. It is also used in anaemia, jaundice and asthmatic condition.

***Nelumbo nucifera* (Nymphaceae)**

Commonly known as kamal, an aquatic herb with stout, creeping rhizome, leaves peltate, petioles long, smooth or with small prickles, flower large white or roasy. The plants possess astringent, cardiotonic, hypotensive properties.

A paste of rhizomes is applied in ring worm and other cutaneous infections. Root paste is used in piles. Flowers are used against diarrhoea, fever, liver ailment and cardiac problems. Seeds are used in skin related problem. Paste of young leaves is applied on forehead to get relief from headache.

***Nymphaea nouchalli* (Nymphaeaceae)**

Commonly known as water lily, a large aquatic herb with tuberous rhizomes, peltate leaves, flower solitary, fragrant, variable in colour, deep red or pure white, fruit a spongy berry. Flowers are astringent and cardio tonic.

Seeds are used as a cooling medicine in cutaneous diseases. The decoction of leaf is used in irregular mensuration. The rhizome is used in dysentery and dyspepsia.

***Eichhornia crassipes* (Ponditeriaceae)**

Commonly known as jalkumbhi or water hyacinth, a perennial aquatic plant with prostrate and densely branches stems. The inflorescence can have one to thirty conspicuously attractive flowers, mostly lavender to pink in colour rarely white. Plant possesses astringent and antioxidant properties. Leaves are said to be used as a carotene rich table vegetable. Young leaves are used as manure.

***Ipomoea aquatica* Forsk (Convolvulaceae)**

Commonly known as kalmi saag. Plant is widely distributed perennial herbs with long hollow stem rooting at the node, flower white or pale purple with dark purple eye. Dry young leaf juice has purgative property.

Young leaf decoction is used as blood purifier. The young terminal shoot and leaves are used as vegetables and in salad. Buds are used in the treatment of ring worm.

***Centella asiatica* (Apiaceae)**

Commonly known as brahmi, creeping herbs with long stolon rooting at the node, reniform, orbicular, crenate leaves, long petiole with white or pinkish white flowers.

It has very rich medicinal values. It is commonly used as green leafy vegetables. Fresh leaves are chewed and taken to relieve from acidity and peptic ulcer. Leaf juice is used in diarrhoea, amoebic dysentery, stomach trouble, leprosy, gastric disorder. It is commonly used for wound healing and memory improvement.

***Trapa natans* Linn. (Trapaceae)**

Commonly known as singhara or paniphal, rooted aquatic herb, stem elongate and submerged, leaves dimorphic, flower solitary, white or lilac. Plant has nutritive, stomachic, astringent properties.

Fruit is eaten as raw or cooked. It contains iron, calcium, starch, phosphorus, manganese etc. Dry fruit is used as flour.

***Ludwigia adscendens* L. (Onagraceae)**

Plants having stem prostrate or ascending, bearing silver white spongy, spindle shaped pneumatophores, flower solitary in leaf axils.

Whole plant is boiled in water, after cooling this water is advised to take thrice a day in fever and cough. It is also used in pursuti fever, headache, pain in ear.

***Alternanthera sessile* (Amaranthaceae)**

Terrestrial plant with small white flower in dense globose head. It has ophthalmic properties.

Leaf extract is useful in various types of eye trouble. It is used as green vegetable. It helps in blood purification and it cures liver disorder. It is good laxative also. Young shots contain protein and iron which is highly nutritive.

CONCLUSION

During the research work a number of plants were known which has immense potential to cure the various diseases and used by tribal with great believes. From the fact it is concluded that edible and medicinal properties of aquatic plants species in Basargadh Pond, Tupudana, Ranchi will change the status of plants from worst weed to important medicines and food which are useful for mankind and help to conserve the pond ecosystem.

ACKNOWLEDGEMENT

Authors are thankful to the local people kabiraj and herbal practitioners who shared their important informations regarding the ethanomedicinal values of various aquatic plants and their uses in various diseases.

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RUJOST, ISSN: 2319-4227, Vol.7, No.1, January, 2022, pp. 31-34

A Biaanual Peer Reviewed Referred Ranchi University Journal of Science & Technology

Botany

Marsilea quadrifolia- An edible and remedying fern

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Abstract: A survey has been made on the importance of one such unexplored well known plant *Marsilea quadrifolia* (Common name - Sushni Saag) commonly used by the local people of Ranchi, Jharkhand for its edible and curative properties. *Marsilea quadrifolia* is a pteridophytic plant that is used by local people. Vegetable sellers use to sell this saag in their local market. This saag is used for various therapeutic purposes for numerous other ailments of nervous system and tribes also use this for its nerve relaxant nature. Sushni saag contains high nutritional value.

Keywords: *Marsilea quadrifolia*, Plant parts, Ethnomedicinal, Tribes, Sushni saag.

INTRODUCTION

European Water Clover is a belligerent and encroaching non-native aquatic species[1] of Europe and Asia. *Marsilea quadrifolia* was found in deep water on bottommost, abundant in Clay and silt. It forms more or less monospecies group.[2] *Marsilea quadrifolia* is registered in the Red Data Book of the International union for Conservation of Nature (IUCN) (Tuba, 1995).[3] *Marsilea quadrifolia* is described as a rare species in Red List floras in Europe.[4] *Marsilea quadrifolia* is eaten by various tribal communities like Mudhuvars of Anamailais hills, Western Ghats, Coimbatore district Tamil Nadu, Pulaiyars, Malasars in India as per their periodic availability.[5] *Marsilea quadrifolia* have an intense antibacterial, antioxidant and Cytotoxic effect and have potentiality to use in medicine.[6] Medicinal Plants contain Secondary metabolites like Alkaloids, Flavonoids etc. Alkaloids form Crystalline salts when combine with acids, as alkaloids are nitrogenous organic compounds.[7] *Rana* sp. (Frogs) is very fond of *Marsilea quadrifolia*.[8] For maintaining the riverine ecosystem aquatic and semi aquatic plants play a major role.[9] *Marsilea quadrifolia* has been recorded in the riverine beds of six major rivers of Bengal[10] (Dwarkeswar, Kansai, Keleghai, Rupnarayan, Shilabati and Subarnarekha). It has been used for the wetland restoration and for nutrient mitigation from the fresh water.[11]

Marsilea quadrifolia is a pteridophyte belonging to the family Marsileaceae and it is commonly known as European water Clover. In India it is known as Sushni Saag, and it is extensively distributed all over India. It is an aquatic and amphibious plant whose roots are submerged in mud, soil or in shallow pools. The plant prefers to grow in Sandy and loamy soil, or it can even grow in water.

The plant body is sporophytic, looks like a four leaved Clover plant and is differentiated into roots, stem and leaves. Rhizome of this fern is branched and show indefinite growth. The rhizome is branched and the branch arises from the leaves. Leaves show circinate venation when young. At the tip of each petiole there found four leaflets which are of equal size and hence, they are commonly known as four leaves Clover.

This fern is commonly known as Sushni in Jharkhand, West Bengal and Bangladesh. This fern is available in the vegetable markets as a green leafy. The name “Sushni” in Bengali means ‘don't sleep’, because of its sleeping inducing effect. Tribal People and other local people use this saag for relaxing in the night after a prolonged work.

MATERIALS & METHODS

This paper is based on survey on the local people and vegetable sellers of Ranchi that lies between latitude 23°22'N and longitude 85°20'E near to the Tropic of Cancer. It covers an area of 175 km^2 and its average elevation is 651 m above sea level. Ranchi, which is located in the southern part of the Chota Nagpur plateau. Ranchi has a humid sub tropical climate. The survey has been done by using standard ethnobotanical methods like interviewing the local knowledgeable people, vegetable sellers, vaidyas etc. Local people, vegetable seller collects *Marsilea quadrifolia* (sushni saag) from the low-lying moist areas from water bodies. After collection of this sushni saag vegetable Sellers used this saag to sell in their vegetable market. Local people also buy this saag or they collect it from their nearby water bodies.

Fig: Photos of *Marsilea quadrifolia* taken during collection

RESULTS & DISCUSSION

Availability of this saag in the market depends only and only on rain. If the rainy season comes in the first week of June, then this saag is available in the market by the last week of July till the last week of November if and only when soil remain saturated. Their leaves, petioles, whole part of the plants which are sold in the market has numerous culinary uses which is listed in the table1.

The distinct knowledge of the plants as a Source of medicine dates back to the Rig Veda times. Much of the medicinal knowledge about plants is often passed through orally from one generation to another. This knowledge is dissipated due to rapid industrialization and modernization. Backing up the indigenous knowledge through ethnobotanical is important for the preservation and implementation of biological resources. Frequent uses of *Marsilea quadrifolia* for ethnomedicinal interest have been recorded and its uses are mentioned in table 1.

Table 1- Medicinal use of *Marsilea quadrifolia* (Sushni saag)

Plant Part Used	Preparation	Uses
1) Leaves and petiole	It is cooked in oil with pinch of salt & masalas.	Its regular intake lightens the hypertension, sleep disorders and headaches.
2) Entire Plants	The plant is grind in a mixer jar along with some garlic to make a juice.	It cures cold and cough as well as fitful and spasmodic condition of leg and muscles.
3) Shoots	The plant is grind in a mixer jar to make a juice	It is used to cure respiratory problems especially for babies and to cure cough.
4) Whole plant	The plants' part is crushed with little amount of sugar or honey.	It is used to cure infantile diarrhea.[12]
5) Young Stems and leaves	The leaves and petioles are cooked	It is used as a famine food at the time of scarcity of food.[13]
6) Young leaves	The leaves have been crushed to make juices out of it and the juices are used in the nostrils twice a day.	It is used to cure migraine.
7) Whole plant	Whole plant along with roots is made into paste with whole plant of *Centella asiatica* and applied it for 7 days regularly about 2 times in a day around breast.	It improves the lactation after childbirth.[14]

8) Spores	Ground the spores and mix it with flour.	It is used for making bread as it is rich in starch.[15]
9) Entire Plant	100 gm of curd which is prepared from black cow's milk, along with 10 gm of entire fresh plants paste is mixed together and this is given orally in an empty stomach for 1 month once in a day.	It is used to prevent epilepsy.
10) Leaves	Leaves are plucked and squeezed the juice and used as a mouth freshener.	Tribes of Bangladesh used it for lesions a tongue or in the mouth and rheumatism.[14]

CONCLUSION

Marsilea quadrifolia is an important marketable genus which has remarkable role in the lives of local people and vegetable sellers. It is a pteridophyte and is suggest for further phytochemical, pharmacological investigation & nutritional analysis, which might result into the discovery of new drug molecules for human welfare.

This plant is being exploited from the aquatic, semi aquatic and riverine ecosystem. For maintaining the riverine ecosystem aquatic and semi aquatic macrophytes play major role. Encouragement of *ex-situ* propagation of this genus will give rise to regular source of income.

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RUJOST, ISSN: 2319-4227, Vol.7, No.1, January, 2022, pp. 35-40
A Biaanual Peer Reviewed Referred Ranchi University Journal of Science & Technology

Botany

Documentation, collection and organoleptic studies of some plant based gums and resins found in Ranchi district

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Abstract: For time immemorial, various parts of the plants such as stems, leaves, seeds, roots, fruits, flowers, etc. have been used for treating various human and veterinary ailments. Excretory products of plants such as oils, latex and gums are known for their therapeutic potential. Such plants are also widely found in Jharkhand. The present study has been undertaken for collection and documenting these excretory products secreted from such plants as *Prunus persica* (L.) Batsch, *Mangifera indica* L., *Anacardium occidentale* L., *Pinus roxburghii* Sarg., *Moringa oleifera* Lam., *Dalbergia sissoo* Roxb., (found in Ranchi district) as also their organoleptic characters and medicinal uses.

Keywords: Ethnomedicine, Gums, Resins, Therapeutic potential.

INTRODUCTION

Increased use of chemicals for controlling various microbial infections has undesirable consequences for the environment. This has caused exploration of naturally occurring therapeutic potential compounds. In the recent years, there has been growing interest in the development of safer anti-microbial agents from plant products to control pathogenic infections. Stems, leaves, seeds, root, fruits, flowers, etc. are being used for treating various plant diseases. Different parts of the various plants are used for curing many diseases. Excretory products of plants such as oils, latex and gums have also been reported to be used in ethno-medicine to treat several human and veterinary ailments.[1] Gums-resins such as *Ferula foetida regel* (asafoetida), *Commiphora molmol* (myrrh), *Boswellia serrata* (salai), *Commiphora wightii* (guggul), etc. have been reported to be used as medicines.[2-4] Natural gums or resins derived from plant sources have also been reported for treating plant pathogens.[5]

There are three categories of natural resins and gums (NRGs) derived from the plants, namely natural resins, natural gums, and gums-resins.[6] Natural resins and gums are metabolic by-products of tissues either in normal course or often as a result of either injury in the bark or wood or disease by insects. The natural gums and resins are polymeric, biodegradable and non-topologically originated, they have wide variations in their characteristics and properties.[7]

Natural Resins: Resins are amorphous mixtures of essential oils, oxygenated products of terpenes and carboxylic acids and are separated as exudates from specialized structures in a wide range of plants which are insoluble in water but soluble in certain organic solvents. They probably function as plant defenses.[5] Resins are widely distributed across the plant kingdom although a few families are notable in accounting for a large proportion of the resins of commerce (e.g., Leguminosae, Burseraceae and Pinaceae).

Natural Gums: Gums are a group of plant products formed primarily due to the disintegration of plant cellulose and this process is known as gummosis. Gums are produced by members of a large number of families. The important gum yielding trees are *Acacia nilotica* (babul), *Acacia catechu* (khair), *Sterculiaceae urens* (kullu), *Anogeissus latifolia* (dhawra), *Butea monosperma* (palas),

Bauhinia retusa (semal), *Lannea coromandelica* (lendia) and *Azadirachta indica* (neem). Gums are also extracted from seeds of certain plants like *Cyamopsistetra gonoloba* (guar), *Tamarindus indica* L. (tamarind), *Cassia tora* L., etc. *Cyamopsistetra gonoloba* (Guar gum) is the prominent seed-based natural gum.

Gum-resins: Gum-resins are a mixture of both gums and resins and possess the properties of both the groups. They contain traces of essential oils. These are usually dried from the plant growing in dry and arid regions. Some of the commonly used gums-resins are *Ferula foetida regel* (asafoetida), *Commiphora molmol* (myrrh), *Boswellia serrata* (salai), *Commiphora wightii* (guggul),etc.

Gums and resins are low volume, high value product. India is one of the biggest producers of gums and resins in the world. India is a rich centre of plant biodiversity having more than 45,0000 plant species including about 120 gum and resin yielding plants. In India, resins and gums are sold to earn a living by the tribes. In the present day, the use of natural gums are numerous and they are employed by a large number of manufacturing industries including food and pharmaceutical industries.[8] The use of gums and resins in India as medicine can be traced back to the ancient days of Acharya Charak who wrote Charak Samhita Granth.[9]

Several studies have been carried out on various plant secreting gums and resins but majority of the work has been done on *Boswellia serrata, Commiphora mukul, Gardenia resinifera* and *Shorea robusta* against some plant pathogenic fungi.[2,4,5,10,11]

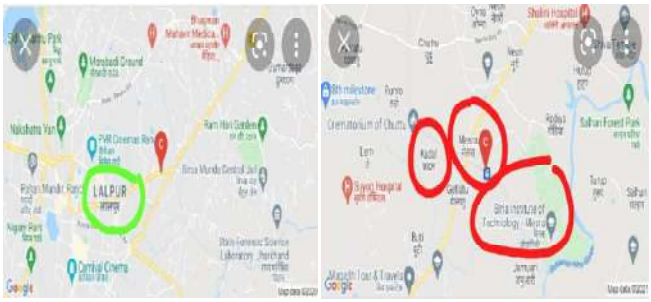
Various screening has been done for antimicrobial activity of plant secreting gums and resins, but most of the studies are being done on gram-positive and gram-negative bacteria.[3,12-14]

Similarly, various compounds have been isolated from plant secreting gums and resins in which they found that maximum of them possessed alkaloids, flavonoids, terpenoids, tannins, saponins and glycosides and many phenolic compounds but concluded that sesquiterpenes is present as a major constituents for the inhibition of bacterial growth.[13,15-17]

The present study deals with the collection of different plant based gums and resins in different areas of Ranchi district. On the basis of their therapeutic uses, this study also deals with the documentation of these excretory products.

StudyArea: Ranchi District of Jharkhand

Plant samples were collected from different areas of Ranchi district. (Figure 1 & 2)



Figures (1&2): Map of the different areas of Ranchi district from where plant samples were collected.

MATERIALS & METHODS

Collection of Plant materials

Plant based gums and resins were collected from different areas of Ranchi district in different seasons on the basis of their availability. Plant twigs were also collected and brought to the laboratory for identification by using 'The Botany of Bihar and Orissa', H. H. Haines (1922). Collected plant twigs were also authenticated by the taxonomists of the University Department of Botany, Ranchi University, Ranchi and herbarium was prepared. Fully dried collected samples were stored in air tight container.

The list of collected plant materials are as follows (Table 1):

Table 1. List of plant based gums and resins collected from different field of Ranchi district.

Sl. No.	Scientific Name	Common Name	Date of Collection	Place of Collection
1.	*Prunus persica* (L.) Batsch	Satalu	10th Sept.,2020	Kedal, Ranchi
2.	*Mangifera indica* L.	Aam	24th Dec., 2020	Kedal, Ranchi
3.	*Moringa oleifera* Lam.	Sahjan	15th Sept., 2021	Lalpur, Ranchi
4.	*Dalbergia sissoo* Roxb.	Shisham	15th Sept., 2021	Lalpur, Ranchi
5.	*Pinus roxburghii* Sarg.	Chir pine	15th Oct., 2021	BIT Mesra, Ranchi
6.	*Anacardium occidentale* L.	Kaju	20th Nov., 2021	Chandwe, Ranchi

Table 2: Images of plants in natural condition and their collected samples.

Sl. No.	Scientific Name	Plant in Natural Condition	Collected Gums and Resins
1.	***Prunus persica* (L.) Batsch**		
2.	***Mangifera indica* L.**		
3.	***Moringa oleifera* Lam.**		
4.	***Dalbergia sissoo* Roxb.**		
5.	***Pinus roxburghii* Sarg.**		
6.	***Anacardium occidentale* L.**		

Table 3: Organoleptic characteristics of plant-based gums and resins.

Scientific Name	Family	Habitat	Types of Products	Colour	Texture, Taste & Odour	Solubility	Reference No.
Prunus persica (L.) Batsch	Rosaceae	Deciduous Tree	Gum	semi-translucent with yellowish or orangish colour.	Gummy crystalline/irregular shapes;	Soluble in water.	18-22
Mangifera indica L.	Anacardiaceae	Tree	Gum	Brown colour	Tasteless; characteristic odour	Slightly soluble in water, practically insoluble in ethanol and acetone.	23-25
Moringa oleifera Lam.	Moringaceae	Tree	Gum	Brownish black	Characteristics odour, mucilaginous in taste.	Sparingly soluble in water forming a viscous solution, practically insoluble in acetone, alcohol and ether.	26-31
Dalbergia sissoo Roxb.	Fabaceae	Deciduous perennial tree	Gum	Reddish brown colour	Characteristics odour, gummy crystalline, tasteless	Soluble in organic solvents.	32-36
Pinus roxburghii Sarg.	Pinaceae	Large Tree	Oleo-resin	Colour varies from light yellow to red, brown, blue or black.	irregular and hard to touch	Soluble in organic solvents.	37
Anacardium occidentale L.	Anacardiaceae	Evergreen shrub or small tree	Resin	Brown resin	Hard to touch, irregular shape, odourless, tasteless.	Soluble in water	38,39

Table 4: List of some plant based gums and resins which exhibit their medicinal potential

Scientific Name	Medicinal uses / Economic importance
Prunus persica (L.) Batsch	used medicinally, improves skin elasticity, diminish the signs of aging, reduce wrinkles and dry skin appearance, boost metabolism and muscle growth, nourish joints and bones, improves cardiovascular health, relieve stress and enhance sleep quality, improves eyesight.[18-22]
Mangifera indica L.	used to treat laxative and antioxidant, for treatment of illness and infections. The pharmaceutical and other industrial applications of the gum gauged as stabilizers, binders, muco-adhesive, disintegrates, sustained and controlled release matrix, also used in dressings for cracked feet and for scabies.[23-25]
Moringa oleifera Lam.	gelling agent, binder, release retardant in tablet formulations, used in herbal medicine, diuretic, astringent, fever, dysentery, asthma, intestinal cancer.[26,29-31, 40-43]
Dalbergia sissoo Roxb.	used for the preparation of AgNPs (Silver nano particles), acts as reducing agents, modified and hydrolyzed gum can be used for pharmaceutical and food industry.[32-36,44]
Pinus roxburghii Sarg.	prevents bad breath, clean tooth, stomach ache, ulcer, anti- diabetic, appetizing, wounds, heel cracks, cuts, to reduce eye swelling.[37]
Anacardium occidentale L.	Used as suspending agent, as a jelling agent in canned food, binder in the production of conventional release tablets. [38,39,45-48]

RESULTS & DISCUSSION

Exploration, collection and documentation of the various plant species secreting gums and resins which exhibit medicinal properties will be helpful for the tribes/ people of Ranchi district. This could help them in getting access to home-based, easily available and cheap medicines. This study will be helpful for compiling the commercial use of the various gums and resins secreting plants such as *Prunus persica* (L.) Batsch, *Mangifera indica* L., *Moringa oleifera* Lam., *Dalbergia sissoo* Roxb., *Pinus roxburghii* Sarg, *Anacardium occidentale* L. with their phytochemical analysis such as alkaloids, flavonoids, glycosides, terpenoids, steroids, tannin and reducing sugars for selected plants.

Name of some plants which secrets gums and resins along with their images in natural habitat and their collected samples are listed in table 2 & 3.

CONCLUSION

Based on the availability in different seasons, the above documented plant-based gums and resins samples were collected, identified and their organoleptic characteristics were studied along with their medicinal values. The present study indicates that various excretory products of the plants are used for their economic importance as well as their medicinal values. This documentation would be beneficial for the local people of Jharkhand residing nearby Ranchi district.

ACKOWLEDGEMENT

We extend our profound gratitude to Prof. (Dr.) Kunul Kandir, Head, University Department of Botany, Ranchi University, Ranchi for her constant support and motivation for this research work undertaken during the pandemic time.

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RUJOST, ISSN: 2319-4227, Vol.7, No.1, January, 2022, pp. 41-44

A Biaanual Peer Reviewed Referred Ranchi University Journal of Science & Technology

Botany

Determination of ash values of some ethnomedicinal plants of family Malvaceae in Ranchi district of Jharkhand

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Abstract: The mallow family (Malvaceae) contains some 243 genera and at least 4,225 species of herbaceous plants, shrubs and trees distributed nearly worldwide. The tribal communities are having very deep knowledge about these plants. These plants are having lots of ethnomedicinal values. The study was conducted to investigate Ash value. Some species of family Malvaceae like *Hibiscus rosa-sinensis* L., *Malvaviscus arboreus* are having medicinal values.

Keywords: Ash value, Malvaceae, Ethnomedicinal plants, Tribal communities.

INTRODUCTION

The present research was carried out to establish detailed study on ash value of *Hibiscus rosa-sinensis* L. and *Malvaviscus arboreus*. Genus *Hibiscus*, with more than 300 species distributed in tropical and subtropical regions have been widely used in several formulae in traditional medicine.[1] Various parts of the plants used in cold, cough and to reduce fever.[2-4] Several studies have focused on economically important plant species.[5,6] These species affect natural ecosystem, structure and function,[7] although they have significant ecological and medicinal benefits too. Ethnobotany is the branch of life science which deals with the study of the dynamic relationship between plants and people.[8] Ethnobotany is multidisciplinary science linked with taxonomy, nutrition, pharmacognosy, phytochemistry, palynology, ecology and conservation biology.[9] The ethnomedicinal uses of plants are practiced by the local people of Jharkhand. Ash values are helpful in determining the quality and purity of crude drugs, especially in powder form. In crude drugs normally ash of leaves usually consisting of carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium. The main objective of this work is to study in detail, the taxonomic and traditional medicinal practices of the family Malvaceae in Ranchi, Jharkhand.

MATERIALS AND METHODS

Plant species were identified with the help of "Botany of Bihar and Orissa vol I-VII".[10,11] In the study of ash value, plants of family Malvaceae were selected from the different areas of Ranchi district. The leaves of *Hibiscus-rosa-sinensis* L. were collected from Ratu Road, Ranchi in January 2021 and the leaves of *Malvaviscus arboreus* were collected from Ratu Road, Indrapuri, Ranchi in March 2021. The fresh leaves were collected then these leaves were kept in hot air oven at 55°C temperature for half an hour. Repeated this activity for 2 or 3 times. After that, dried leaves were grinded into powdered form. About 10 gms of leaves powdered was weighed then put it in an earthen pot and heated for an hour on heater continuously, until free from carbon. The cellular parts degrade, moisture and other foreign materials were removed automatically and ash formed was white. Only the cellular residue was left. It was then cooled and weighed. Difference in weight is the ash value of the material.

Ash Value = Initial weight of Drug - Final weight after heating

The percentage of ash with reference to air- dried drug was calculated using the following formula,

$$\text{Ash Value \%} = \frac{\text{Weight of Ash}}{\text{Weight of leaf}} x\ 100$$

Ash value is a validity parameter to describe and to assess the degree of purity of a drug.[12]

RESULT

In this paper study of Ash value of two ethnomedicinal plants viz *Hibiscus rosa-sinensis* L. and *Malvaviscus arboreus* has been done.

Table 1 : Ash Value of *Hibiscus rosa-sinensis L*

Material	Number of Observation	Weight of Powdered Material	Weight of Ash	Difference	Ash Value %
Powdered plant leaves of *Hibiscus rosa-sinensis L.*	1	10 gms	1.516	8.484	84.84
	2	10 gms	1.364	8.636	86.36
	3	10 gms	1.554	8.446	84.46
	4	10 gms	1.502	8.498	84.98
	5	10 gms	1.480	8.520	85.20
	6	10 gms	1.558	8.442	84.42
	7	10 gms	1.492	8.508	85.08
	8	10 gms	1.520	8.480	84.80
	9	10 gms	1.546	8.454	84.54
	10	10 gms	1.538	8.462	84.62
Total					849.3

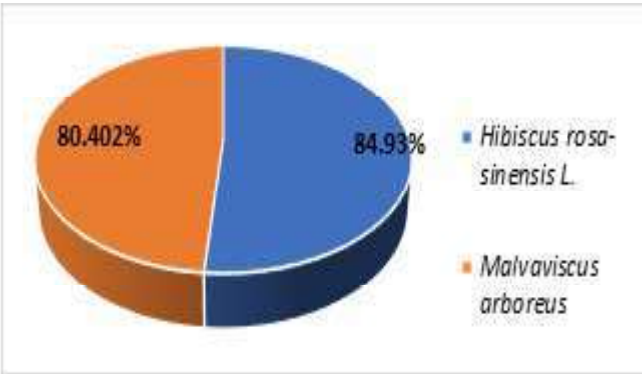
Average Ash Value = 84.93 %

Table 2 : Ash Value of *Malvaviscus arboreus*

Material	Number of Observation	Weight of Powdered Material	Weight of Ash	Difference	Ash Value %
Powdered plant leaves of *Malvaviscus arboreus*	1	10 gms	1.958	8.042	80.42
	2	10 gms	1.924	8.076	80.76
	3	10 gms	1.964	8.036	80.36
	4	10 gms	1.996	8.004	80.04
	5	10 gms	1.972	8.028	80.28
	6	10 gms	1.936	8.064	80.64
	7	10 gms	1.952	8.048	80.48
	8	10 gms	1.944	8.056	80.56
	9	10 gms	1.984	8.016	80.16
	10	10 gms	1.968	8.032	80.32
Total					804.02

Average Ash Value = 80.402 %

Graph 1- Ash value of medicinal plants



In this investigation, Ash value % was calculated which showed highest Ash value % in *Hibiscus rosa-sinensis* L. were 84.93 and lowest ash value % in *Malvaviscus arboreus* were 80.40. Ash value % in *Malvaviscus arboreus* was less than *Hibiscus rosa-sinensis* L.

Fig. 1- *Hibiscus rosa-sinensis* L. plant

Fig. 2- *Malvaviscus arboreus* plant

Fig. 3- Plant powder

Fig. 4- Puff initiation

DISCUSSION

Ash value is useful in determining authenticity and purity of sample and also these values are important qualitative standards. This ash value percentage clearly indicates the best for drug action and effects.

CONCLUSION

From the present observation it can be concluded that the knowledge and usage of conventional medicine for the treatment of various diseases among the rural, tribal and non-tribal communities is still a major part of their life and culture. Tribal people of Jharkhand have a strong faith in the success of traditional medicine. The medicinal plants continued to play a vital role in tribal and non-tribal community of Jharkhand.

ACKNOWLEDGEMENT

I express my sincere gratitude to Dr. Kunul Kandir, Head University Department of Botany, Ranchi University, Ranchi for providing the Laboratory facilities.

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RUJOST, ISSN: 2319-4227, Vol.7, No.1, January, 2022, pp. 45-50
A Biaanual Peer Reviewed Referred Ranchi University Journal of Science & Technology

Botany

Taxonomical study of ethnobotanical plants used in the rituals of Munda tribe of Khunti district

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Abstract: Khunti is the 23rd district of Jharkhand state and it is Munda dominated district. The Munda tribal community believes that almighty God and Deities reside in some sacred plants. So, their all rituals, festivals, cultures and customs are closely related to plants. They celebrate many rituals like birth and naming ceremony of a child, kanbhedi, marriage, godna and death rituals etc. and festivals like Mage, Phagu, Baa Parab, Batauli, Her Puna, Rowa Puna, Karam, Soso Karam, Jom Nawa and Sohrai etc. present study deals with the taxonomical study of ethnobotanical plants used in the rituals of Munda tribe.

Keywords: Khunti, Munda, Sacred plants, Rituals, Festivals, Taxonomical study and Ethnobotanical plants.

INTRODUCTION

Jharkhand is the land of forest where various ethnic groups like Santhal, Oraon, Munda, Ho, Kharia, Bhumij, Paharia, Kharwar, Gond, Kol, Birhor and Savar etc. are residing. A group of people that have same language and customs and that have a leader is called tribe. All tribals are closely related with the nature but their knowledge about different plants is different. The study has revealed the fact that medicinal uses of these plants vary in these districts.[1] For example, *Cassia fistula* is used by Santhals in blood purification, diarrhea, jaundice and liver complain but in Ranchi district the Mundas use this plant in constipation, snakebite, skin disease and rheumatism. It is also found that several plants have similar ethnomedicinal uses with valid scientific name.[2]

Parts of south Chotanagpur commissionary, Khunti is the 23rd district of Jharkhand state. It has a total area of about 2,611 sq.km. and around 40% of the total area is covered by forest. As per 2011 Census of India, Khunti district has a population of 5,31,885 on which 91.49% of population of Khunti district lives in rural areas of villages and 74% of the population being tribal. Khunti is the Munda dominated district.[3]

Munda tribals are closely associated with the nature. They dependent on agriculture for their livelihood. They have deep knowledge about the plants and different crops. They have clear idea about the identification and characterization of plants. They utilize various plant parts like leaf, bark, root, shoot, stem and flowers etc for food, medicine and other ethnic uses. Their all rituals and festivals are related to plants. A study reveals that almost all the festivals are related to a plant or a crop and all these plants have nutritional and medicinal values.[1] According to World Health Organization, more than 80% of the world's population relies on traditional herbal medicine for their primary health care. The knowledge about the plants is transmitted by oral means, folklore and folktale etc. The knowledge about the ethno botanical uses is related to socio-cultural life of tribals. Though most of the tribal people know about the plants and their uses, the scientific study of these plants also necessary.

Some studies have been previously conducted on plants used as medicine and for ritual ceremonies among tribes. Such as, An analytical study of ethnomedicinal and sacred plants of Jharkhand.[2] Taxonomical studies of

ethnomedicinal plants of Jharkhand.[4] Studies on the use of plants and plant parts by the tribals of Malkangiri district, Odisha for different rituals and medicinal uses.[5] Ritual plants used by Indigenous and ethnic societies of District Banswara (South Rajasthan), India.[6] Use of plant diversity in household and rituals by tribal people of Dhenkanal district, Odisha, India.[7] Some studies have been done on traditional knowledge of tribals of Khunti district. Studies on underutilized weeds of family Amaranthaceae used as edibles by the Munda tribe of Jharkhand, India.[8] Diversity and traditional knowledge on some less known edible wild herbaceous plant resource from district Khunti, Jharkhand, India.[9] Studies on indigenous traditional knowledge of some aquatic and marshy wild edible plants used by the Mundas tribe of district Khunti, Jharkhand, India.[10] There is no any report available on the plants used in the rituals of Munda tribe of Khunti Disrict. The objective of the study is to motivate the people to come forward for the utilization, cultivation and preservation of these plants.

MATERIAL & METHODS

The proposed study was based on personal interviews of different village headmen, spiritual leaders, priests, knowledgeable experienced persons and some local people for recording local names and their habitat, parts of the plant in households use, as medicine and for ritual purposes. Four blocks of the district (Khunti, Karra, Torpa and Murhu) were selected for study which have rich forest area. Field trips were conducted at regular intervals in different seasons and occasions in the year 2020-2021. The data collected through questionnaire in local language (Mundari) from different personals. During the study daily activities, rituals and festivals were being observed. Identification of plants was done with the help Flora of Botany of Bihar and Orissa by Haines, Part I-VI[11] and latest edition of International Code of Botanical Nomenclature.[12,13] The plants collected are listed with their scientific names followed by family, their local names in Mundari and the parts used in rituals, household use and medicinal purpose.

RESULT & DISCUSSION

Table 1 - List of plants used in the rituals and festivals of Munda tribe and their uses.

Sl. No.	Scientific name	Family	Local name	Plant parts and their Uses
1	*Achyranthes aspera* L.	Amaranthaceae	Chirchiti (Mundari), Chichita (Hindi), Devil's horsewhip (English)	Juice of whole plant - to remove abscess, wound, dog bite, snake bite, piles, Root – during labour pain, vomiting
2	*Brassica nigra* L.	Brassicaceae	Mani (Mundari), Sarson (Hindi), Mustard (English)	Seeds and oil - cold, back pain, ring worm, for vomiting when poisons are eaten.
3	*Curcuma longa* L.	Zingiberaceae	Sasang (Mundari), Haldi (Hindi) Turmeric (English)	Rhizom - worm in stomach, wound, cancer, toothache, piles, fever, blood purification and colouring food stuff.
4	*Cynodon dactylon* L.	Poaceae	Dubla tasad (Mundari), Dub (Hindi), Bermuda grass (English)	whole plant, Juice of whole plant (white leaf)- strengthen the skull of baby.
5	*Eleusine coracana* L.	Poaceae	Kode (Mundari), Ragi (Hindi), Finger millet (English)	Seedlings are used in kodeleta festival to make kichdi, seeds and leaves - rich source of iron, minerals, fibers and vitamins, lower the risk of type II diabetes.
6	*Guizotia abyssinica* Cass	Asteraceae	Maga (Mundari), Surguja (Hindi), Niger (English)	Seeds and oil – edible oil, paint, illuminant, used to cure asthama and lungs problems.
7	*Haldina cordifolia* Roxb	Rubiaceae	Karam (Mundari and Hindi), Haldu (English)	Long twig is used during Karam festival. Bark used in fever, burning and anthelmintic
9	*Semecarpus anacardium* L.	Anacardiaceae	Soso (Mundari), Bhilwa (Hindi), Marking nut (English)	Branch – planted in paddy field, plant ash is used in snake and scorpion sting.
10	*Shorea robusta* Gaert.	Dipterocarpaceae	Sarjom (Mundari), Sal (Hindi and English)	Whole plant - blood dysentery, leucorrhoea.

Present study reveals with 10 plant species belonging to 8 families used by the Munda tribe in different rituals and festivals. The religion of the tribal community is the resultant of traditions and beliefs that have come down to them from their ancestors. The traditional use of plants for various occasions is strictly based on folklore. They are expert in utilizing different plants both wild as well as cultivated plants for their livelihood.

1. *Achyranthes aspera* L. Sp. Pl.1:204,1753. Haines, Botany of Bihar and Orissa V:805-806.1924.

Description: Wild, perennial, erect herb. Stem branched, nodes and internodes are prominent, green but violet or pink at nodes. Leaves opposite decussate, petiolate, acute or acuminate, hairy all over. Inflorescence a spike with reflexed flowers arranged on long peduncle.

Flowering & fruiting time: September - April

2. *Brassica nigra* L. W. D. J. Koch, Deutschl. Fl.(ed.3)4: 713-714, 1833. Haines, Botany of Bihar and Orissa I-II:25, 2008. (Reprint)

Description: Annual herb,3-4 m tall with taproot, upper leaves linear oblong, glabrous, flowers in elongate racemes, regular petals yellow, seeds dark reddish brown to black, oval to spherical.

Flowering & fruiting time: June - October

3. *Curcuma longa* L. Sp. pl. 1:2 (1753). Haines, Botany of Bihar and Orissa V-VI:1186.1924.

Description: Annual herb 30 -90 cm tall, leafy shrubs, rhizome deep orange colour. Leaves 30 -50 cm glabrous oblong entire slightly wavy margin, apex acute. Flowers whitish yellow bracteates, spike short, sepals hairy.

Flowering & fruiting period: September - December.

4. *Cynodon dactylon* (L.) Pers., Syn.pl. (person) 1: 85 (1805), Haines, Botany of Bihar and Orissa V-VI:1012.1924.

Description: Creeping perennial grass, rooting at nodes, stem erect, leaves lanceolate, flowering spikes 3-4 branches at tip, light green.

Flowering & fruiting period: September - April

5. *Eleusine coracana* Gaertn., Frruct. Sem. Pl.i.8.t.1. (1788). Haines, Botany of Bihar and Orissa V-VI:1015 - 1016, 1924.

Description: Annual grass, culms erect, laterally flattened, 60-120cm tall or long, profusely tillering in addition to branches sent out at the rounded nodes in succession, inflorescence a whorl of 2-8, digitate, straight or slightly curved spikes arranged alternately on rachis, each containing 4-7 seeds, caryopsis nearly globose to somewhat flattened, reddish brown to nearly white or black.

Flowering & fruiting time: August - November

6. *Guizotia abyssinica* (L.f.) Cass, Dict.Sci. Nat.(ed.2)59: 248, 1829. Haines, Botany of Bihar and Orissa III-IV:483.2006. (Reprint)

Description: Herbaceous annual, 0.5-1.5 m tall, stems pubescent to tip, leaves opposite, sessile, subcordate to ovate lanceolate, serrate, biseriate scales, flowers yellow, arranged in corymbs, heads with 40-60 tubular hermaphroditic florets, surrounded by a marginal row of ligulate florets.

Flowering & fruiting time: September - December

7. *Haldina cordifolia* (Roxb.) Ridsdale Blumea 24(2): 361 – 362, 1978. Haines, Botany of Bihar and Orissa III- IV:421-422.2006 (Reprint)

Description: Large deciduous tree up to 40 m tall and 2.2 m in diameter. Leaves opposite which are broadly oval in shape with heart shaped base and pointed tip. Flowers are bisexual yellow in color in round heads, seeds many with tail at one end and bifid wings on another end.

Flowering & fruiting time: July - December, October - March

8. *Oriza sativa* L. Sp.Pl.1:333,1753., Franch. & Sav., Enum.Pl.Jap.2.155,sphalm. (1877). Haines, Botany of Bihar and Orissa V-VI: 1025.1924.

Description: Annual grass and grows to about 1.2 meter in height. The leaves are long and flattened and are borne on hollow stems. The fibrous root system is often broad and spreading. The panicle or inflorescence is made up of spikelets bearing flowers that produce the fruit or grain.

Flowering & fruiting time: July - December

9. *Semecarpus anacardium* L.f. Suppl.Pl.182, 1781. Haines, Botany of Bihar and Orissa I-II: 222. 2008. (Reprint)

Description: Moderate size deciduous tree, 12-15meter height, leaves are large, simple, ovate-oblong, curvaceous covered with five pale pubescence. Flowers are small, dull greenish yellow. Fruit is a drupe of 2-2.5 cm long, obliquely ovoid, smooth and shinning, orange colour and black when ripe.

Flowering & fruiting time: December - January, February - June.

10. *Shorea robusta* Gaertn. Suppl.Carp.48, (t.186,f.1) (1805). Haines, Botany of Bihar and Orissa I-II:56. 2008. (Reprint)

Description: A large deciduous tree with grey bark. leaves simple, ovate-oblong, acuminate, tough, flowers yellowish in lax axillary or terminal panicles, fruits indehiscent, ovoid with 5 equal wings, seeds ovoid with fleshy unequal cotyledons.

Flowering & fruiting time: March -April, June - July.

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Achyranthes aspera **L.**

Guizotia abyssinica **Cass.**

Brassica nigra **L.**

Haldina cordifolia **Roxb.**

Curcuma longa **L.**

Oriza sativa **L.**

Cynodon dactylon **L.**

Semecarpus anacardium **L.**

Eleusine coracana **L.**

Shorea robusta **Gaert.**

CONCLUSION

It can be concluded that the Khunti district is rich in wide variety of plants and the tribal people are not only familiar with the knowledge of plant species in their surroundings but also understand the importance of plants in their life. Their sociocultural activities and rituals show the interdependency. They are related with the cultivated crops as well as wild plants. Their traditional knowledge about these plants should be conserve.

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RUJOST, ISSN: 2319-4227, Vol.7, No.1, January, 2022, pp. 51-56

A Biaanual Peer Reviewed Referred Ranchi University Journal of Science & Technology

Botany

Review on soil bacteria *Rhizobia* as Plant Growth Promoters

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Abstract: Plant growth promoting rhizobacteria are beneficial bacteria, which are able to establish a symbiotic or nonsymbiotic association with plants in the rhizosphere. *Rhizobium i*s a soil habitat, gram negative bacterium that is associated symbiotically with the roots of leguminous plants life. *Rhizobium* is the most important PGPR, which is able to develop a symbiotic association with its specific host plant and increase its growth and yield by biologically fixing atmospheric nitrogen. Presently, *Rhizobium* is labeled into species *Rhizobium*, *Bradyrhizobium, Mesorhizobium, Azorhizobium* and *Sinorhizobium. Rhizobium* promotes universal growth and improvement of the plant via direct and indirect mechanisms. Loss of soil fertility, fluctuating climatic factors and increasing pathogen and pest attacks are today's agriculture challenges. Eco-friendly approaches like biofertilizers, biopesticides and crop residue return are needed for sustainability and environmental safety for agricultural production. In modern agriculture multiplicity of beneficial effects of microbial inoculants, particularly plant growth promoters (PGP) emphasize the need for further strengthening the research and their use. Bio inoculants of rhizoid could correctly enhance the agricultural yield and productiveness which indicates that *Rhizobium* is a powerful plant growth promoting microbes.

Keywords: *Rhizobium*, Nitrogen fixation, Leguminous, Symbiotic, Agrochemical, *Bradyrhizobium*, *Rhizosphere*, Symbiotic, PGPR, Biofertilizer.

INTRODUCTION

The *Rhizosphere* is the volume of soil surrounding and underneath the affect of plant root and *rhizosphere* is the plant root surface and strongly adhering soil debris. Beneficial free- dwelling soil microorganism is usually referred to as plant growth promoting *Rhizobacterium* (PGPR). PGPR inhabit the rhizosphere for nutrients from plant root exudates. Among the soil microorganism there is a particular organism called *Rhizobia* that have useful impact at the growth of legumes. *Rhizobium* in soil inhibit bacteria that shape the foundation nodules in which symbiotic organic nitrogen fixation occurs. In the soil the bacteria are free living and motile, feeding on the slays of useless organism. Free living *Rhizobia* cannot restore nitrogen and they have an exceptional shape from the micro- organism found in root nodules. They are everyday in structure appearing as instantly rods in root nodules the nitrogen-fixing shape exists as irregular cells referred to as bacteria which might be frequently membership and Y-shaped.[1] By reaction, they help in increased plant growth through soil nutrient enrichment by nitrogen fixation, phosphate solubilization, siderophore production and phytohormones production and also increased plant protection by influencing cellulase, protease, lipase and β-1,3 glucanase productions and enhance plant defense by triggering induced systemic resistance through lipopolysaccharides, flagella, homoserine lactones, acetoin and butanediol against pests and pathogens. To reduce the utilization of chemical fertilizers which disturbs the environment, biofertilizers are organized commercially. The soil contains many styles of microorganism-microscopic types of animal existence such as bacteria actinomycetes, fungi and algae, soil microorganism are vital due to the fact they affect the soils physical, chemical and biological homes. For instance, the technique of decay breakdown and disappearance of useless plant and animal materials occurs because of the motion of many unique

types of microorganism. When *Rhizobia* stay in the soil, they may be referred to as saprophytes. Many soils contain *Rhizobia* that stay at the natural rely without legume companion (known as native *rhizobia*) at the same time as the ones the farmers upload as inoculant are known as induced *Rhizobia*. The population of local can vary various which include species and many distinct strains within every species. Numbers can variety from zero to greator than one million *Rhizobia* consistent with gram (gm) of soil. *Rhizobium* inoculant was first made in USA and commercialized by private exposure in 1930s and strange situation at the that time chronicled by Smith.[1,2] The *Rhizobial* inoculants are commonly applied to seeds of legume crops to ensure effective nitrogen-fixation by *Rhizobium,* thereby making this essential at nutrient available to crop. The inoculants are often used together with agrochemical (pesticide) which besides containing essential nutrients also contains contaminants and toxic elements.[3] Pesticides might also impact nodulation and biological nitrogen fixation in legumes by using affecting *Rhizobia*, plant or each.

Characterization of *Rhizobia*:

Strains of *Rhizobium*, namely *Tephrosia* (*Tephrosia purpurea*), Tick clover (*Desmodium tortuosum*), Karanj (*Pongania pinnata*), Shisham (*Dalbergia sissoo*), Lupin (*Lupinus albus*), Gulmohar (*Delonix regia*) were isolated from non-cultivated wild legume plants.[4] (Figure 1) On different biochemical test for screening of this 7 species, IAA, HCN production and Ammonia production test was observed positive among all the isolates of *Rhizobium* species while negative result was observed in phosphate solubilisation. The bacteria are pleomorphic form (bacteriods) which are normally involved in the fixation of atmospheric nitrogen into utilizable form by plant. Molecular + C content of DNA is 59.64 Tm. On the basis of dry matter, the bacteria contain 52-55% carbon and 4-5% nitrogen.[5] Cells of alfalfa, clover, pea, soybean *Rhizobia* are characterized by low content of basic nitrogen.[6] Movement in this bacterium is due to thread like structure called flagella.

Figure 1- Root nodules of non cultivated wild legume plants[4]

In laboratory, *Rhizobia* are grown on a special medium called Yeast Extract Mannitol Agar (YEMA) medium.[4] They are grouped into two main genera-the Fast-growing *Rhizobium* species and slow growing *Bradyrhizobium* species. When cultured on YEMA, the *Rhizobium* species produce visible growth in 2-3 days (Figure 2).

Figure 2- Colony morphology of *Rhizobium* species on YEMA and Nutrient Agar[4]

They produce an acid growth reaction, which can be detected by addition of bromothymol blue to the medium. *Rhizobia* isolated from pea, bean, clover, alfalfa, chickpea and leucaena are all fast growers. The soybean and cowpea are slow growers.[4] Temperature required for the growth of *Rhizobium* on YEM is 25-42°C and pH range for this genus of bacteria is 4-9. The extensive literature with nutritive requirement was reviewed by Williams.[7] (Table 1). Fast growing responds to the additions of biotin. Slow growing strains of cowpea and soybean are not stimulated.

Table 1- Nutritional requirment.[7,8]

Vitamin	*R.melliloti*	*R.trifolli*	*R.leguminoserum*	*R.phaseoli*	*R.lupini*	*R.japonicum*	*Rhizobium sp*
Biotin	S,+	+	+	+	+	-	-
Thiamine	-	-	-	-	-	-	-
Riboflavin	-	S,+	-	+	+	-	-
Nicotinic acid	-	+	-	+	+	-	-
B-Alanine	-						
Panthothenic acid	s						

"s" means vitamin is synthesized, "+" vitamin is essential, "-" vitamin is not essential.

Classification of *Rhizobium*:

Rhizobium has been classified in Bergey's Manual of Determinative Bacteriology in such diverse families as Azotobacteriaceae, Mycobacteriaceae, Myxobacteriaceae, & Pseudomonadaceae. Bergey's Manual of Determinative Bacteriology, was first published in which cataloguing of information of identifying bacteria was included. Much Revision, by the American Society of Bacteriology (now American Society for Microbiology) provided such references which are followed by Bergey's Manual of systematic Bacteriology. Speciation of *Rhizobium* is based on the cross-inoculation grouping given by the classical studies of Fred.

The foundation for pass-inoculation grouping lies in the capability of an isolate of *Rhizobium* to form nodules on roots of a restricted species of legumes which are related to each other. Based in this precept, *Rhizobia* that may form nodules on roots of certain legumes were collectively taken as a species.

Cross Inoculation groups of *Rhizobium*:

Scientists have studied the matching system for many important food storage, and tree legumes. They have categorized *Rhizobia* and their legume partners into Cross Inoculation Groups. Each of these consists of the entire legume species that will develop nodules when inoculated with *Rhizobia* obtained from any other member of the same group. (Table 2)

Table 2: Cross Inoculation Groups[10]

Rhizobium sp	Cross inoculation grouping	Legume Types
R.leguminosarum	Pea group	Pisum, Vicia, Lens
R. phaseoli	Bean group	Phaseolus
R.trifoli	Clover group	Trifolium
R.meliloti	Alfa group	Melilotus, Medicago, Trigonells
R.lupini	Lupini group	Lupinus,Orinthpus
R. japonicum	Soyabean group	Glycine
Rhizobium sp.	Cowpea group	Vigna, Arachis

Rhizobia were frequently observed to go-infect or interchange between companies.[4,10] Rhizobiologists have come to treat cross inoculation grouping is a handy and great way to categories *Rhizobia* into species. It is likewise feasible to distinguish *Rhizobia* on the premise of increase on a defined substrate, as speedy growers and sluggish growers. Studies had been performed on morphological and physiological person (colonial individual, vitamin, carbohydrate and nitrogen vitamins, antibiotic sensitivities and infective attributes) of *Rhizobia* to discover if a higher method of rhizobial classification can be proposed. *Rhizobia* are able to produce acid or alkali on YEMA medium. Based on this criterion, the fastgrowing *R. phaseoli, R. trifolii, R. leguminosarum, and R. meliloti* could be grouped as acid producers while the slow-growing *R. japonicum, R. lupine, and Rhizobium sp.* (cowpea) could be grouped as non-acid producers.[11] The slow-grouping, which are nonacid-producing *Rhizobia,* are associated with primitive tropical legumes growing in alkaline environment.[11]

The base composition of pure DNA (expressed as molar percent-age of gunanine and cytosine) of several *Rhizobia* has been analyzed and a suggestion has been made to regroup *Rhizobia* as fast-growing *peritrichous* strains having a low percentage (G + C) composition in the range of 58.6-63.1 percent which belong to *R. leguminosarum and R. meliloti.* On the contrary, it has been suggested that the sub-polarly flagellated, slow-growing strains having higher percentage (G+C) mostly in the range of 62.8-65.5 % come under *R. japonicum*.[12] However, more studies are needed in this direction before we can come to any definite conclusions on the use of DNA base composition data in the classification of nodule

bacteria.[13] As per the ninth edition of Bergey's Manual of Determinative Bacteriology the genus *Rhizobium* is classified into following classification.[14]

Genus I: *Rhizobium*

Rhizobium leguminosarum (*biovars trifolii, phaseoli, viceae), R. meliloti, R. lotifast-* developing, *Densiflorus, and Anthyllis vulneraria* (however also nodulate *Orinthopus sativum)*. Include the short-developing lines nodulating *Cicer, Sesbania, Leucaena, Mimosa,* and *Lablab*.

Genus II: *Bradyrhizobium*

Slow-growing polar, or sub-polar flagellated traces nodulating soybean, *Lotus uliginosus, Pendulatus, and Vigna*. Including those are sluggish-growing, traces nodulating *Cicer, Sesbania, Leucaena, Mimosa, Lablab and Acacis*. The diverse cultures can be exact as *Bradyrhizobium sp.* (Vigna), *Bradyrhizobium sp.* (Cicer) and many others. *Rhizobia* currently consist of 61 species belonging to 13 different genera *Rhizobium, Mesorhizobium, Sinorhizobium, Bradyrhizobium, Azorhizobium, Allorhizobium, Methylorhizobium, Burkholdera, Cupriavidus, Devosia, Herbaspirillum, Orchobactrum and Phyllobacterium.*[15]

Plant growth promoting rhizobacteria

The complexity of the soil ecosystem is established by numerous and diverse interactions among its physical, chemical and biological components.[16]

Especially, the variable genetic and functional activities of the heterogeneous microbial populations have a vital effect on soil functions, as such microbes are considered powerful forces for specific enzyme mediated fundamental metabolic processes.[17] The unique physio-chemical and biological characteristics of the soils that are associated with the roots, compared to the soils away from the root and root surface are responsible for the enhanced microbial diversity together with the increased numbers and activity of the microorganisms in the rhizosphere. [18].

During plant-microbe interplay, plant roots exude the natural materials which are used up by using root related microorganisms as vitamins in addition to natural fabric is likewise supplied to the soil micro biota through the dying of dead and decay vegetation as both growth substrates and structural components or signal molecules. Microbial activity in the rhizosphere influences the rooting sample and deliver of nutrients to the flowers. Interaction of plant life with rhizosphere bacteria impacts the general crop yield and agricultural productivity commercially.

Role of *Rhizobium* as a PGPR is as follows:

Nitrogen fixation is the process in which atmospheric nitrogen (N_2) is converted into ammonia (NH_3) and is subsequently available for plants. This is perhaps a symbiotic process in which plant supplies the *Rhizobium* with energy in the form of nutrients and *Rhizobia* fix dinitrogen from atmosphere for plant uptake. The reduction of atmospheric dinitrogen into ammonia is second most important biological process on earth after photo synthesis.[19] It has been estimated that 1gm of soil may contain a community of 109 micro-organisms, with *Rhizobia* representing 0.1% of soil microbes or 106 *Rhizobia* g^{-1} soil.[20]

Process of Biological N_2 fixation regarding reduction of N_2 into NH_3 is an energy dependent method. It requires 16 molecules of ATP and a complicated set of enzymes to interrupt N_2 bonds in order that it could integrate with hydrogen.

Stress and Biocontrol Action of *Rhizobium* against certain pathogens:

Rhizobacteria are a group of microbes which effectively play a major role in controlling plant pathogen and suppressing the diseases induced by them. They can suppress a broad spectrum of bacterial, fungal and nematode disease. Greenhouse and field tests indicated that *Bradyrhizobium* have variation in the fungicide tolerance, peanut *Bradyrhizobium* strains from different peanut cultivators have been previously reported.[21] *Rhizobacteria* have been shown to suppress disease by inducing a resistance mechanism in the plant called "Induced Systemic Resistance". Induced Resistance is the state of enhanced defensive ability developed by plants when appropriately stimulated.[22] Greater application of PGPR is possible in agriculture for Biocontrol of plant pathogens and biofertilization.[23] Strains of *S. meliloti* are adversarial to *Fusarium oxysporum* and *Rhizobia* hostile to *F. solani f.* sp. *Phaseoli* isolated from industrial snap bean, appeared to have a very good capability for controlling *fusarium rot*.[24,25]

DISCUSSION

Rhizobia is symbiotic *diazotrophic* soil micro-organism infecting the roots of leguminous plant life to form root nodules to restore molecular atmospheric

nitrogen (N_2) with the aid of nitrogenase enzyme, turning it right into an extra readily usable shape for plants. Bioavailability of nutrients within the soil is enriched with the aid of Rhizobial movement due to metallic solubilization and siderophore activity. The combined hobby of phytohormones, enzymes, and siderophores contributes towards the boom and improvement of the concerned plant along with smooth nutrient uptake and phytoremediation. Besides, *Rhizobia* resource in biocontrol through antibiosis, parasitism, or competition with exclusive pathogens for important nutrient uptake. This has made it an essential candidate for sustainable agriculture in various economies across the globe. Nitrogen fixation by *Rhizobia* is of great importance in agriculture in several ways. The *Rhizobial* strains that nodulate groundnut roots have been robotically considered as *Bradyrhizobium* spp. comprising slow growing lines able to nodulating numerous legumes.[26]

CONCLUSION

Rhizobium is the maximum widely recognized species of bacteria which can be engaged in symbiotic courting with leguminous vegetation. They gain their vitamins from the plants and produce nitrogen fixing root nodules through a technique known as biological nitrogen fixation. *Rhizobia* can be labeled into slow and rapid growing. Presently, *Rhizobium* is classified into *Rhizobium, Bradyrhizobium, Mesorhizobium, Azorhizobium* and *Sinorhizobium*. It has been seen that microbial biofertilizers are a powerful supplement in agriculture towards usage of chemical fertilizer. Farmers can stimulate biological nitrogen fixation via applying an appropriate *Rhizobia* to legume crops, a system known as Inoculation. Biofertilizers fixes atmospheric nitrogen to a stage of 20-40 kgs hectare and overall allows in crop yield.

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RUJOST, ISSN: 2319-4227, Vol.7, No.1, January, 2022, pp. 57-60

A Biaanual Peer Reviewed Referred Ranchi University Journal of Science & Technology

Botany

Leea macrophylla Roxb. ex Hornem. a wild edible medicinal plant: A review

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Abstract: Medicinal plants are the moneyed bio-resource of drugs of a number of traditional systems of medicines. Medicinal plants can also be used as nutraceutical, food supplements, folk medicines, pharmaceutical intermediates, and chemical entities for synthetic drugs. A good number of researches on medicinal plants have enriched the science of modern medicine over the last decades. *Leea macrophylla* (Vitaceae) locally known as 'Hathikana', 'Hasthikarna palasa', is a shrub that has been used in herbal medicine as a cure for number of disorders. It is a wild edible plant with enormous ethnomedicinal importance. Several studies have proven that the plant possesses potential antimicrobial, anti-oxidant, anti-inflammatory, analgesic, neuropharmacological, anti-cancer and anti-diabetic activities. Tribal communities used the plant parts as a remedy for a number of ailments as well as nutritional products.

Keywords: *Leea macrophylla*, Hathikana, Bio-resource, Modern medicine, Enormous ethnomedicinal, Wild edible.

INTRODUCTION

India is well known for its rich heritage and known repository for medicinal plants since ancient times. Traditionally used plants and their products have been widely evaluated for pharmacological properties have been increased throughout the world now a day's. It is believed that the drug of natural origin plays a vital role in healthcare without any side effects.[1] Medicinal plants typically contain mixtures of different chemical compounds that may act individually, additively or in synergy to improve health.[2] As per W.H.O. report, about 75% - 80% peoples from developing countries relies on herbal medicines for better health and because of these medicinal plants are growing worldwide. Hereof, it is constitutive to study the uses of plants and other associated knowledge should develop for researchers for introducing new phytoproducts as well as the mechanisms in understanding the traditional knowledge for scientific validation.

Genus Introduction

Leea genus contains 70 species and is placed under Vitaceae family, distributed throughout Northern and Eastern Australia, South and Southeast Asia, New Guinea, and parts of Africa.[3] Of these, 11 species are mentioned in the database of the Botanical Survey of India.[4-5] The tropical plant genus *Leea*, named after the 18th century English nurseryman James Lee, is the closest relative to the botanical family of the grapes, Vitaceae. It was originally described by Van Royen, but was formally published by Linnaeus in 1767, with *Leea aequata* designated as the type species. *Leea* genus was formerly placed in Sapotaceae and was thought to be related to either Meliaceae or Sterculiaceae. It was also more recently associated with Rhamnales until this was refuted by molecular evidence.[6-8] In contrast, according to some taxonomists, *Leea* was originally assigned to the family Ampelideae but was transferred to the Leeaceae and then again to Vitaceae.[9-14]

Plant Introduction

One species of the genus *Leea* of family Vitaceae, namely *Leea macrophylla* Roxb. ex Hornem. (Fig.1) has been ascribed with abundant therapeutic claims for its ethnomedicinal and economical uses. *Leea* is often placed in its own family, Leeaceae, on the basis of morphological

differences between Leeaceae and Vitaceae. In India, it is distributed in Sub-Himalayan tract and Western Ghats, mounting up to 2250 m in the Himalaya, Uttar Pradesh, Bihar, West Bengal, Sikkim, Assam, Meghalaya, Odisha, Madhya Pradesh, Maharashtra, Andhra Pradesh, Karnataka, Tamil Nadu, Kerala, and Andaman.[15] *Leea macrophylla* Roxb. ex Hornem. (Fig.1) is an erect herbaceous shrub widely distributed to Sub-Himalayan tract and Western Ghats of India. The leaves of Plant look like an Elephant's Ear. Hence, it is traditionally named as 'Hathikana' or 'Hastikarnapalasa' by the local tribal people. This traditional name of this plant might be come from the morphological structures of leaf which looks like an Elephant's ear. *Leea macrophylla* (Large leaf *Leea*) (Fig.1) is widely distributed to Western Ghats and Sub-Himalayan region identified under Indian habitat growing up at altitude of 2000-2500m relatively hotter parts of India. It is an erect herbaceous shrub attains the height about 1ft. – 3ft. with tuberous roots and elephant ear shaped leaves.[16]

Fig.1: *Leea macrophylla* Roxb. ex Hornem.

Taxonomical Classification

Kingdom	:	Plantae
Division	:	Tracheophyta
Class	:	Magnoliopsida
Order	:	Vitales
Family	:	Vitaceae
Genus	:	*Leea*
Species	:	*L. macrophylla* Roxb. ex Hornem.

Common Names

Sanskrit	:	Dhola, Jino, Morata, Hasthikanda, Samudrika, Hastiparni, Hastikarnapalasa
Hindi	:	Hathikana, Hatikana, Dholsamudra, Samoodraka
Marathi	:	Gajakarni, Dinda
Bengali	:	Dholsamudra
Rajasthani	:	Dalavad, Lalpatta
Kannada	:	Mandala gadde
Telugu	:	Peddapayagillaaku
Malayalam	:	Njallu
Assamese	:	Kath tenga
Nepali	:	Ekle galeni

MATERIALS & METHODS

A thorough literature review survey of the plant *Leea macrophylla* with focus on Indian species was carried out, and information was gathered using scientific publications, conference proceedings from Science Direct, Google Scholar, Springer Links, and Books, Journals, etc. Besides, bibliographies of referred articles of the plant *Leea macrophylla* Roxb. ex Hornem. were also referred.

Ethnomedicinal and Traditional Uses

The plant has various ethnopharmacological uses and almost all parts of the plant possess potential curative properties. Decoction of leaf juice administers as local anti-inflammatory agent to cure of a number of pain disorders like boils, arthritis, gout, and rheumatism. It can be used in cuts and wounds remedy. Like leaves, roots are used in a number of disorders like fracture and cut injury. Roots also possess anthelmintic, astringent, styptic and anti-septic properties. Chowdhary *et al*. (2008)[16], showed therapeutic uses in the treatment of cancer, dysentery, body-ache. During survey in Ranchi District, it was reported that the Tribal people used the plant parts in cold, cough, headache, body pain and a number of ailments. Tribal people also use the leaves as vegetables in their food menu. Traditional practitioners used leaves, seeds and root in ayurvedic preparations since ancient times in the preparation of seasonal tonic known as 'Modaka' preparation.[17]

It has also significant uses in goiter, gastric tumor, lipoma, and tetanus. The leaves are used in gastric tumor, goiter, lipoma, tetanus[18] and in urinary disturbances.[19,20] The tuberous roots are astringents and alexipharmic; traditionally used to kill guinea worm, and pounded is applied to obstinate sores to promote cicatrisation. Plant is also used to heal pain and to stop bleeding.[19] The leaves are traditionally used in snake bites, arthritis, tonsilitis, tetanus, rheumatism, nephrolithiasis, pain, sore and blood effusion.[21] Leaves have hepatoprotective, anti-amnesic, and neuroprotective properties. The dry powdered root is often mixed with clarified butter to get anti-aging properties. Newly, it has proved that, the plant contains anti-oxidant components. Several chemical constituents like phenolics, saponin, glycoside, carbohydrate, and proteins were obtained in the phytochemical studies with the different extracts of seed of *Leea macrophylla.*[22]

CONCLUSION

The review compresses the updated knowledge related to the potential phytochemical and pharmacological activities, ethnobotanical uses and scientific information for many diseases and disorders of a traditionally used wild edible plant *Leea macrophylla* Roxb. ex Hornem., which belongs to the family Vitaceae. Traditionally the plant parts are used to cure a number of ailments for example cough, common cold, rheumatism, goitre, lipoma, headache, body pain, fracture, gastric tumor, tetanus, arthritis, etc. On the basis of these traditional uses, a number of scientific studies were performed and they all revealed potential activities of the plant species like anti-oxidant, antimicrobials, analgesic, antidiabetics, neuropharmacologicals, anticancer etc. The data available in the present research work may help to check potency as well as efficacy of the drug. This review will be helpful for further studies on this plant. More research is required for the beneficial for commerce and trade of the drug *Leea macrophylla*.

ACKNOWLEDGEMENT

Authors manifest their sincere thanks to Prof. (Dr.) Kunul Kandir, Dean, Faculty of Science and Head,

University Department of Botany, Ranchi University, Ranchi for providing necessary support and valuable suggestions. Author is grateful to the traditional healers, for co-operation and sharing their precious knowledge and information.

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RUJOST, ISSN: 2319-4227, Vol.7, No.1, January, 2022, pp. 61-64
A Biaanual Peer Reviewed Referred Ranchi University Journal of Science & Technology

Botany

Decline in biodiversity of Orchids in Ranchi district and its adjoining areas

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Abstract: Orchids are one of the most beautiful Creation of God. Orchid belongs to Orchidaceae family and order -Asparagales. Due to their medicinal values and ornamental uses most of the authors have been attracted to study more about it. Jharkhand which is known as the 'Land of Forest' is full of floral species including orchids but due to different activities done for public welfare most of the species is extinct. Ranchi which is the capital of state Jharkhand was chosen for study since it has humid subtropical climate due to its position and the forest around the city. Data collection from different Sources (Botanical Survey of India, Forest and climate change Department, Govt. of Jharkhand, Department of Forest etc.) and previous data available from various studies were compared from present investigation undertaken between 2018 to 2021. Result indicated that Ranchi district has diverse habitat for Orchids from epiphytic, lithophytes, terrestrial to even saprophytic members. But due to coal mining, deforestation, road construction, forest firing etc has led to decrease in the GPS data of different species. Although there has been decline in different species biodiversity the genus *Vanda* forms a dominant species of this region.

Keywords: Orchids, Asparagales, Subtropical, Epiphytic, Lithophytes, Saprophytic, Mining, Deforestation.

INTRODUCTION

Theophrastus a student of Pluto noted the round praised bulbs of one common European Orchid and named it the Greek word for testicle, later this name was applied not only to one genus but also to the entire family Orchidaceae which is one of the second largest family of the flowering plants. There are 22,500 species distributed in wide range of ecological habitat's throughout the world. A plant with complex flowers that are often showy or bizarrely shaped having a large specialized lip and frequently spur Orchids occur world-wide specially as epiphytes in tropical forest hothouse plant the flowering stem of a cultivated Orchids. The Orchidaceae are diverse and widespread family characterised by colourful flower that blooms having pleasant smell.

There are four Veda in Sanskrit literature: - Rig Veda, Samaveda, Ayurveda and Atharvaveda, written between 400 and 1600 B.C that deals with various uses of plant drugs. In the field of Ayurveda which is considered as an Upaveda (applied knowledge) the Indian Orchids were brought to notice by the great Indian medicine man 'Charak' in 125 A.D. In his book 'Charak Samhita' he has described about Orchids like VANADA which provides description of present known Orchid like *Flikengriya*, *Malaxis*, *Eulophia* having tremendous horticulture and medicinal value.[1] However, it is believed that Chinese were the first to cultivate and describe Orchids and their medicinal uses.[2]

There are about 1,141 species of Orchids belonging to 186 genera. In India the history of Orchid study starts from Lindley (1857, 1858)[3,4] followed by Hooker (1888-1890)[5] who did a legendry work in floral field of British India, including the information on the Orchidaceae of India and published a book on Indian Orchids (Hooker 1895)[6]. After this King and Pantling (1898)[7] studied Orchids of Sikkim Himalayas on Orchids of NW Himalayas. Various other studies about Orchids have also been done in different regions of India.

Various studies on plants and Orchids have also been mentioned by different authors such as Prain (1903)[8], Duthie (1920)[9], Haines (1921–1924)[10], Raizada (1975)[11], and Sharma and Sarkar (2002)[12], flora of Bihar Singh *et al.* (2001)[13]. 63 species of orchids with 26 new ones were recorded by Kumar *et al.* (2002)[14]. Recently in 2012 Government of Jharkhand also estimated the Orchid diversity and their distribution. The common orchids of the state include *Acampe praemorsa* (Roxb.) Blatt. & McCann, *Aerides multiflora* (Roxb.), *Aerides odorata* (Lour.), *Bulbophyllum crassipes* (Hook. f.), *Dendrobium crepidatum*, Lindl. & Paxton, *Dendrobium formosum*, Roxb.ex Lindl., *Rhynchostylis retusa* (L.) Blume, *Vanda tessellata* (Roxb.) Hook.ex G. Don etc.

According to Integrated Wildlife Management Plan in West Singhbhum, in 2012 Thalkobad area of the Saranda Forest present in Jharkhand represented a very special habitat for Orchids. It is believed to be the home of *Bulbophyllum*, an epiphytic Orchid represented by a single species, *Bulbophyllum crassipesis*. This species is present only in the Saranda Forest area in Jharkhand state and Saranda forest is the last home of its wild population. During the further investigation *Pecteilis triflora* was the species recorded only from two places in India, one being Saranda forests and the other in the Western Himalaya in Tons Valley of Uttarakhand.

MATERIALS & METHODS

The major equipment's used for the study were DSLR camera, Mobile phone, G.P.S etc. For the collection of species and data journey was started from Ranchi followed by visiting the adjoining areas including historical, geographical, floral and faunal land forests of this district. Several photos of the Orchids were also taken with the help of DSLR cameras and collection of the species were also done for its further study in the laboratory. Wherever required questions from Baidyas and knowledgeable person were also asked to gain more information about the orchids. Several questions have been mentioned below:

Questionnaire No. 1

Vaidyas, Herbal Practioner, patient, tribal

Name - Bahira

Age - 80 year

Main Occupation - Jari-Buti collection and cure the villagers

Cast - Munda

Place - Jamun Toli near Bhut Bungalow Village Chama, P.O.-Chama, Block- Chanho, District – Ranchi, Jharkhand

Medico Botanical Studies: –

Question –How did you get the knowledge about the medicinal plant and its curative properties?

Ans- From ancestors

Question –Common name and the parts used for curing diseases

Common Name – Bando

Parts used -Aerial Roots juice are uses in Eye drop

Mahesh Bhagat, Vill- Ramdga (Harinduba) P.O.Chama, Block Chanho, District Ranchi (Jharkhand)

Age 50 years

Main Occupation – Jari- Buti Cure disease of Villagers

OBSERVATION

From the above findings Orchid of *Vanda* species were present in large scale including the species *Vanda tessellata*, *Vanda spathulata* while *Vanda nana*, *Dendrobium*, and *Phalanopsis* were rarely present. The images of several species have been shown below. Deforestation, clearance of forest for human need such as coalmines, road construction and greed's by forest firing and cutting by tribal and merchant is leading to depletion of Orchid species in Jharkhand.

Images showing different *Vanda* species

***Vanda tesselata* on *Madhuca indica* (Mahua)**

***Vanda nana* on *Butea monosperma* (Palas)**

***Vanda tesselata* on *Soria robusta* (Sal Tree) near Mcclluskieganj.**

***Vanda* on *Scheichera oleosa* (Kusum) in bundu Tamar Road**

***Vanda* species on *Artocarpus heterophyllus* (Jackfruit)**

RESULT & DISSCUSSION

After discussion with the villagers, forest guards, forest officers, our Guide Dr. A.K. Choudhary and Scientist of Botanical Survey of India, Dr. Avishek Bhattacharya, Sharat Mishra and Director of National Research Centre for Orchid and different Scientist Kalaivanan sir etc. it was found that orchids that were mostly found near Ranchi clusters were of *Vanda* species and most of it had very useful medicinal values. Host also acted as an important source of habitat for the survival of these species. *Madhuka indica* indicated about 35 % of the *Vanda* species followed by *Magnifera indica* (15%), *Soria robusta* (15%) and *Scheichera oleosa* (10%) as indicated in Fig no. 1.

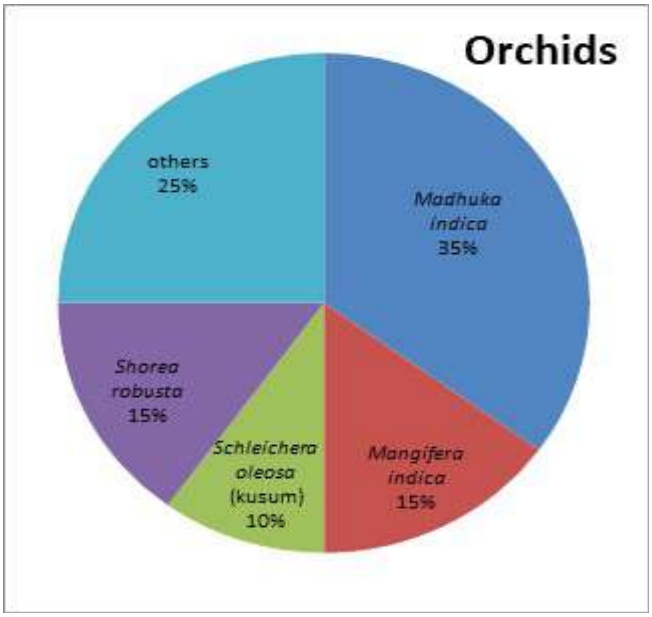

Fig no. 1: *Vanda* species findings in Ranchi district and adjoining area

CONCLUSION

As we know that Jharkhand is full of flora and fauna but Orchids which are really major equipment's of baidyas in villages for curing diseases have been undergoing great decline. The natural habitats of these species have been abruptly misused for public welfare. Road construction, forest fire, mining, deforestation, etc are the major problems for depletion of these species. Protection and conservation of these plants should be initiated so that their disruption can be restored and their scientific study and propagation could be preserved.

ACKNOWLEDGEMENT

Authors are thankful to Prof. (Dr.) Kunul Kandir, Dean, Faculty of Science and Head, University Department of Botany, Ranchi University, Ranchi for providing necessary support and valuable suggestions. Authors are also thankful to Baidyas for sharing their knowledge and information.

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RUJOST, ISSN: 2319-4227, Vol.7, No.1, January, 2022, pp. 65-74

A Biaanual Peer Reviewed Referred Ranchi University Journal of Science & Technology

Botany

Effect of 2, 4-Dichlorophenoxy Acetic Acid (2, 4-D) and Benzyl Amino Purine (BAP) concentration on callus induction in two traditionally grown grain legumes of Jharkhand *Cyamopsis tetragonoloba* L. and *Vigna radiata* L.

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Abstract: Present work was conducted to determine the most appropriate concentration of two growth hormone 2, 4-Dichlorophenoxy Acetic Acid with combination of benzylaminopurine for callus induction of two varieties of secondary grain legumes viz; cluster bean (*Cyamopsis tetragonoloba* L.) and mung bean (*Vigna radiata* L.). Experimental material was collected from traditional grain legume growing local tribal farmers near Ranchi district of Jharkhand and authenticated. the obtained seeds were cultured in a modified MS medium supplemented with varying concentrations of 2, 4-Dichlorophenoxy Acetic Acid 2,4-D and Benzyl Amino Purine (BAP) *viz.,* 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l. The explant were inoculated and incubated in growth chamber at 25 ± 2ºC, humidity of 85-90% and kept in dark. Incubated explant was continuously monitored for callus induction. It was found that swelling of the explants were observed after 12-13 days (approx. 2 weeks) in *Cyamopsis tetragonoloba* and after 30 days (approx. 4 week) in *Vigna radiata*. The callus initiation begun after 3 weeks in cluster bean and after 6 weeks in mung bean. The initial calli just after three weeks in cluster bean were whitish not very compact and nodular. In mung bean callus was very compact yellowish and nodular. In case of cluster bean callus colour turn brown when not subculture after 4 to 6 days of callus induction. The embryogenic callus induction frequency varied from 75 % to85.60 % in mung bean and cluster bean respectively. Data generated were analyzed using Chi-square and showed a significant difference among the different 2, 4-D concentrations (Pd"0.05) and BAP concentration. The maximum callus induction from cotyledon explants was evident in cluster bean on a medium supplemented with 2, 4-D 2 mg/l and BAP 1.5 mg/l, and in mung bean on a medium supplemented with 2,4-D 1mg/l and BAP 1.5 mg/l. It was found that the induction rate increased. Low amount of callus induction was also found in cluster bean at the concentration of two hormonal combination, first 1.0 mg/l (2,4 –D) 1.5mg/ml (BAP) second 1.5 mg/l (2,4-D) 1.5mg/ml (BAP). Low amount of callus induction was also found in Mung bean at the concentration of two hormone combination, first 0.5mg/l (2,4-D) 1.5mg/ml (BAP) second 1.0mg/l (2,4-D) 1.5mg/ml (BAP). In this experiment optimisation of callus induction in two secondary viz; cluster bean (*Cyamopsis tetragonoloba* L.) and mung bean (*Vigna radiata* L.) traditionally grown in Jharkhand, that is best concentration supplemented with 2, 4-D 2 mg/l and BAP 1.5 mg/l, and in mung bean on a medium supplemented with 2,4-D 1mg/l and BAP 1.5 mg/l, temperature 25 ± 2ºC, humidity 85-90%, light intensity 1000 lux.

Keywords: Callus induction, BAP, 2, 4-D, Grain legumes

INTRODUCTION

Secondary grain legumes are those plants used as food in the form of unripe pods, immature seed or mature dry seed, directly or indirectly. Not only do grain legumes provide variety to the human diet but they also supply dietary protein for many populations lacking animal or fish protein. In general, they are rich in lysine but poor in methionine content, thereby complementing the reverse amino acid pattern found in cereals. Additionally, virtually all of the grain legumes fix their own nitrogen, thereby reducing, in many situations, the cost of nitrogen inputs by farmers. The, especially soybeans and peanuts, are excellent sources of vegetable oils used in the production of cooking oil, margarine, mayonnaise, and salad dressings. Grain legumes are part of a healthy, balanced diet and have been shown to have an important role in preventing illnesses. Grain legumes are low fat source of protein, with a high fiber content and low glycemic index.

Grain legumes are indispensable for ensuring nutritional security, diversifying agriculture and achieving ecological sustainability. Apart from being rich source of proteins, legumes such as dry beans, dry peas, chickpea, pigeon pea, lentil, soybean, groundnut, etc., are also important sources of micro- and macronutrients as well as health promoting secondary metabolites.[1] In addition to contributing to a healthy, balanced diet, grain legumes nutritional qualities makes them particularly helpful in the fight against some non-communicable diseases. The World Health Organization estimates that up to 80% of heart disease, stroke, and type 2 diabetes and over a third of cancers could be prevented by eliminating risk factors, such as unhealthy diets and promoting better eating habits, of which grain legumes are an essential component. Grain legumes can help lower blood cholesterol and attenuate blood glucose, which is a key factor in against diabetes and cardiovascular disease. Eating grain legumes as a replacement to some animal protein also helps limit the intake of saturated fats and increases the intake of fibres the role of legumes in the Covid 19 pandemic both in recovery and post recovery cannot be ignored. Grain legumes are included in all 'food baskets' and dietary guidelines. The World Food Programme (WFP) for instance includes 60 grams of grain legumes in its typical food basket, alongside cereals, oils and sugar and salt.[2] Grain legumes have three or four times more protein content than rice and ten to fifteen times more than potatoes.[3] Grain legumes contain 51% carbohydrate, 26% protein, 3% minerals, 3% vitamins and 10% moisture.[4] The high lysine content makes it a good complementary food for rice based diets because lysine is the first limiting amino acids.[5]

Mung bean (*Vigna radiata* L.) also known as green gram is one of the most important grain legumes, widely cultivated in a large number of countries. It has tremendous value in agriculture as a good source of plant protein for its high digestibility, good flavour, and high protein content and free from flatulent effects which are common to grain legumes.[6] It is cultivated most extensively in India, Myanmar, Bangladesh, Sri-Lanka, Pakistan, Thailand, Philippines, China, Japan, Korea, Iran, Indonesia, parts of East and Central Africa, West Indies, USA and Australia.

Cluster bean (*Cyamopsis tetragonoloba* L. Taub), popularly known as guar, is commercially grown in several countries like USA, Australia, Brazil, South Africa, India and Pakistan. In the developing world, it is primarily grown as a drought hardy crop under rain fed conditions. It has recently gained the status of an industrial crop, due to the presence of gum (galactomannan) in its seed. Owing to its unique biochemical properties, guar gum is used in wide range of industries, from paper and cosmetics to mining and explosives.[7] These two legumes are very high in fiber, containing both soluble and insoluble fibers. Soluble fiber helps to decrease blood cholesterol levels and control blood sugar levels, and insoluble fiber helps with digestion and regularity. Grain legumes provide important amounts of vitamins and mineral. Some of the key minerals in grain legumes include: iron, potassium, magnesium and zinc. These are also particularly abundant in B vitamins; including folate, thiamin and niacin. These two legumes typically contain about twice the amount of protein found in whole grain cereals like wheat, oats and barley and rice, and in most developing countries constitute the main source of protein for most populations. Cluster beans have also been shown to be helpful in the prevention of certain cancers, because of their fiber content but also because of their mineral and amino-acid contents, in particular folate in economically and socially backward state Jharkhand. Several studies have shown that legumes are been associated with long-lived food cultures such as the Japanese (soy, tofu, natto, miso), the Swedes (brown beans, peas), and the Mediterranean people (lentils, chickpeas, white beans) and that they could be an important dietary factor in improving longevity. Over 60 percent of total utilization of grain legumes is for human consumption. But the importance of grain legumes in human diets varies from region to region and country to country, with a general trend of higher consumption in lower income nations. The share of food use in total utilization of grain legumes in the developing countries is over 75 percent, compared to 25 percent in the developed countries. Grain legumes are locally adapted and can be grown by local farmers for their own nutrition as well as for sale, which is important to improve food security. They are highly accepted crops, which can keep well in storage.[8,9]

Grain legumes, because of their role in improving sustainability, notably through soil management, also impact food security. Soil degradation is a major threat to food security in many areas. Africa is particularly impacted by soil degradation, yet grain legumes are part of traditional diets and often grown by small farmers. By improving the

crop patterns using grain legumes, farmers can improve their yields and limit the long-term threat to food security that soil degradation represents.[10]

There is a very significant yield gap in grain legumes in many developing countries - productivity is low and improving it could help enhance food security tremendously. The average global yield for grain legumes in 2010 was estimated at 819kg/ha. India's yields were even lower, at 600kg/ha while Canada and the US had yields more than three times that of India, at around 1800kg/ha. France achieves an even higher yield, of around 3800kg/ha, even though on a much smaller planted surface. Grain legumes, the important constituents of sustainability-based cropping systems and energy-limited vegetarian diets have long been the subject of scientific research. Tremendous technological strides were made in the so-called orphan crops, in terms of both varietal improvement and generation of basic information despite recalcitrancy and high genotype dependency, in vitro culture techniques such as organogenesis.

In Jharkhand, grain legumes are considered to be the poor man's meat, particularly because of its high protein content and low price as compared to animal proteins like meat, fish, egg, milk and milk products. In Jharkhand traditionally, guar and mung bean have been used as a forage crop, for green manuring, and as a human food. Besides its use as grain legumes and vegetables the tribal and small stockholding farmers of Chotanagpur plateau region also use these two grain legumes as medicine. Both the plants have short life cycle of 60-70 days, cultivation improve the soil condition and increase the income of small landholders.[9] In Chotanagpur plateau region both these legumes are well adapted to the rain fed & irrigated. Smallholder farmers do not adopt hybrid seed because hybrid seed need fertilizer. Though chemical fertilizers increase crop production; their overuse has hardened the soil, decreased fertility, strengthened pesticides, polluted air and water, and released greenhouse gases, thereby bringing hazards to human health and environment as well. Besides this, chemical fertilizers can cause root burn or fertilizer burn, as chemical fertilizers do not allow enough water intake for the plants. Dry up the plant. On other hand indigenous seeds are hardy, pest resistant, withstand unfavorable conditions in the area of their origin require less water and nutritional input, fit in better in the organic method of farming and may even have special characteristics such as nutrition, fragrance, colour and various medicinal value. Many smallholder farmers or tribal farmers of Jharkhand save seeds selectively after seeing the vigour and growth of individual plants. This is an old tradition and need to be continued but the gradual substitution of the natural environment by modern civilization has made exploitation, as well as the discovery of new substances, of such economically important commodities increasingly more difficult. The supply of certain important raw and natural plant of both the grain legumes guar and mung are today or may in the near future be limited. It has therefore become increasingly important to find and develop alternative resource. Successful plant regeneration from tissue culture of legumes is rather limited.[11] Considering the above facts the present investigation was undertaken to find out suitable explants and medium for regeneration, to develop a stable, reproducible and efficient protocol for the in vitro regeneration of mung bean & cluster bean. Tissue culture and regeneration of plants open new potentials for improving the crops.[12] Although it has been recently reported the high potential of cotyledonary nodes for direct shoots regeneration.[13] The somatic embryogenesis resulting into regeneration can produce large number of guar plants in very short time duration.[14] The somatic embryogenesis was reported in few species of *Vigna.*[15] The development of shoots from different explants of mung bean and callus has been reported such as from cotyledon[16] and cotyledonary node.[17] An alternative methods to the multiplication of vegetative seed superior, healthy, identical genetic and disease-free can be using tissue culture techniques in vitro. However, some factors work to determine the success of in-vitro propagation, including the type of ex-plants and substance of growth promotor or regulator used. Growth promotor often used in tissue culture for initiation of callus and increase production of secondary metabolites like auxin and cytokinin.[18]

Auxin is usually used to induce the formation of callus, culture suspension, roots, and to stimulate elongation and cell division. Cytokines are compounds that can increase cell division, growth, and development and the boost of the leaf. The combination of 2, 4 -D (auxin) and BAP (cytokines) will stimulate the growth and development, division of cells and increases the synthesis of proteins and affecting the growth of callus and the production of secondary metabolites. Encouraging

awareness of the nutritional value of grain legumes can help the local tribal adopt healthier diets. In developing countries, where the trend in dietary choices tends to go towards more animal based protein and cereals, retaining grain legumes is an important way to ensure diets remain balanced and to avoid the increase in non-communicable disease often associated with diet transitions and rising incomes. In this paper, the result of a study carried out to determine the most suitable 2, 4-D and BAP concentration in vivo callus production in traditionally grown varieties of mung bean & cluster bean collected from the traditional tribal farmers of Jharkhand and to develop a stable, reproducible and efficient protocol for in vitro callus production of these legumes.

MATERIALS & METHODS

A local variety of *Cyamopsis tetragonoloba, Vigna radiata* seeds were collected from traditional tribal farmers in village of Jharkhand.

Treatments

Isolation and sterilization of seed of both the leguminous crop were excised. Surface sterilization of the explants was carried out in the following steps:

i. Explants were first washed on a running tap water.
ii. They were treated with house hold detergent for five minutes.
iii. They were washed again on a running tap water to remove all traces of detergent.
iv. They were then washed with double distilled water in the laminar flow hood.
v. They were further sterilized by dipping into 70% ethanol for 2 minutes.
vi. They were then again washed three times with triple distilled water in the laminar flow hood to remove all traces of ethanol.
vii. Seeds were treated with 0.1% of $HgCl_2$ for 5 minutes.
viii. They were finally rinsed 3 times with sterile triple distilled water to remove all traces of $HgCl_2$.[19,20]

Media Preparation

One liter of Murashige & Skoog (MS) medium supplemented with different concentrations of 2,4-Dichlorophenoxy Acetic Acid (0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0mg/l), BAP(0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0mg/l) and 30% sucrose was prepared. The media was solidified using 8g agar. The pH of the media was adjusted to 5.8 using potassium hydroxide (KOH). The prepared media was autoclaved at 121°C for 15 minutes.[19-21]

Inoculation

The seeds were aseptically germinated on half MS medium.[22] Cotyledons, hypocotyls and root tips were excised aseptically from 5 to 7 days old seedlings and cultured on MS medium containing different concentrations and combinations of BAP (0, 1.0, 1.5, 2.0, 2. 5 and 3.0 mgL^{-1}) and 2,4-D (0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mgL^{-1}).

Incubation

Explants were incubated at 27°C and kept in darkness for 6 weeks in different concentration of 2,4-D and BAP to induce callus formation.[19,20]

Observations and Data Collection

Percentages of callus formation, embryonic callus were studied and determined using the following formula:

$$1.\ \text{Percentage of callus formation} = \frac{\text{Number of explants forming callus}}{\text{Total number of explants}}$$

$$2.\ \text{Percentage embryonic callus} = \frac{\text{Number of embryonic callus}}{\text{Total number of callus}}$$

RESULTS

(1) APPREANCE OF CALLUS

Swelling of the explants was observed two week after inoculation. However, callus initiation was observed in the third week after inoculation. Four weeks later, callus initiated at the cut edge of the explants and developed into a full grown callus. The callus was morphologically found to be yellowish-white, compact, dry and nodular (Plate I). The response of the two grain legumes to treatments and subsequent callus induction varied, with different concentration. Table 1 show the average time when the callus appears ranges from 8-20 days after planting. Of the 25 treatments, five treatments 2,4-D and BAP concentrations did not give any signs of callus appearing up until 30 days of observation, namely on levels without 2,4-D and BAP, levels without 2,4-D and 2, 0 mg / l BAP, 2.0 mg / l 2,4-D and without BAP, 2.0 mg / l 2,4-D and 0.5 mg / l BAP, and 2.0 mg / l 2, 4-D and 2.0 mg / l BAP

The mean result for *Vigna radiata* indicated that for highest percentage of callus formation, 2,4-D concentration of 1.5mg/l with BAP concentration1.5mg/ml gave the best result (Table 1,Table 4, Fig 1). However,

the mean result of *Cyamopsis tetragonoloba* showed that highest percentage of callus formation was observed at 2.0mg/l 2,4-D concentration and 1.5 mg/l BAP concentration. (Table 2, Table 3, Fig 1)

Table 1- Morphogenic responses of growth of callus taken from 7-50 day old aseptically grown seedling of *Vigna radiata* to plant growth regulators.

No. of seeds	2, 4-D con.	BAP con.	Growth of callus observation							
			1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week
10	0	0	No response	No response	No response	Swelling of explant	Swelling of explants	No response	No induction of Callus	No induction of Callus
10	0.5	1.5	No response	No response	No response	Swelling of explant	Swelling of explants	Low amount of callus formation	Callus decolouration dehydration	Callus decolouration dehydration
10	1	1.5	No response	No response	No response	Swelling of explant	Swelling of explants	Low amount of callus formation	Low amount of callus formation	Callus decolouration dehydration
10	1.5	1.5	No response	No response	No response	Swelling of explant	Swelling of explants	Low amount of callus formation	good amount of callus formation	Callus decolouration dehydration
10	2	1.5	No response	No response	No response	Swelling of explant	Swelling of explants	No induction of Callus	No induction of Callus	No induction of Callus
10	2.5	1.5	No response	No response	No response	Swelling of explant	No response	No response	No induction of Callus	No induction of Callus
10	3	1.5	No response	No response	No response	No response	No response	No response	No induction of Callus	No induction of Callus

Table 2- Morphogenic responses of growth of callus taken from 7-40 day old aseptically grown seedling of *Cyamopsis tetragonoloba* to plant growth regulators.

S. No.	No. of seeds	2, 4-D con.	BAP con.	Growth of callus observation					
				1st week	2nd week	3rd week	4th week	5th week	6th week
1	10	0	0	No response	Swelling of explant	Swelling of explant	Not induce any response	Not induce any response	Not induce any response
2	10	0.5	1.5	No response	Swelling of explant	Swelling of explant	Swelling of explant	Swelling of explant	Not induce any response
3	10	1	1.5	No response	Swelling of explant	Swelling of explant	Low amount of callus formation	Low amount of callus formation	Callus become brown and dry
4	10	1.5	1.5	No response	Swelling of explant	Swelling of explant	Low amount of callus formation	Low amount of callus formation	Callus become brown and dry
5	10	2	1.5	No response	Swelling of explant	Swelling of explant	Low amount of callus formation	Good amount of callus formation	Callus become brown and dry
6	10	2.5	1.5	No response	No response	No response	Swelling of explant	Not induce any response	Not induce any response
7	10	3	1.5	No response	No response	No response	No response	No response	No response

Table 3- Effect of 2,4-D and BAP on hypocotyls derived callus of *Cyamopsis tetragonoloba* on MS medium (35 days after growth).

(a) Where BAP is constant.			(b) Where 2, 4-D is constant.		
Concentration of synthetic hormone		**Growth of callus**	**Concentration of synthetic hormone**		**Growth of callus**
2,4-D (mg/l)	BAP(mg/l)	Callus frequency	2,4-D(mg/l)	BAP(mg/l)	Callus frequency
0	0	0%	0	0	0%
0.5	1.5	10%	0.5	1.5	10%
1	1.5	20%	1	1.5	20%
1.5	1.5	40%	1.5	1.5	40%
2	1.5	70%	2	1.5	70%
2.5	1.5	0%	2.5	1.5	0%
3	1.5	0%	3	1.5	0%

Table 4 : Effect of 2,4-D and BAP on hypocotyls derived callus of *Vigna radiata* on MS medium (35 days after growth).

(a) Where BAP is constant.			(b)Where 2, 4-D is constant.		
Concentration of synthetic hormone		**Growth of callus**	**Concentration of synthetic hormone**		**Growth of callus**
2,4-D(mg/l)	BAP(mg/l)	Callus frequency	BAP(mg/l)	2,4-D(mg/l)	Callus frequency
0	0	0%	0	0	0%
0.5	1.5	10%	0.5	1.5	10%
1	1.5	30%	1	1.5	20%
1.5	1.5	80%	1.5	1.5	80%
2	1.5	10%	2	1.5	30%
2.5	1.5	0%	2.5	1.5	0%
3	1.5	0%	3	1.5	0%

COMPERATIVE DATA OF FREQUENCY OF EMBRYOGENIC CALLUS OF BOTH GRAIN LEGUMES

Table 5 : Result for Embryogenic callus of *V.radiata* on different callus frequency

Callus frequency	**Embryogenic callus Frequency**
10%	0%
20%	0%
30%	33.30%
80%	75%

Table 6: Result for Embryogenic callus of *C. tetragonoloba* on different callus frequency

Callus frequency	**Embryogenic callus Frequency**
10%	0%
20%	0%
40%	50%
70%	85.60%

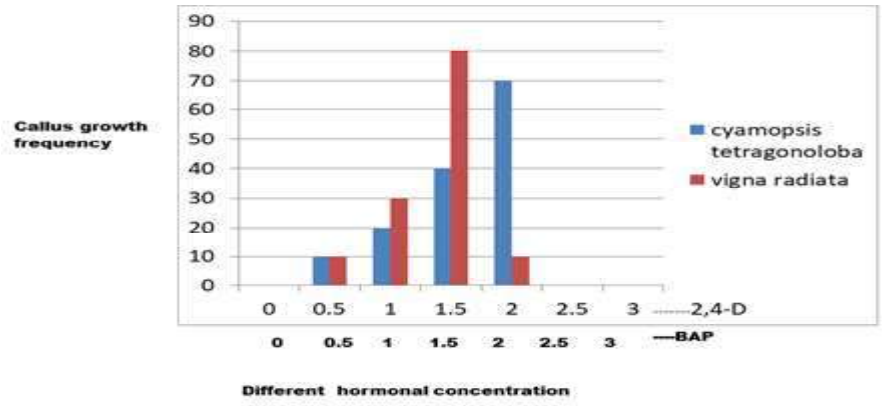

Figure: 1 frequency of callus induction from Hypocotyle derived explant of *Vigna radiata* and cotyledon derived explant of *Cyamopsis tetragonoloba* influenced by different concentration of growth regulator.

Plate 1 : Callus initiation from Hypocotyl explants in *Vigna radiata* (in 6th -7th weeks)

Plate 2 : Callus initiation from cotyledon explants in *Cyamopsis tetragonoloba* (in 4th -5th weeks)

The time when the callus appears is the time of formation of the first callus in ex-plants. The appearance of the callus is characterized by tissue swelling, white spots or white bumps resulting from tissue injury of ex-plants. The ex-plants began to form callus can be seen in Plate 1 and Plate 2.

Ex-plants Form a Callus

An ex-plant forming callus is an ex-plant that visually shows the characteristics formed callus that is marked by the bumps in the ex-plant section. Based on observations of ex-plants forming callus, it indicates that there are 6 administrations of concentrations of 2,4-D and BAP which formed callus. Table 3 and Table 4 shows that there were 6 treatments given and one control d treatment. BAP concentration was kept fixed that is 1.5 mg/land 2,4-D concentration vary in both legumes callus induction. Observation for 30 days showed that the administration of 2,4-D concentrations and BAP was able to stimulate formation of callus. When observing the callus, 80% of the ex-plants began to form a callus. But until the last observation, which developed to form a callus was only found in 4th treatments in Guarbean and 3rd treatment in Mung.

DISCUSSION

Young guar bean (*Cyamopsis tetragonoloba*) cotyledon explants was reported to provide good explants source for callus induction within 4-5 weeks and young mung (*Vigna radiata*) hypocotyl explant was reported to provide good explants source for callus induction within 6-7 weeks. Changes in ex-plants that were characterized by tissue swelling and the color of the ex-plants to brownish-yellow were a sign that the callus was starting to appear. The swelling of ex-plants was a response from plants that results in most carbohydrates and proteins that will accumulate in the injured tissue.[23] This may be as a result of their physiological state which provides actively dividing cells.[19,24-26] The time when the callus appears was influenced by several factors, including the source of plants used as the ex-plants and growth regulators used.

Presence of 2,4-D which is a suitable growth hormone responsible for callus induction in both the selected beans which has been reported in various species in plant tissue culture work[27-29] this conclude that in the tissue-culture, morphogenesis of ex-plants depend on the interaction between the auxin and the given cytokinins and those already contained in the ex-plants. The concentration of the two growth regulators is often used to control the shape and amount of growth of culture, both in callus growth and organogenesis.[30] It was reported that related to the percentage of live ex-plants of guar bean (*Cyamopsis tetragonoloba*) plants with 2,4-D and BAP treatment showed that BAP concentrations of 1.5 mg/l and 2,4-D 2 mg/l were treatments with the highest percentage of live explants each reaching 70%. And the lowest in the treatment of 0.5 mg/l 2,4-D and BAP 1.5 mg/l was 10%. The percentage of live ex-plants of Mung (*Vigna radiata*) plants with 2,4-D and BAP treatment showed that BAP concentrations of 1.5 mg/l and 2,4-D 1.5 mg/l were treatments with the highest percentage of live explants each reaching 80%. And the lowest in the treatment of 2 mg/l 2,4-D and 1.5 mg/l BP was 10%.

Thus, there was optimum Callus induction with increase in 2,4-D concentration.[31,32] With regards to highest percentage Callus formation, the two legumes responded in different ways. However, (2 mg/l) 2,4-D and (1.5mg/l) BAP concentrations gave better embryonic callus growth of cotyledonary explant in *Cyamopsis tetragonoloba*, and (1.5 mg/l) 2, 4-D and (1.5mg/l) BAP concentrations better embryonic callus growth in *Vigna radiata.*

ACKNOWLEDGEMENT

The authors are grateful to the Faculty In charge of Biotechnology Department of Botany, Ranchi University, Ranchi for support and permission to use their Plant Tissue Culture Laboratory Facilities for this work.

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RUJOST, ISSN: 2319-4227, Vol.7, No.1, January, 2022, pp. 75-82

A Biaanual Peer Reviewed Referred Ranchi University Journal of Science & Technology

Botany

Pharmacognostical study of *Bixa orellana* L.

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Abstract: *Bixa orellana* L., known since ancient times, is a multi-use plant popularly known as Lipstick tree or Sinduri. *B. orellana* is the only species of Bixaceae family. The pharmacognostic investigation was carried out in term of organoleptic, microscopic and physical parameters. Studies revealed that leaves possess microscopically spongy parenchyma shows the presence of calcium oxalate crystal. Fluorescence analysis of leaf powder with different reagents was carried out, which showed evidences of presence of different prominent phytoconstituents which are responsible for various medicinal properties. These findings will be useful towards establishing pharmacognostic standards on identification, purity and quality.

Keywords: Pharmacognosy, Leaves, Stomata, Bark, Fluorescence, Moisture content.

INTRODUCTION

Bixa orellana L., also known as sindur, were frequently, commonly known as Annatto in English, Sinduri in Sanskrit, is indigenous and native to tropical America, but now cultivated in many tropical countries including India.[1]

Bixa orellana is an evergreen shrub or small tree, 2-8 m high; trunk up to 10 cm in diameter. It produces bright green leaves, white or pink colour flower and red or orange colour capsule, seed enclosed within capsule.[2-4]

Traditionally, medicine is a fundamental part of the health system in just beginning countries. It has been traditionally used by the people as a dye to paint hair and body, as insect repellent or protectant from ant and sunburn.[5] Annatto possesses various pharmacological activities like anti-diarrheal, anti-inflammatory, antioxidant, hypoglycemic, anti-bacterial. The pulp surrounding the seed is used as mosquito repellent, haemostatic, anti-dysentric, diuretic and useful to treat epilepsy, kidney and some skin disease. The decoction of leaves is used to prevent vomiting and nausea; to treat urinary difficulties and stomach problems, sore throat, jaundice, snake bites, dysentery, gonorrhoea.[6,7] Root and root bark is perspirant and anti-pyrectic in action which is used as antiperiodic and for controlling Asthmatic Paraoxysm.[8,9] The entire plant is used against fever and dysentery. A decoction of the leaves is used to stop vomiting and nausea; treat heartburn, prostrate and urinary difficulties, stomach problems and internal inflammation, arterial hypertension, high cholesterol, cystitis, obesity, renal insufficiency and to estimate uric acid and as a mild diuretic.[9]

Bixa is used traditionally cooking as colouring agent. It also used for colouring edible materials such as butter, ghee, margarine, cheese and chocolate. The *Bixa* dye also known as annatto dye is extracted from the outer covering of the seed of *Bixa orellana.*[10-12] It also implemented in textile industries for dyeing of jute, wool, cotton and silk. In the global market, it has a preference next to saffron as natural food grade colourant due to its non-toxicity as well as its tendency to preserve natural food colour.[13] The seeds are used to treat fever, skin diseases, and gonorrhoea.[14] The seed derived oil is used to cure leprosy and the decoction is used to treat jaundice.[15-17]

Bixa orellana L. is found in some places of Jharkhand, but research on its medicinal potential is still lacking. Though this plant has received considerable from

Latin American countries but there are few reports from Indian scientists. *Bixa* plant survey and taxonomical identification, phytochemical and pharmacognostical investigation is still in scarcity. Therefore, in the present investigation, attempt would be made to make taxonomical identification of the plant. Besides this, attempt would also be made to undertake phytochemical and pharmacognostical study on *Bixa orellana*.

Classification

Kingdom	–	Plantae
Sub kingdom	–	Tracheoblonta
Super division	–	Spermophyta
Division	–	Magnoliophyta
Class	–	Magnoliopsida
Sub Class	–	Dillenildae
Order	–	Violales
Family	–	Bixaceae
Genus	–	*Bixa*
Species	–	*B. orellana* L.

METHEDOLOGY

The leaves were collected from the Birsa Agricultural University, Kanke, Ranchi, Jharkhand and authenticated as *Bixa orellana* L. from subject experts of Taxonomy University Department of Botany, Ranchi University Ranchi. After authentication of plant the leaves and bark were collected in bulk and washed under running tap water to remove adhering dirt. Then leaves and barks were shade dried and lastly in hot air oven to remove some dirt. The dried material was made into coarse powder by grinding in mechanical mixer grinder and stored in a closed air tight container for further use. The fluorescence analysis was carried out by treating the powder with different reagents as per standard procedures.[18-20]

RESULTS

Macroscopy

Leaves simple, alternate opposite, ovate, cordata, acute apex, unicostate reticulate venetion, green or dark green in colour, 19-22cm length, 12-15cm breadth, 9-10cm petiole (fig. 5 & 6).

Bark smooth, outer part brown or dark brown in colour, inner part of bark was red or orange in colour.

Flowers were pink or white in colour, terminal branched panicles, 7-30 flowered, covered with reddish brown scales, cluster, medium sized flower, dichasial cyme, open, 5 petals, quincuncial, persistence calyx, ovary superior, many stamens (fig. 3).

Fruit a spherical or elongated broadly like ovoid capsule, immature capsule is green further than becomes mature red or orange in colour, seeds numerous (fig. 8).

Table 1- Organoleptic character of different plant part of *Bixa orellana*

S.no.	Organoleptic character	Result		
		Seed	Leaf	Bark
1.	Colour	Red	Green	Brown
2.	Odour	Odourless	Odourless	Odourless
3.	Taste	Slightly bitter	Bitter	Tasteless
4.	Shape	Ovoid angular	Ovate	
5.	Size	3-5mm	19-22 cm	

Microscopic

Stomatal number and Stomatal index:-

It is the average number of stomata per square mm of the epidermis of leaf. Stomatal index was calculated as the percentage of number of stomata present per number of epidermal cells and each stoma was counted as one cell.[13,21]

$$I = \frac{S}{E+S} x\ 100$$

I = Stomatal Index
S = No. of Stomata per unit area
E = No. of epidermal cells in the same unit area

Table 2- Determination of stomatal number and stomatal index of *Bixa orellana* leaf

Leaf	Upper Epidermis	Lower Epidermis
Average stomatal number	21.8	23.8
Average stomatal index	16.41	16.05
Average epidermal cells	110	121.2

MOISTURE CONTENT

Five gram of accurate weight of leaves, flowers, barks and stem of *B.orellana* was placed in hot air oven in petridish and dried at the temperature 72°C for 6 hours

and weighed. Drying and weighing was continued at 2 hour interval until differences between two successive weighings and constant weight was reached.[18]

The % moisture content was determined as follows by using this formula:

$$\% \text{ moisture content} = \frac{(Y_1 - Y_2)}{Y_1} X\ 100$$

Table 3- Moisture contents in different plant part of *Bixa orellana*

S.No.	Plant part	Initial weight (Y1)	Final weight (Y2)	Y= Y1-Y2	**% Moisture contents**
1.	Leaf	5g	2.2	2.8	**43.12±1.54**
2.	Bark	5g	1.5	3.5	28.44 ± 0.26
3.	Flower	5g	0.9	4.1	**16.4±0.49**
4.	Stem	5g	1.6	3.4	27.64±0.57

FLUROSCENCE ANALYSIS

The fluorescence analysis of the drug with specific fluorescent colours and also to find out the fluorescent impurities and it can be used as diagnostic tool for testing adulteration.[11-13] Leaf and bark powder were taken in different tubes. To each of these a fixed volume of different organic solvents like distilled water, ethanol, methanol, glacial acetic acid, sulphuric acid, nitric acid; hydrochloric acid and ammonia were added. The leaf and bark powder are 5g and different type of solvent measured 20 ml. Then all the tubes were left for 30 min and observed under the visible and UV light for the visualization of characteristic colour reaction was compared with a standard colour chart and colours were noted.

Table no. 4: Fluorescence analysis of *Bixa orellana* Leaf and bark powder

S.No.	Reagents	Day Light		UV254nm		UV365nm	
		Leaf	Bark	Leaf	Bark	Leaf	Bark
1.	Such Powder	Light Green	Brown	Light Green	Brown	Brown	Black
2.	Distilled water	Transparent	dark red	Green	Black	Transparent	black
3.	Methanol	Green	Dark red	Green	Black	Green	black
4.	Nitric Acid	Orange	Dark red	Green	black	Green	Black
5.	Ethyl alcohol	Light orange	Light orange	Light green	Green	Dark green	Light green
6.	Sulphuric Acid	Black	Black	Black	Black	Black	Black
7.	Sulphuric acid 50%	Black	Black	Black	Black	Black	Black
8.	Acetic acid glacial	Soil colour	Light yellow	Black	Light green	Black	Transparent
9.	HCL	Dark Brown	Red	Green	Dark green	Green	Black
10.	HCL 50%	Transparent	Orange	Green	Green	Green	Green
11.	Ammonium hydroxide	Orange	Orange	Light green	Green	Black	Green
12.	Formic acid	Black	Light orange	Darkblue	Light green	Black	Transparent

Fig. 1- Habitat

Fig. 2- Fruits

Fig. 3- Flower

Fig. 4- Collection of plant part

Fig. 5- Length of Leaf

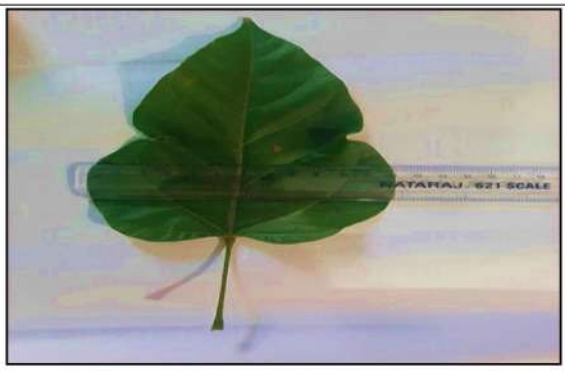


Fig. 6- Breadth of Leaf

Fig. 7- Capsule

Fig. 8- Seeds enclosed in capsule

Fig. 9- T.S. of leaf mid rib region

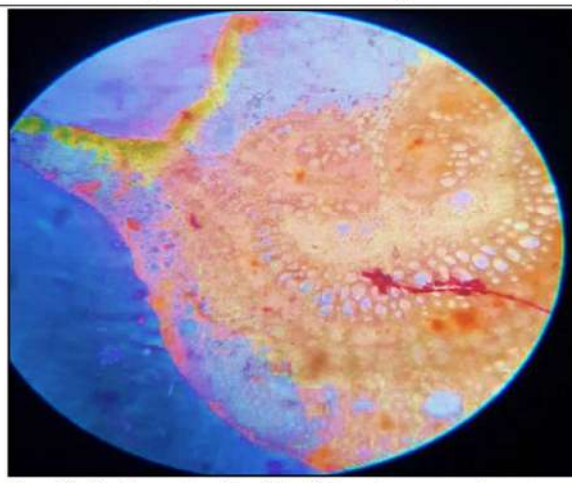


Fig. 10- Epidermal cells of leaf showing parenchymatous tissue

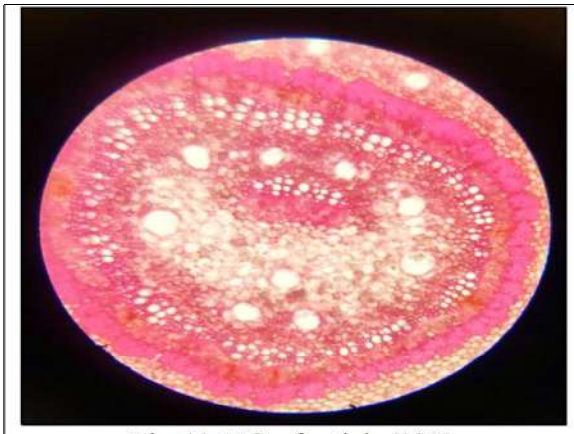


Fig. 11- T.S. of petiole (10X)

Fig. 12 T.S. of petiole (40X)

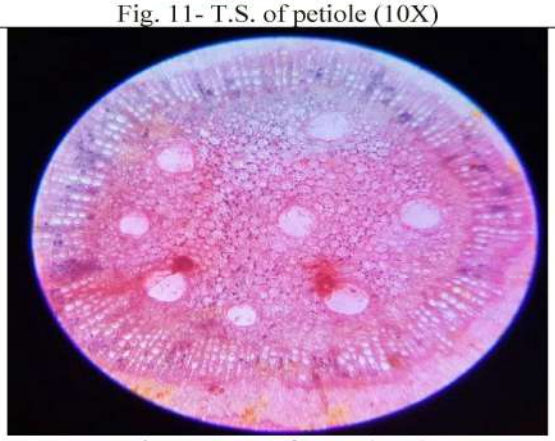


Fig. 13- T.S. of Stem (10X)

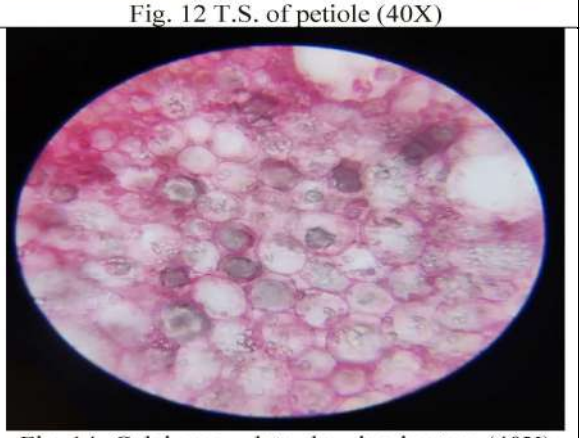


Fig. 14- Calcium oxylate showing in stem (40X)

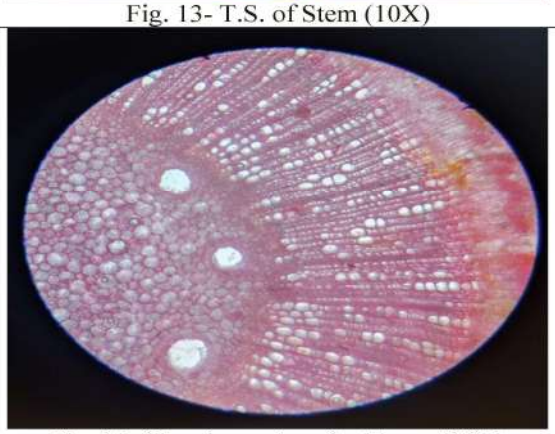


Fig. 15- Showing xylem & phloem (10X)

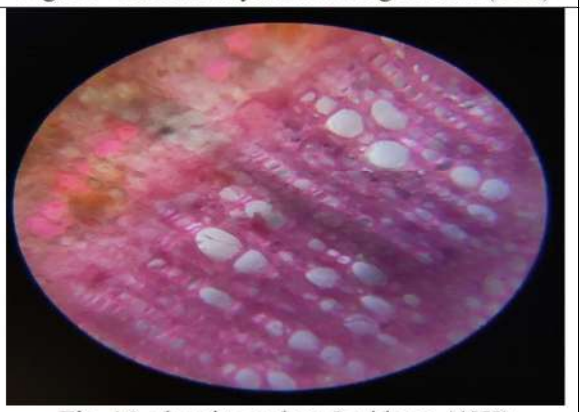


Fig. 16- showing xylem & phloem (40X)

Fig 17: Powder of leaf

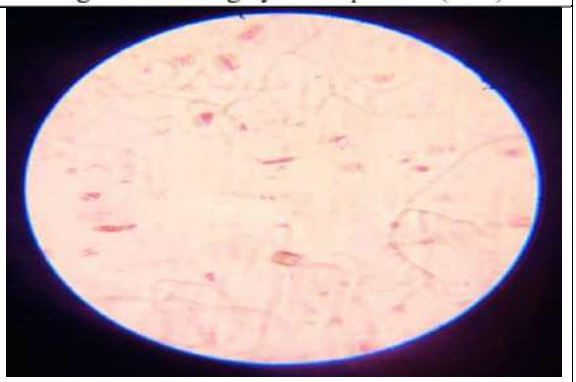


Fig 18: showing trichomes in powder analysis

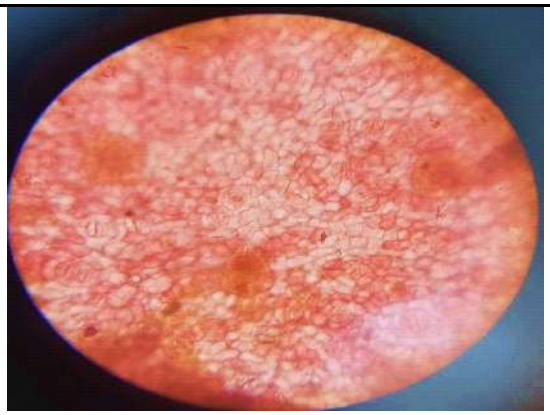


Fig. 19: showing Stomata on lower leaf

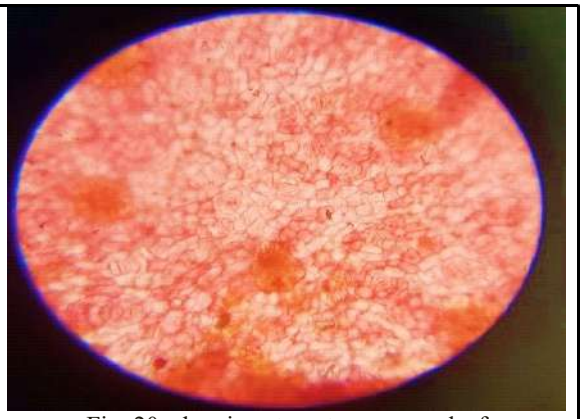


Fig. 20: showing stomata on upper leaf

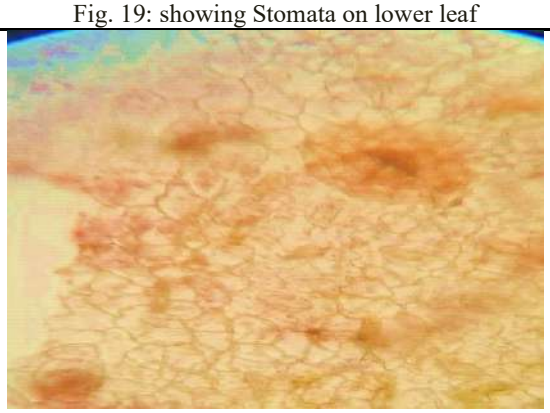


Fig. 21: shape of stomata

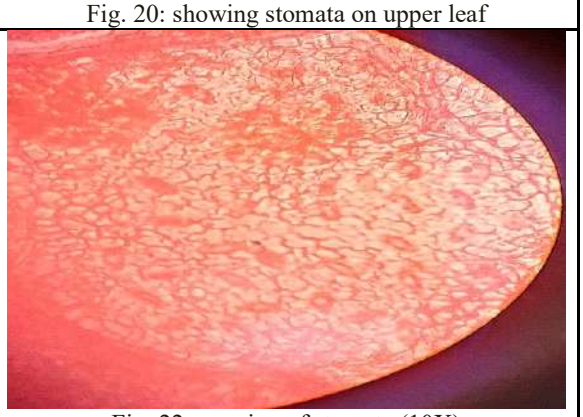


Fig. 22: opening of stomata (10X)

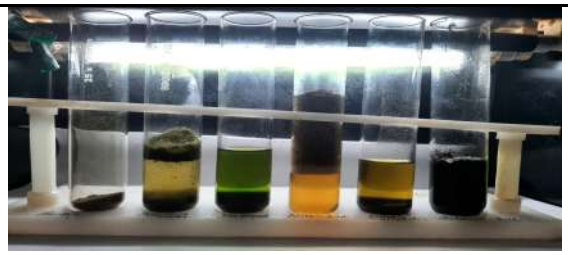
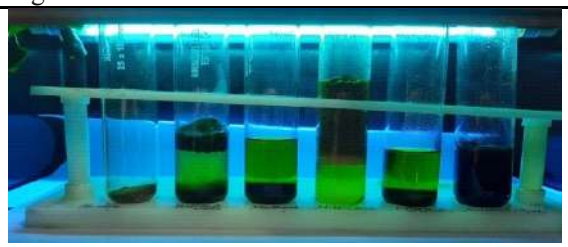
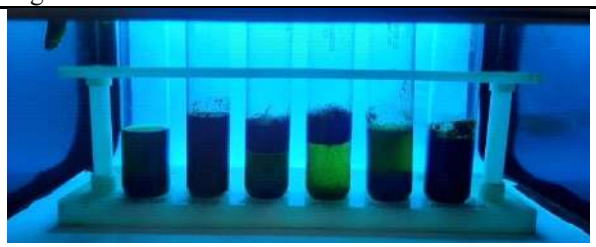
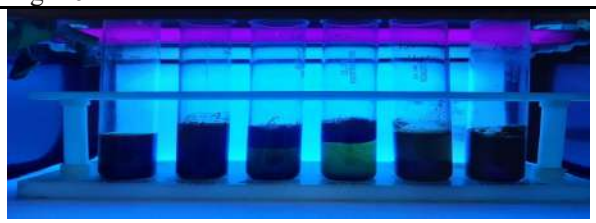
Day light	 Fig: 23	 Fig: 24
254nm	 Fig: 25	 Fig: 26
365nm	 Fig: 27	 Fig: 28

Fig : 23-28 Fluoresence Analysis of Leaf in different solvent

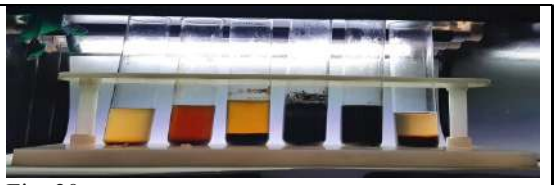
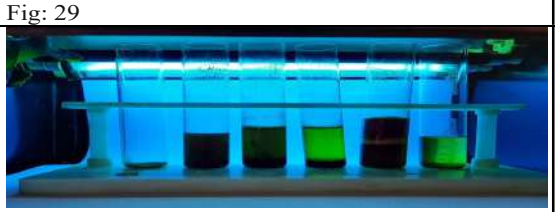
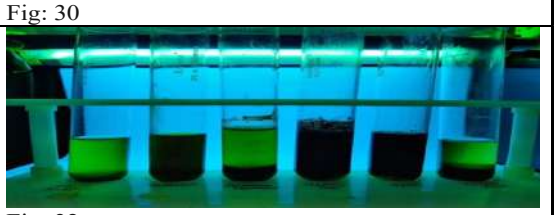
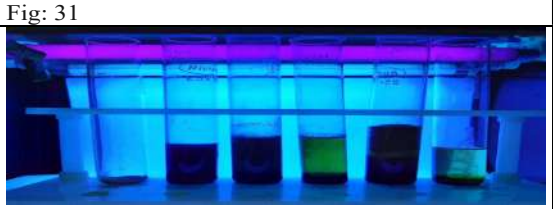
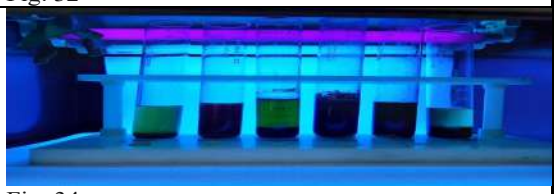
Day light	 Fig: 29	 Fig: 30
254nm	 Fig: 31	 Fig: 32
365nm	 Fig: 33	 Fig: 34

Fig: 29 -34 fluorescence analysis of bark in different solvent

DISCUSSION & CONCLUSION

Bixa orellana L. has been in use since many years in different countries for different medicinal purposes. Many of their therapeutic properties have proven for different biological and pharmacological activities like antimicrobial, antiviral, antibacterial, antimalarial, skin hydration effect etc. But from leaves very few chemical constituents have been isolated though it is using as popular medicine by general public in India. Though the pharmacological activities have been carried out by many researchers, the Pharmacognostical studies on the leaves have not been carried out till date in Jharkhand as per the existing literature.

In organoleptic evaluation, appropriate parameter like taste, odour, size, shape and colour of the leaves, leaves powder, bark and bark powder were studied. Morphology of *B. orellana* was studied in climatic region of Jharkhand. Microscopically, the leaf was simple in composition, opposite in arrangement, apex and base were acute, margin was entire and average leaf size is 9 ± 22 cm (length) and 12 ± 15cm (breadth). Fresh leaves were green in colour and characteristic in odour with a slightly bitter taste. The leaf powder was light green colour with characteristic odour and bitter taste (table 1). Moisture content was higher in leaf and lower in flower (table 3). Micro morphological features revealed that the leaf powder contains numerous calcium oxalate crystal present in leaf and stem. The powders also show the presence of xylem and phloem. Multicellular, long and covering trichomes seen were lignified (fig.18).

The bark was smooth and brown in colour and powder of bark characteristic in odour with as tasteless.

The leaves were greenish in colour, smooth and thick texture and possess number of trichomes on both leaf surfaces. The microscopy studies shows the presence of vascular bundles, collenchyma, spongy parenchyma and palisade cells. Palisade cells were closely packed, elongated and compactly arranged. The stomata show anomocystic shape. (fig. 21)

The average stomatal number and stomatal index of lower epidermis & the upper epidermis of the leaf shown in (table 2). The result shows the upper epidermis of the leaf contain more stomatal number than lower epidermis. The result of moisture content shows higher percent in leaf and lower in flower (table 3). The fluorescence analysis of the powdered drug of *B. orellana* leaf and bark in various solvents was performed under normal and UV light to detect the fluorescent compounds (table 5 & 6). The fluorescence analysis of the drug helps to identify the drug with specific fluorescent colours, and also to find out the fluorescent impurities. Thus, the study of fluorescence analysis could be used as a diagnostic tool for testing adulteration result were shown in table 4.

ACKNOWLEDGEMENTS

We are thankful to the University Department of Botany, Ranchi University, Ranchi for providing the

facilities to carry out the work successfully. We would also like to extend our gratitude to Dean, Faculty of Science and Head of the Department, Prof. (Dr.) Kunul Kandir, for necessary encouragement.

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RUJOST, ISSN: 2319-4227, Vol.7, No.1, January, 2022, pp. 83-92

A Biaanual Peer Reviewed Referred Ranchi University Journal of Science & Technology

Botany

A concise review on Alpha -amylase from bacterial source

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Abstract: This review highlights the progress made in microbial alpha amylase which is among one of the most important industrial enzyme in various industries like food and beverages, textile, paper, pharmaceutical, detergent etc. In this article family, source, characteristics, production aspects, industrial application of α-amylase had discussed.

Keywords: α-amylase, α-1,4 glycosidic bond, SmF, SSF, *Bacillus* sp.

INTRODUCTION

Amylases are the enzyme that catalyses the hydrolysis of starch molecule into dextern, maltose and ultimately into glucose units. They hydrolyse the internal α-1,4-O-glycosidic bonds in polysaccharides with the retention of α-anomeric configuration in the product. Mostly alpha amylases are metelloenzyme which requires calcium (Ca^{2+}) ions. Amylases are one of the most used enzymes in industrial sector because of its usability. These enzymes have the wide variety of applications in sectors like paper, sugar syrup, starch liquefaction, brewing industries, pharmaceuticals and many more. They are present in micro as well as macroorganisms.[1] Amylases production has reached upto 65% in the industries and still increasing day by day.[2] They are also preset in saliva of humans as well as saliva of some other mammals where it starts the process of digestion. Alpha amylase is also produced by pancreas. Both pancreatic and salivary amylase hydrolyses dietary starch into disaccharides and trisaccharides which are further converted into glucose by some other enzymes. Presently there are more than 30 different amylolytic and related enzymes are known. Amylases can be classified into different groups named alpha, beta, and debranching amylases.[3] They act on random location on starch molecules and alpha amylase act on anywhere in a long carbohydrate chain and produce maltotriose and maltose from amylose, maltose, glucose and limit dextrin from amylopectin. α-amylase are best known endoamylases which are faster than beta amylases. They act best on pH 6.7-7.0. The alpha amylases form is also found in plants, fungi (ascomycetes and basidomycetes) and bacteria (bacillus). β-amylases can also be obtained from plants, fungi and bacteria. Beta amylases act on the non-reducing end and hydrolyses the second alpha-1,4 glycosidic bond and cleave two glucose units (maltose) at a time. When fruits ripes β-amylase breaks down starch into maltose which gives fruits its sweet flavor. Alpha and beta amylase both present in the seed, while β-amylase present in an inactive form prior to germination, α-amylase and protease appears when germination starts to begun. *Bacillus* genus is most used bacteria for the production of α-amylase and for this purpose many strain like *B. subtilis*, *B. cereus*, *B. licheniformis*, and *B. amyloliquefaciens* are isolated and screened.[4] Some of the *Bacillus* strains are also used in raw starch degradation.[5-7] These enzymes can also be categories according to their degree of hydrolysis of substrates into two different categories; liquefying (30-40%) and saccharifying (50-60%). This division is very helpful in describing the various properties of α-amylase.[8] This results in different length of oligosaccharide produced by α-amylase.

AMYLASE FAMILY

Enzymes are protein, enzymes are biocatalyst that speed up the chemical reactions in living things in

biological system. There are more than 30 different types of amylolytic and related enzyme and the alpha amylase belongs to the clan GH-H of family glycoside hydrolases, transferases and Isomerases.[9] There is a large variety of enzymes that are able to act on starch. These enzymes are divided into four groups: endoamylases, exoamylases, debranching enzymes and transferases.[10]

- Internal α-1, 4 bonds are cleave by endoamylases which produce alpha anomeric products.
- α-1,4 or α-1,6 bonds of the external glucose residue leave by exoamylase which produce alpha or beta anomeric products.
- α-1,6 bonds hydrolized by debranching enzymes leaving long linear polysaccharide.
- And transferases cleaves the α-1,4 glycocidic bond of donor molecule and transfer part to a glycocidic acceptor molecule making a new glycocidic bond.

Wide variety of saccharides are metabolised by glycoside hydrolases. Based on their mode of reaction and family they belong they are divided into classes. Most enzymes which convert starch belongs to GH 13 family. Further this GH 13 family is divided based on their larger unit called clan, a three dimensional structure of catalytic module. A clan may consist of more than two families which may have same three dimensional structure of catalytic domain with limited sequence similarities which indicate that protein structure is better preserved then amino acid sequence during evolution. α-amylase family belongs to 8th clan, G-H-H.[11] The conception of grouping this enzyme as α-amylase was done in 1992.[12] This group of family have following characteristics number :

- They must have four highly conserved regions in their primary structure.
- They must act on alpha glycosidic linkage and hydrolized them to produce monosaccharides and oligosaccharide.
- They must have residues of Asp and Glu, Asp as their catalytic site.
- They must possess a $(\beta/\alpha)_8$ or TIM barrel catalytic domain.

CATALYTIC DOMAIN

β-3, β-4, β-5 and β-7 strands are the four conserved sequence of the catalytic domain $(\beta\text{-}\alpha)_8$-barrel domain identified and is used to define α-amylase family. Prototype of two dimensional structure of α-amylase consist of three domains name A, B and C. N terminal TIM barrel structure is the domain A. While domain B consist of long loop that protrudes between β strand 3 and α-helix 3 where as domain C have β sheet structure link to domain A. Four highly conserved regions of TIM barrel closely related to active site present in all α- amylase.[13]

SOURCES OF ALPHA AMYLASE

α-amylase are the ubiquitous enzyme produced by animals, plants and microbes, helping in their carbohydrate metabolism. Amylases from these sources are used as food additives from centuries. In brewing industry barley amylase is being used. In the preparation of oriental food fungal by lasers are used. In industries fungal and bacterial amylases are mainly used because they are cost effective and it required less time and space for the production and their process optimization and modification is also easy to modify.[14]

Among bacteria *Bacillus* species is widely used for production of amylases. There are many species like *Bacillus subtilis*, *Bacillus stereothermophilus, Bacillus licheniformis* and *Bacillus amyloliquefaciens* are some of the good producers of α- amylase. Also filamentous fungi have been widely used for α-amylase production for centuries. Fungi belonging to genus *Aspergillus* have been most commonly employed for α-amylase production. Genetically modified microorganisms are also good producers of α-amylases with noble characteristics like thermal stability.

FERMENTATIVE PRODUCTION OF α-amylase

To fulfil the high demand of α-amylase in the various industry low cost medium is required for the production of this bacteria for the production both SSF (solid state fermentation) and SmF (submerged fermentation) could be used for amylase, traditionally these have been obtained from submerged cultures because of ease of handling and greater control of environmental factors such as pH and temperature. Mainly synthetic media have been used for amylase production to submerged for fermentation.[15-18] The content basically in the synthetic media suggest nutrient broth, soluble starch as well as other components are very expensive although this could be replaced with cheaper agriculture by products for the production of low cost medium. SSF resembles natural microbiological process such as composting and insulin which can be utilised in a controlled way to produce a desired product from the very long time SSF has been used to convert moist agriculture

polymeric substrate like sweet, rice, soy extract into fermented food products including industrial enzymes.[19] During SSF the microorganisms are grown on moist solid substrate with negligible free water.[20] This solid substrate provides support or both support or nutrition to the microorganism.

During SSF the metabolites so produced are concentrated and purification with low cost.[21-24] In journal SSF is preferred over submerged fermentation because of its simple technique, low capital investment, lower level of catabolite repression and end product inhibition, water output, better product recovery, and high quality production.[25] Different type of substrates are used in solid state fermentation such as wheat bran, mason flour mill, rice husk, cotton seed meal, soya bean meal, and pearl millet and rice bran etc.[26]

SSF technique is generally used to process fungi and bacteria.[27,28] The production of α-amylase is by solid state fermentation is limited to genus *Bacillus*. *B. subtilis, B. polymyxa, B. mesentericus, B. vulgarus, B. coagulans, B. megateriumand, B. licheniformis*. Less fermentation time was required for α-amylase from bacteria in solid state fermentation technique.[29] This will lead to considerable reduction in the capital and repetitive expenditure. Also the utilisation of these agriculture wastes provides alternative substrate and help in solving soil pollution problems.

PROCESS OPTIMIZATION

Different parameters and manipulation of media were used to optimize for the large scale production of enzyme to meet the industrial demand.[1] Various chemical and physical parameter affect the production of α-amylase like temperature, pH, incubation time, carbon sources, surfactant, nitrogen sources, phosphate, different metal ions, moisture and agitation with regards to solid state fermentation and submerged fermentation respectively.

TEMPERATURE

Temperature is directly related to the growth of organism. Hence optimum temperature is required depending upon whether the culture is mesophilic or thermophilic. Most of the study on α-amylase production in case of fungi have been done with mesophilic fungi with the temperature range of 25-37°C.[30,31] From *Aspergillus ficuum* α-amylases was produced at 30°C.[32] Yeast likes *S. cerevisiae* and *S. kluyveri* to produce α-amylase add 30°C.[33] An optimum temperature for α-amylase production has been reported at 50-55°C for the *Talaromyces emersonii, Thermomonospora fusca* and *Thermomyces lanuginosus.*[34,35] Where as bacterial amylase can be produced at much wider range of temperature 37-60°C the most common bacteria for α-amylase production are *B. licheniformis, B. subtilis,* and *B. amyloliquefaciens, B. stereothemophilus. Thermococcus profundus* and *Thermatoga maritime* are reported to produce α-amylase at 80°C.[36] The optimum temperature for *Rhodothermus marinus* which is a marine thermophilic bacteria produces α-amylase at 61°C. α-amylase from Antarctic psychrophile *Alteromonas haloplanktis* was reported to exhibit optimum activity for is reported at 4°C.[37]

pH

One of the most important factors which determine the morphology and growth of the microorganism is that microorganism is sensitive to hydrogen ion concentration present in the medium. It has been reported that fungi require aesthetic be it while the bacteria required the neutral page for its maximum activity. Concentration of hydrogen iron in the medium directly affect the synthesis and secretion of α-amlase it also affects its stability.[38] *Aspergillus* species like *A. oryzae, A. ficuum* and *A. niger* produce α-amylase at pH= to 5.0 - 6.0 in SmF.[32,39,40] Some of the strain such as *Saccharomyces cerevisiae* and *S. kluyveri*give significant enzyme production at pH 5.0.[33,41] Bacteria like *B. licheniformis, B. subtilis,* and *B. amyloliquefaciens* produce α-amylase add pH 7.0.[1,42,43] While *Rhodothermus marinus* produces α-amylase at pH range 7.5-8.[44]

CARBON SOURCES

Galactose, glycogen and insulin are the best suitable carbon sources as a substrate for the production of α-amylase by *B. licheniformis* and *Bacillus* sp.1-3.[45,46,10] Glycerol as well as starch are known to increase enzyme production in some of the bacteria like *B.subtilis* IMG 22, *Bacillus* sp. PS-7 and bacillus sp. I-3.[6,47,48] Soluble starch has been reported as the best suitable substrate for the production of α-amylase by *B. stearothermophilus.*[49] For both liquid and solid fermentation agriculture waste are being used to reduce the cost of fermentation media. These agriculture waste consist of nitrogen and carbon sources which is required for the growth and metabolism of organisms. These nutrient sources includes orange waste, potato, pearl millet start, wheat, corn and rice as flower.[40,43,50]

NITROGEN SOURCES

One of the best nitrogen source for α-amylase production is soya bean meal by bacillus sp.1-3.[6,31,47] It has been reported that peptone increase the enzyme activity while yeast extract does no effect on α-amylase production. Strains like *B. stearothermophilus* and *B. amylolyticus* shows maximum α-amylase production when the media is supplemented with 1% peptone, 0.5% yeast extract at 0.5% maltose under shaking conditions.[51] It has also been reported that peptone is better nitrogen source for enzyme production by *B. licheniformis* SPT 278 than ammonium hydrogen phosphate which is the best among inorganic nitrogen sources.[52]

SURFACTANTS

The role of surfactant in the fermentation medium is to increase the secretion of protein by increasing cell membrane permeability. Hence surfactant is used for production of α-amylase.In the fermentation medium addition of tween 80 (1.3%) increases the α-amylase production by two folds in *Thermomyces lanuginosus.*[53] It has been reported that 5% PEG 600 and PEG 3000 increases the enzyme production by 31% in *B. amyloliquefaciens* and 21% increase in *B. subtilis.*[54]

METAL IONS

Food growth of microorganism the production medium can be supplemented with salt of certain metal ions and hence and increased enzyme production (α-amylase are known to be metalloenzyme). Calcium ions are naturally present in these enzymes so the addition of calcium chloride in the fermentation medium can increased the enzyme production.[31,55] It has been reported that calcium chloride (0.1%) (and sodium chloride 0.1%) had increase the α-amylase production in solid state fermentation using amaranths green as substrate.[56]

PURIFICATION

Production of pure enzyme is done through downstream processing which constitute a major percentage of overall production cost. In downstream processing the purification is done after fermentation which depends upon the cost of the process, market, quality of final product and the technology which is being used. α-amylases enzymes are purified by any chromatographic technique. Some of the purification technique for α-amylase list are given in Table 1.

ENZYME CHARACTERISTICS

The rate at which α-amylase hydrolysis starch depends upon properties like temperature, pH, nature of substrate, enzyme concentration, presence of calcium iron, substrate concentration. These properties of α-amylase should match its application. These properties diversify the applications in the need to search for noble α-amylase**.**

EFFECT OF TEMPERATURE

It is desirable that α-amylases should be active at high temperatures of gelatinization (100–110°C) and liquefaction (80–90°C) to economize the process; therefore, there has been a need and continual search for more thermophilic and thermostable α-amylase.[14] The Ca^{2+} is necessary for enzyme folding and enzyme stability. Secondary calcium binding sites have also been reported, which enhanced the thermostability. Saboury reported the presence of 17 different secondary binding sites for calcium in α-amylase of *B. amyloliquefaciens*, which were responsible for stabilization of the enzyme against thermal and surfactant denaturation.[50] Different sources of α-amylases exhibiting considerable temperature stability are given in Table 2.

EFFECT OF pH

Natural pH of starch slurry is generally around 4.5. It is known that gelatinized starch is more susceptible to degradation than nongelatinized starch. The extreme conditions required for such pretreatment necessitate the use of an enzyme that is resistant to high temperatures and low pH. Acid hydrolysis of peptide bonds at low pH has been reported to occur most often at the C-terminal side of Asp residues, with the Asp-Pro bond being the most susceptible. This may be due to the facts that the nitrogen of proline is more basic than that of other residues, and Asp has an increased propensity for a–b isomerization when linked on the N side of a proline.[40] (Table 3)

METAL IONS

Most of the amylases are found to be dependant on divalent metal ion like Ca^{2+}, Zn^{2+}, Mg^{2+}, Fe^{2+}, Mn^{2+} etc[21]. α-amylase activity of alkaliphilic *Bacillus* sp. ANT-6 is increased in the presence of calcium iron.[14] Calcium ions also provide stability of the enzyme by sorting out of hydrophobic residues in the protein.[6] Zinc iron has been reported to inhibit thermostable α-amylase from thermophilic *Bacillus* sp. This will further suggest that the inhibition with the iron determine the thermal stability of enzyme. Zinc ion decreases the activity of ANT-6 enzyme.[14]

INHIBITORS

In the study of the characteristics of enzyme it is important to know the effect of metal chelators and chemical compounds. The inhibition effect of these metal chelators indicates that the requirement of certain metal irons for enzyme activity. Several studies have done on the effect of chemical modifiers for enzyme activity of α-amylase like in the case of marine *Vibrio* sp.[58] The result's showed that the amino acids like Trp, Lys, Asp/Glu, and His affect the enzyme activity.

Table 1: Different methods of purification of α-amylase

Method	Adsorbent	Yield %	Purification Fold	Reference
Affinity adsorption chromatoraphy	β-cyclodextrin-iminodiacetic acid-Cu^{2+}	95	-	57
Substitute affinity method	Insoluble corn starch at 4°C	78	163	58
Expanded bed chromatography	Alginic acid-cellulose cell beads	69	51	59
High speed counter current chromatography	PEG4000-aqueous two-phase system	73.1	-	60

Table 2: Effect of temperature on α-amylase activity

Organism	Temperature Range/ °C	Temperature Optimum/ °C	Residual Activity	Reference
Aspergillus tamarii	50-60	55	90 (65 °C for 3 h)	61
Bacillus sp. I-3	65-100	70	50 (80 °C for 2.5 h)	6
Cryptococcus flavus	50-60	50	60 (60 °C for 60 min)	62
Pyrococcus furiosus	80-100	100	50 (98 °C for 13 h)	36
Lactobacillus manihotivorans	50-60	55	70 (50 °C for 1.0 h)	63
Thermobifida fusca NTU22	50-60	60	70 (60 °C for 3 h)	64
Scytalidium *thermophilum*	55-65	60	0 (55 °C for 25 min)	65

Table 3: Effect of pH on enzyme activity.

Organism	pH Range	pH Optimum	Residual Activity	Reference
Bacillus sp. I-3	5.0–5.5	7.0	80 stable	6
Bacillus sp. ANT-6	9.0–13	10.5	55 (pH=10 for 15 h)	14
Bacillus KSM-K38	6.0–11.0	8.0-9.5	80 (pH=11 for 30 min)	66
Bacillus sp. PS-7	5.0– 8.0	6.5	96 (pH=5.0 for 90 min)	47
Lactobacillus manihotivorans	4.0–6.0	5.5	90	63
Cryptococcus sp. S-2	5–7	6.0	-	67
Aspergillus kawachii IFO 4308	2.0–6.5	5.0	90 (pH=2.0 for 30 min)	68

APPLICATIONS

The first enzymes which are to be commercially produced were α-amylase. The first industrial production of amylase was established by Dr. J Takamine from *A. oryzae* which is known as "Taka diastase" which was consumed as digestives. In 2004 the global market of this enzyme reached upto $2 billion. Also there is approximately 3.3% average annual growth. There is 40% share of carbohydrases comprising amylases, pectinases, cellulases and isomerase and 90% carbohydrase produced are utilized in food and beverage industries.[69] It is estimated that annual sale of amylases will reach upto $11 million.[70]

Sector	Uses	Reference
Food industry	Production of maltose syrup, glucose syrup, high fructose corn syrup, crystalline glucose.	71,72
	Reduction of viscosity of sugar syrups, haze formation in juices	
	Solubilization and saccharification of starch for alcohol fermentation in brewing industries.	
	Retardation of staling in baking industry	
Paper industry	For appropriate coating of paper reduction in viscosity of starch	
Detergent industry	Used as additive to remove starch based dirts.	
Textile industry	Wrap sizing of textile fibers.	
Pharmaceutical industry	Used as digestive aids	

Table : Uses of amylases

CONCLUSION

α-amylase are most widely used enzymes which have many industrial use from food, textile industry to paper industry. The demand of α- amylase is is increasing continuously as there is increase in requirement of this enzyme in various industries. It's wide range of application spectrum and with specificity of the enzyme, many research is being currently occurring on developing thermotolerant and pH tolerant α-amylase from microbes. There is also modification of these enzymes genetically or applying site directed mutagenesis to achieve desired characteristics in the enzyme. Mostly production of α-amylase is done through submerged fermentation but nowadays solid state fermentation is being looked as a potential tool for production specially applying agro industrial residue as substrate.

ACKNOWLEDGEMENT

I express my gratitude to Prof. (Dr.) Kunul Kandir, Head and Dean, University Department of Botany, Ranchi University, Ranchi, for her support and motivation to do research work and providing all the necessary facilities and Dr. Ladly Rani, Assistant Prof. University Department of Botany, Ranchi University, Ranchi, for her guidance and encouragement.

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RUJOST, ISSN: 2319-4227, Vol.7, No.1, January, 2022, pp. 93-98
A Biaanual Peer Reviewed Referred Ranchi University Journal of Science & Technology

Chemistry

Microbial analysis of waste water from industry and reduction of chemical oxygen demand by using selective organisms

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Abstract: One of the major problems faced today is pollution and amongst all water is one of the most polluted natural resources at present. And one of the major factors causing this pollution is the industrial wastes containing hazardous substances that are disposed into the water bodies which further joins other bodies and this chain ends up only at oceans hence polluting everything in its way. This can be prevented by initial analysis of industrial waste before being dumped. Analysis such as pH test, colour, SS-BOD, oil and grease content and COD amongst others will help determine the quality level of the waste which can further be sent to waste water treatment plants before being discharged in case its not safe for regular disposal. There are several physico-chemical methods in place to bring down the contamination level of the waste water but owing to limitations of results and costs these are not implemented everywhere. Using microorganisms such as *Pseudomonas aeruginosa, Bacillus subtilis, Enterobacter aerogenes* which help in reduction of COD (chemical oxygen demand) significantly. These organisms are fit to reduces COD by 60-70% within few days for wastes generated from pharmaceutical, textile, milk and food industries.

Keywords: waste-water, analysis, COD (Chemical Oxygen Demand), reduction, microorganisms

INTRODUCTION

One of the major challenges that exists for human kind is pollution and amongst all, the resources that exists naturally on earth, water is the most polluted resource. Maximum portion of the entire pollution found in water bodies come from wastes generated and discharged by industries. The nature of the pollutant discharged depend upon the process involved in the origination of the waste. Industries like pharma, liquor, milk and food discharge highly hazardous effluents that are poisonous and harmful to both land and aquatic creatures.

Though the level of untreated waste from pharma is small but that small amounts to a lot of harmful non-biodegradable organic products. Milk industry amounts to 4 to 11-million-meter-cube of waste per year and this is close to double the amount of milk produced. Most of the milk industries use a processing unit that uses "clean in place" system which pumps cleaning products (solutions) across all equipment, and these solutions are mostly acidic (sodium hydroxide, nitric acid). The textile wastes contain metals like zinc, copper and many more, which are capable of harming environment. Dye's wastes can even cause serious issues like haemorrhage, nausea and ulceration of skin.

Conventional methods are becoming more and more challenged in waste water treatment as contamination, population and activities increase. Hence what is required is - Advanced Oxidation Techniques to be implemented in industrial waste treatment.

Chemical Oxygen Demand Reduction

Chemical Oxygen Demand (further referred as COD) determines the requirement of oxygen that can be oxidized with the help of a strong chemical oxidant. It is an extremely noteworthy and necessary parameter, that measures water bodies based on their organic strength. In the below study, microorganisms are used to reduce the COD from waste water making it suitable for discharge.

The dichromate reflux method is suitable over other oxidant used procedures of the open reflux method. Organic content gets oxidized by potassium dichromate with catalyst being silver sulphate with hydrochloric acid being present to produce carbon dioxide and water. The excess potassium dichromate is titrated with ferrous ammonium sulphate, the consumed dichromate gives oxygen which is required for organic matter's oxidation.

The involved reactions are as follows:

i. $2K_2Cr_2O_7 (+) 8H_2SO_4 \rightarrow 2K_2SO_4 (+) 2Cr_2(SO_4)_3 (+) 8H_2O (+) 3O_2$
ii. $C_6H_{12}O_6 (+) 6O_2 \rightarrow 6CO_2 (+) 6H_2O$
iii. $Cr_2O_7^{--} (+) 6Fe^{++} (+) 14H^+ \rightarrow 6Fe^{+++} (+) 2Cr_3^+ (+) 7H_2O$

METHOD

Pure culture (organisms living without the presence of other organisms) of the three bacteria- *Enterobacter aerogenes*, *Pseudomonas aeruginosa* and *Bacillus subtilis* were taken.

A suspension of the culture and also sub-culture of the organisms in separate nutrient media was prepared.

Nutrient-broth that contains different concentrations of the samples like 5%, 15% etc. were also prepared and were sterilized.

5 ml of culture suspension of each organism in different samples were poured but with same concentration of nutrient-broth. Then incubated at 37ºC for 48 hours. After the incubation, samples from each organism inoculated at 5% concentration sample were taken and were placed on nutrient agar plate. Then it was incubated at 37ºC for 24 hours. Growth of particular organism in each plate were monitored and observed.

Another culture suspension from this latest nutrient plate was prepared and 5ml of this culture was added to a new concentration sample of nutrient-broth and the same steps were repeated and the growth in this case was observed as well.

Preparation of the Sample

200ml sample was taken into a 500ml conical flask and was sterilized at 15 p.s.i. at 121°C. After autoclaving, the sample were left to cool down to room temperature. Then the sample was introduced to 30ml of culture suspension prepared from a 15% concentration sample of nutrient-broth. The flask was warmed at room temperature on shaker at a rpm of 200. Post 24hour, sample were taken for analysis of COD reduction. This was continued for a period of 5 days.

Calculation

$FAS=K_2Cr_2O_7$

$N_1V_1=N_2V_2$

Where,

N_1 = Normality of FAS
V_1 = Volume of FAS used for titration of Dichromate
N_2 = Normality of Potassium Dichromate
V_2 = Volume of Potassium Dichromate taken for titration

On using the above values, FAS normality can be determined.

RESULTS & DISCUSSIONS

Ten samples for industrial wastes (effluents) were tested for parameters like: pH, chloride, colour, TDS and TSS, BOD, COD and oil-grease content. It was found that few samples had permissible amount whereas the limits on few samples made them unfit and hence were suggested to go for further treatment before the unloading. The overall results are indicated in table 2.

pH:- This is a logarithmic value used to determine the nature of a solution, whether it is acidic, alkaline or neutral. The pollutant in almost all the chemical industries are large scale and is either in acidic or alkaline form in manufacturing units. Variations in pH value of the effluent can alter the rates of reactions (mostly biological) and also the survival of various micro-organisms. In the current study, pH of final outlet samples is all within range.

Chloride:- Chloride is present in all water's bodies with varying concentrations. Chloride in excess of 250 mg/L brings a salty taste in water. Organisms not accustomed to high chlorides content may be subjected to some or great extent of laxative effects.

Colour:- Uses of colored water is limited and the severity of the situation can lead to complaints and probable breach of discharge limits. The intensity of the situation with colour is severe and can cause disturbances in water bodies.

TDS or Total Dissolved Solids and TSS or Total Suspended Solids:- This is a measure for salinity in the water. Many salts like carbonate, sulphate, Ca, Mg, Na, K, Fe, and Mn etc. are present. A high content of dissolved solids affects the water density and influences regulation of freshwater in organisms.

BOD (Biochemical Oxygen Demand):- It is the measure of biodegradable material (organic) present in wastewater. BOD can be stated as the oxygen requirement

of the micro-organisms in balancing the biologically degradable organic matter under aerobic conditions.

COD (Chemical Oxygen Demand):- This analyses the measurement of oxygen depletion capacity of any water which is contaminated with organic effluents. Typically, it gets the equivalent amount of required oxygen to oxidize organic compounds in water.

Oil and Grease:- Oil forms a coat on the surface of water bodies causing reduction of oxygen in the water bodies which affects the organism living beneath. Due to coating sunlight penetration is also affected and hence affects the photosynthesis process directly. In either land or water animals, coating of oil or grease on the water surface can hamper the properties of fur and feathers.

Table 1- Materials and methods of physical, chemical and microbial parameters

PARAMETERS	MATERIALS	METHODS
PHYSICAL PARAMETERS		
pH	Electric pH meter	Electrometric method
Color	Color comparator	Visual comparison method (APHA ed. 22, pg. 2 – 5)
TDS & SS	Filter assembly , filter paper	Filtration & Gravimetric method (APHA ed. 22, pg. 2- 65,66)
CHEMICAL PARAMETERS		
BOD	Magnesium sulphate, Calcium chloride, ferric chloride, starch solution, sodium sulphate, phosphate buffer, seed culture	Alkali azide method , titrimetric method (APHA ed. 22, pg. 5- 4)
COD	Concentrated H_2SO_4 with $AgSO_4$ Standard potassium dichromate(0.25N), Ferrous ammonium sulphate (0.25N), $HgSO_4$ powder	Open reflux method (APHA ed. 22, pg. 5 – 16)
O & G	Hexane, sodium sulphate, HCL, separation funnel	Alkali Azide Method, Titrimetric method (APHA ed. 22, pg. 5 – 38)
Chloride	Potassium chromate, $AgSO_4$ (0.014N)	Argentometric Method (APHA ed. 22, pg. 4 – 72)
Sulphate	Conditioning reagent, $BaCL_2$, D/W	Turbidimetric method (APHA ed. 22, pg. 4-188)
Ammonical nitrogen	Alkali solution, boric acid, phenolphthalein, H_2SO_4 (0.02N)	Ammonia distillation & titrimetric method (APHA ed. 22, pg. 4 – 110)
MICROBIAL PARAMETERS		
Total coliforms	Lauryl Tryptose Broth, Brilliants Green Bile Broth	Multiple tube fermentation method (APHA ed. 22, pg. 9 -66)
Fecal coliforms	EC Broth	Multiple tube fermentation method (APHA ed. 22, pg. 9 – 74)

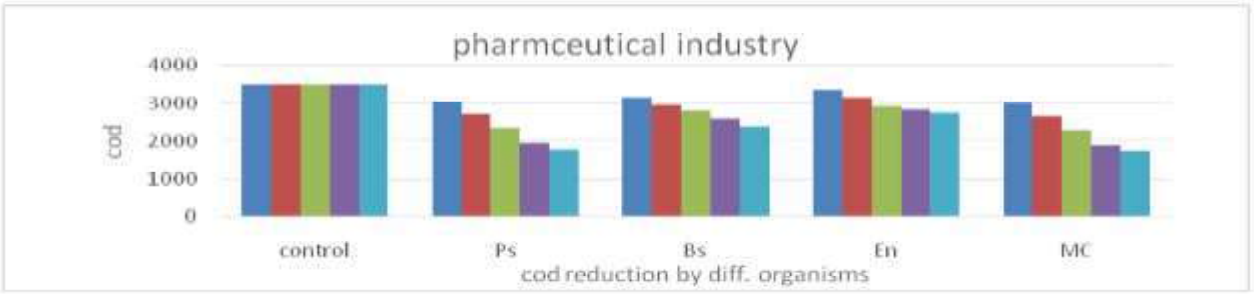
Table 2- Results of all parameters

Samples	pH	Colour	Chloride	Sulphate	BOD	COD	O&g	Nh3n	TDS	Ss
DAIRY										
1	7.84	blackish	1890	240	744	1712	2	0.56	4092	16
2	8.62	light black	1072	150	4	12	3.8	19.18	2340	22
TEXTILE										
3	12.09	blackish	990	570	80	275	2.8	2.82	3364	158
4	7.54	greenish	1485	1070	211	456		31.02	5693	313
FOOD &FOOD										
5	8.1	greyish	608	502	110	321		29.93	2328	128
6	5.84	light yellowish	507	123	946	1338	2.2	13.68	2230	640
PHARMACEUTICALS										
7	7.84	colourless	204	160	54	155	16.3	19.18	928	64
8	6.98	blackish	700	237	568	5452	3.4	53.58	1780	4280

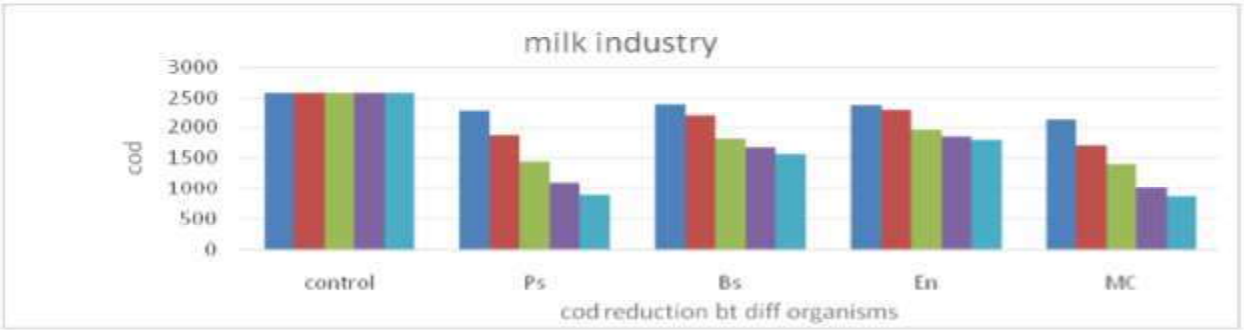
Table 3- Results of COD reduction

SAMPLE		1ST DAY	15TH DAY	% REDUCTION
Pharmaceutical industry	*Pseudomonas* spp.	3096	1548	50%
	Bacillus spp.	3096	2388	24%
	Enterobactor spp.	3096	2726	18%
	MIXCULTURE	3096	1920	62%
Milk industry	*Pseudomonas* spp.	2279	878	64.75%
	Bacillus spp.	2388	1285	50.0%
	Enterobactor spp.	2365	1802	24.0%
	MIXCULTURE	2111	743	71.03%
Food industry	*Pseudomonas* spp.	1518	582	68.9%
	Bacillus spp.	1726	992	46.9%
	Enterobactor spp.	1776	1305	30.3%
	MIXCULTURE	1490	479	74.7%
Textile industry	*Pseudomonas* spp.	1843	1028	49.2%
	Bacillus spp.	1922	1435	29.0%
	Enterobactor spp.	1972	1584	26.0%
	MIXCULTURE	1827	744	63.3%

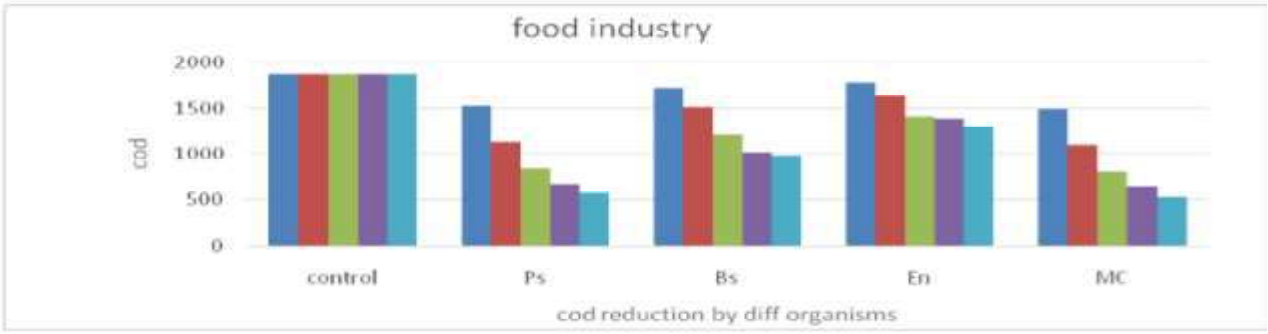
COD REDUCTION GRAPHS



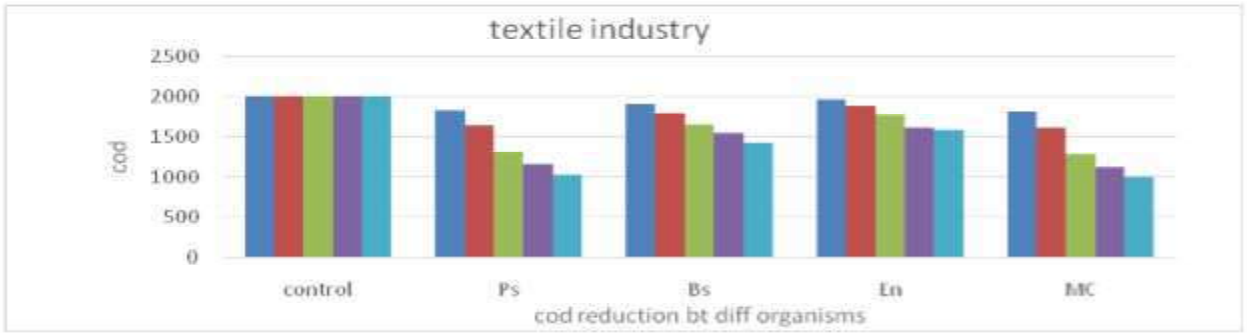
GRAPH: –Depicts that COD reduction of pharmaceutical waste water by different organisms are different such as *Pseudomonas*. shows reduction during 1st day - 2%, 3rd day - 13%, 6th day - 22%, 9th day - 32%, 12th day - 44%, 15th day - 50%. *Bacillus* spp. shows reduction during 1st day - 2%, 3rd day - 10%, 6th day - 15%, 9th day - 20% 12th day – 26%, 15th day - 24%. *Enterobacter* spp. shows that 1st day - 1%, 3rd day - 4 %, 6th day - 10%, 9th day - 17%, 12th day - 19%, 15th day - 18%. Mix culture shows during 1st day - 2%, 3rd day - 13%, 6th day - 24%, 9th day - 34%, 12th day - 46%, 15th day - 62%.



GRAPH: –Depicts that COD reduction of milk industrial waste water by different organisms are different such as *Pseudomonas*. shows reduction during 1st day - 2%, 3rd day - 11%, 6th day - 27%, 9th day - 44%, 12th day - 58%, 15th day - 65%. *Bacillus* spp. shows reduction during 1st day - 2%, 3rd day - 7%, 6th day - 15%, 9th day - 30% 12th day – 35%, 15th day - 39%. *Enterobacter* spp. shows that 1st day - 1%, 3rd day - 8%, 6th day - 10%, 9th day - 24%, 12th day - 28%, 15th day - 30%. Mix culture shows during 1st day - 2%, 3rd day - 18%, 6th day - 33%, 9th day - 45%, 12th day - 60%, 15th day - 66%.



GRAPH: –Depicts that COD reduction of food industrial waste water by different organisms are different such as *Pseudomonas*. shows reduction during 1st day - 2%, 3rd day - 19%, 6th day - 39%, 9th day - 55%, 12th day - 64%, 15th day - 69%. *Bacillus* spp. shows reduction during 1st day - 2%, 3rd day - 8%, 6th day - 19%, 9th day - 35% 12th day – 45%, 15th day - 47%. *Enterobacter* spp. shows that 1st day - 1%, 3rd day - 5%, 6th day - 13%, 9th day - 25%, 12th day - 26%, 15th day - 30%. Mix culture shows during 1st day - 2%, 3rd day - 20%, 6th day - 41%, 9th day - 57%, 12th day - 65%, 15th day - 72%.



GRAPH: –Depicts that COD reduction of textile industrial waste water by different organisms are different such as *Pseudomonas*. shows reduction during 1st day - 2%, 3rd day - 9%, 6th day - 18%, 9th day - 35%, 12th day - 42%, 15th day - 49%. *Bacillus* spp. shows reduction during 1st day - 2%, 3rd day - 4%, 6th day - 10%, 9th day - 18% 12th day – 23%, 15th day - 29%. *Enterobacter* spp. shows that 1st day - 1%, 3rd day - 2%, 6th day - 6%, 9th day - 12%, 12th day - 19%, 15th day - 21%. Mix culture shows during 1st day - 2%, 3rd day - 9%, 6th day - 20%, 9th day - 36%, 12th day - 44%, 15th day - 50%.

CONCLUSION

From the present study of the industrial effluent sample, it was concluded that microorganisms are able to reduce the COD on utilization of organic matter. It was clear from the study that COD content of effluents post shaking condition was reduced about 60%-70% by mix culture and individually reduced COD about 30%-50%, while *Bacillus* sp. and *Enterobacter* sp. reduces COD around 35% and 25% respectively.

It is well concluded that above used mixed culture or even individual organisms, the three bacteria, are highly effective in reducing COD and can be rigorously used at industrial level for effluent treatment.

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RUJOST, ISSN: 2319-4227, Vol.7, No.1, January, 2022, pp.99-104
A Biaanual Peer Reviewed Referred Ranchi University Journal of Science & Technology

Characterization Techniques for properties of nanomaterial and its significance: A review

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Abstract: Nanomaterials have attracted huge interest in today's world because of their small size, surface effect as well as potential applications in traditional materials, medical devices, electronic devices, coatings and other chemical industries. Particles of the size of 1-100 nm have different applications in various areas such as food packaging, catalyst industry, polymer paint industry, drug delivery, cancer diagnostics and treatment. The catalytic properties of nanoparticles improve due to their small shape, size, and morphology diverge from those of bulk materials. Characterization techniques are required to explore material properties and microscopic structures. Characterization of material helps to determine the composition and structure of materials, and also give information that whether the method was successful or not. Characterization is also important to ensure that the prepared particles are at a nanoscale. In characterization of nanomaterial some techniques are qualitative and some are quantitative. The techniques used to characterize the nanoparticles are: XRD, SEM, EDX, TEM, AFM, UV -Visible Spectroscopy, Thermo Gravimetric Analysis/Differential Thermal Analyzer (TGA/DTA). In this review we have tried to summarize the present techniques that are available for the characterization of nanomaterials.

Keywords: XRD, SEM, EDX, TEM, AFM, TGA/DTA, STM, ICP, nanomaterial,

INTRODUCTION

Nano scale science and technology is one of the fields that encompasses nearly every discipline of science and engineering.[1] In this decade the field of nanotechnology continuously expanding, people are discovering additional applications that benefit from the use of (NMs). An increasing number of NMs are being investigated for use in food supplements, food products and food packaging materials.[2] Such examples include liposomal systems which are under development to deliver essential nutrients while minimizing oxidative damage,[3] antimicrobial NMs viz. zinc oxide and silver oxide being used in food contact materials to prevent spoilage of product and to increase shelf life.[4] Nano clays being used in polymeric food packaging to improve the gas barrier properties and extend the shelf life of beverages. Thus risk factor increasing by different types of NMs and their widespread applications. Therefore, risk assessments challenging and necessitate ever-improving regulations and guidelines,[5] instead of this for assessment of catalytic properties, polymeric properties. In-depth and thorough characterization is essential to fully understand and evaluate. Characterization is also important to ensure that the prepared particles are at a nanoscale. In material science, the term "characterization" refers to the general and broad processes through which the properties and structure of the material are explored. Characterization helps to determine the composition and structure of materials, and also allows us to assess whether the method was successful or not. Some techniques are qualitative, whereas some are quantitative. This review paper will focus on wide use and effective techniques available to characterize nanomaterial.

Parameters of Nanomaterial to be Characterized

Nanomaterial exists in different shape, size, chemical compositions ranging from micelles to metal (oxide)s, from

synthetic polymers to large biomolecules.[6] Each of these materials features a completely different chemistry, which can be analyzed by a different method including optical spectroscopy, X-ray fluorescence and absorbance, Raman spectroscopy, and solid-state NMR.[7] However, often the behavior of nanoparticles is largely governed by their nanometer dimensions. As such, throughout nanoparticle characterization, the investigation of size, shape, surface charge and porosity is a fundamental step for fully understanding and predicting their behavior. These essential parameters are the focus of our review. Nanomaterial's from the wider class of nanoparticle consisting nanostructured objects that might present dimensions in the micro and millimeter regime, such as nanostructured films or nanotubes.[8-10] Size and shape affect the nanoparticle functionalization capacity, fluid drag and diffusion, optical properties, and uptake into cells.[11] Surface charge, besides controlling the stability of a colloidal suspension and its tendency toward aggregation,[12] also plays a major role in shaping the interactions between nanoparticles and the environment.[13,14] Finally, owing to their increased surface to volume ratio, nanoparticles possess a large external surface area that can be functionalized for different applications.[15-18]

METHODS

Different methods of characterization of nanomaterial's

1. Spectroscopy-based characterization techniques
2. Microscopy- based nanoparticle characterization
3. X-ray related characterization techniques
4. Dynamic light scattering
5. Elemental composition analysis
6. Zeta Potential
7. Thermo Gravimetric Analysis/Differential Thermal Analyzer (TGA/DTA)

1. Spectroscopy-based Characterization Techniques

Spectroscopy-based characterization techniques method consists of a deuterium or tungsten lamp for the ultraviolet and visible region wavelengths respectively, sample and reference beams, a detector, and a mono-chromator.

Raman Spectroscopy

Raman spectroscopy is a spectroscopic technique that makes use of Raman scattering or inelastic scattering of monochromatic laser light and it is used to study the rotational, vibrational, and other modes of a system. It may be used as a tool to identify the phases and phase transitions of various nanoparticles and other nanostructured materials, determine which regions of a nanomaterial are amorphous or crystalline, there are any defects present in the nanomaterial, determine the size of nanomaterials.[19]

Fourier Transform Infrared Spectroscopy

FTIR analysis is used for the identification of organic, inorganic, and polymeric materials utilizing infrared light for scanning the samples. FTIR is useful in identifying and characterizing unknown materials, detecting contaminants in a material, finding additives, and identifying decomposition and oxidation.[20]

Ultraviolet- Visible Spectroscopy

It is based principle of Beer-Lambert law. When we expose the sample to UV light which give the UV spectrum. Quartz Cuvettes are used for sample holding and are kept inside the instrument for introducing samples to the light path. The bandwidth of the spectrum, magnitude, and wavelength peak of the plasmon resonance linked to the nanoparticle depends on the composition of the material, environment, shape and size of the material.[21-23]

2. Microscopy- based nanoparticle characterization

Optical microscopy helps to observe materials at a micron level with reasonable resolution. Aberrations and limited wavelength makes further resolution difficult to achieve in optical microscopes. Therefore, other imaging techniques like AFM, SEM, TEM, and STM have been technologically advanced to detect materials with submicron size. These techniques produce a highly magnified image.[24]

Scanning Electron Microscope

In scanning electron microscopy (SEM), an electron beam is directed towards the specimen instead of a light beam, as in the case of an optical microscope. The SEM scans the surface of the sample with high-energy electron beams. Thus, SEM differs from conventional light microscopes as they uses light waves to create a magnified image. In SEM, when the electron beam strikes the specimen surface, it interacts with the surface.[20,25]

Transmission Electron Microscopy

When an electron beam is transmitted through the sample, it interacts with the sample, and the transmitted electrons are used to form the image by magnifying and focusing them using an objective lens. This method is an

invaluable tool for studying the nanomaterial properties of materials due to the high resolution it offers. With this high resolution, it is possible to image crystal structures, defects in the crystal, and individual atoms.[26]

Atomic Force Microscopy

AFM is a powerful and versatile microscopy technology used to study the samples at a nanoscale. It takes an image in a three-dimensional topography and provides various kinds of surface measurements, meeting the needs of engineers and scientists. AFM can generate images at an atomic resolution with angstrom scale resolution height information with less sample preparation.[27]

Scanning Tunneling Microscopy

Surface images are produced using this instrument with an atomic scale lateral resolution. A fine probe with a tip does the scanning over the conducting sample's surface with the aid of a piezoelectric crystal, and the subsequent tunneling current is observed.[28]

3. X- ray related characterization techniques

We will discuss here two techniques which is based on x-ray related characterization are XRD and XPS.

X-ray diffraction

XRD patterns can be used for determining the element proportions if the sample is in mixture form. The degree of crystallinity, deviation of a particular element from its ideal composition, and its structural state can also be derived from the data analysis. Based on Bragg's equation it is used for measuring the angle of diffraction where d denotes the spacing between the planes, θ denotes the angle of incidence, n denotes an integer, and λ denotes the beam wavelength.[29]

X- ray Photoelectron Spectroscopy

XPS is a quantitative spectroscopic surface analysis technique useful for estimating the elemental composition of a material. The kinetic energy measurements and the number of electrons that have escaped from the material surface provide the XPS spectra.[30,31]

4. Dynamic Light Scattering

Dynamic Light Scattering Technique may use to determine the size, shape and diffusion coefficient of nanoparticle.

Dynamic Light Scattering

Characterization of colloidal solutions and nanoparticles is carried out with DLS. Light scattered from a laser, which travels through the colloidal solution, is measured by DLS. Modulation of the intensity of the scattered light is analyzed as a function of time, and from this information we can derive the particle' size.[32]

5. Elemental composition analysis

Energy Dispersive X-ray Spectroscopy -

Energy dispersive X-ray spectroscopy (EDS or EDX) is an analytical technique used for the elemental analysis or chemical characterization of a sample. It is a micro-analytical technique conventionally used in scanning electron microscopy (SEM) for the local determination of chemical elements in solid samples.[33]

Inductively Coupled Plasma Mass Spectrometry and Inductively Coupled Plasma Optical Emission Spectroscopy

ICP-MS is a powerful analytical technique for elemental characterization. With the rapid advancement of instrumentation. Additionally, because ICP-MS has a resolution at about 1.0 atomic mass unit, it can provide isotope information, which can be critical in the analysis of nanomaterial's for several toxic metal contaminants such as arsenic and chromium.[34,35]

6. Zeta Potential

Zeta potential is a measure of the effective electric charge on the nanoparticle's surface and quantifies the charge stability of the colloidal particles. Nanoparticles or colloidal particles will have a surface charge in suspension. As soon as the electric field is applied, a particle starts moving because of the interaction between the electric field and the charged particle.

The magnitude of the zeta potential provides information about the particle stability. The higher magnitude represents increased stability due to increased electrostatic repulsion.[18]

- Particles tend to aggregate in the range 0-5 mV.
- Minimally stable particles are in the range 5-20 mV.
- Moderately stable particles are in the range 20-40 mV.
- Highly stable particles are in the range 40+ mV.

7. Thermo Gravimetric Analysis/Differential Thermal Analyzer (TGA/DTA)

Thermo Gravimetric Analysis (TGA) is a thermal analysis technique which measures the weight change in a material as a function of temperature and time, in a controlled environment. This is very useful to investigate

the thermal stability of a material, or to investigate its behavior in different. It is suitable for use with all types of solid materials, including organic or inorganic materials. Differential thermal analysis (DTA) is a calorimetric technique, recording the temperature and heat flow associated with thermal transitions in a material.[36]

CONCLUSION

The synthesis of nanomaterial and their application to human welfare and solving other problems of our time requires advancement in nanomaterial characterization. The properties of nanomaterial like size, shape, surface charge, and porosity of nanomaterial are directly connected to function and their effects on health and the environment. Measuring these properties is important for translating potential benefits of nanomaterial into specific applications. Characterization is also the first step to ensure that synthesized compounds possess the desired properties. This review described the role of several different techniques for the characterization of nanomaterial.

RECOMMENDATIONS

1. More than one technique may be used to characterize the same quantity.
2. If possible the obtained results should be compared with published data.
3. Measured parameters should be calibrated against a standardized reference.

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RUJOST, ISSN: 2319-4227, Vol.7, No.1, January, 2022, pp.105-108
A Biaanual Peer Reviewed Referred Ranchi University Journal of Science & Technology

Chemistry

Microwave assisted synthesis of some Chromium (III) complexes of cis-4-cyclohexene-1,2-dicarboxylic acid with TBC

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Abstract: Microwave has proved to be convenient tool in the hands of chemists for organic synthetic processes in the recent past. In the present work, the products obtained by the oxidation of cis-4-cyclohexene-1,2-dicarboxylic acid in tetrahydrofuran (THF), 1,4-dioxane and dichloromethane by ditertiary butyl chromate (TBC) in varying molar ratios by microwave dielectric heating conditions are studied. The analysis of compounds was done by chemical as well as instrumental methods including FTIR and DTA/TGA mass loss pattern. The results clearly indicate the advantages of Microwave Assisted Organic Synthesis (MAOS) over conventional synthetic methods with respect to reaction efficiency and product yield, less pollution and higher energy efficiency.

Keywords: Microwave, cis-4-cyclohexene-1,2-dicarboxylic acid, ditertiary butyl chromate (TBC), synthesis tetrahydrofuran (THF), and FTIR spectroscopy

INTRODUCTION

Spencer (1947)[1] introduced microwave dielectric technique as a source of energy. Gedye *et al*. (1986, 1987)[2,3] applied microwave dielectric heating to organic synthesis and now there are more than 2000 papers that describe the application of this technique for synthesis of new compounds. Microwave Assisted Organic Synthesis (MAOS) and Microwave Organic Reaction Enhancement Synthesis (MORE) have gained popularity as a non conventional eco-friendly technique for rapid organic synthesis in conformity with 12 principles of Green chemistry.[4] Lidstrom *et al.* (2001)[5] have reported a detailed survey of MAOS and MORE. Keeping in view, the increasing importance of MAOS and MORE in organic synthesis, the authors have undertaken the oxidation of cis-4-cyclohexene-1,2-dicarboxylic acid in different solvents with ditertiary butyl chromate by microwave heating.[6] TBC has proved to be a versatile oxidant under aqueous and non aqueous media as substantiated by the volumes of work done after its first use by Oppenauer and Oberrauch (1949).[7] The oxidation products of organic substrates formed may serve as ligand to give adducts or complexes of chromium in different oxidation states.[10-13] cis-4-Cyclohexene-1,2-dicarboxylic acid is a white crystalline solid[8-9] having carboxyl groups at position 1 and 2 of cyclohexane and double bond at 4 position. It is used in synthesis of pesticides[6] and fungicides. In this present paper, we have reported the synthesis and study of some complexes of chromium in lower oxidation states with cis-4-cyclohexene-1,2-dicarboxylic acid by oxidizing it with TBC to explore the versatility of oxidising agent and expand the horizon of cis-4-cyclohexene-1,2-dicarboxylic acid chemistry.

MATERIALS & METHODS

The chemicals used in the present work were of A.R. grade obtained from commercial sources. The chemical used were cis-4-cyclohexene-1,2-dicarboxylic acid, tetrahydrofuran (THF), dichloromethane, 1,4-dioxane, tertiary butyl alcohol (TBC), acetone, chromium (VI) oxide, silver nitrate, potassium persulphate, ammonium iron(II) sulphate (Mohr's salt), potassium dichromate, barium diphenylamine-4-sulphonate.

TBC which has been used as the oxidant in the present work was prepared in situ by dissolving a weighed amount of chromium (VI) oxide in 10 ml tertiary butyl alcohol. Weighed amount of cis-4-cyclohexene-1,2-dicarboxylic acid was dissolved in 10 ml of solvents i.e., THF, 1,4-dioxane and dichloromethane in clean and dry beaker with constant stirring. The substrate: oxidant ratios used were 1:1, 2:1 and 3:1; cis-4-cyclohexene-1,2-dicarboxylic acid was found to be freely soluble in all the three selected solvents. Oxidation of this substrate with TBC was performed in three sets, each for the three solvents. The two solutions were mixed together to prepare a homogeneous reaction mixture, stirred continuously for a considerable time using a magnetic stirrer [REMI RS-12 Rotatory Shaker] and any change in consistency was noted. The mixture was then heated in an LG MG-3937C 20-Litre, 2450 MHz 800W Microwave Oven (MW irradiation 160W, variable oxidation time). Initial and final temperature of the reaction mixture was recorded to specify the exothermic/ endothermic nature of the reaction under investigation. The reactions were found to be exothermic, in general, except for those in 1,4-dioxane in which they were endothermic as indicated by a drop in temperature of the reaction mixture. The nine products formed in each of these cases were washed with acetone, dried and weighed, bottled and labelled as 111CHDCA, 121CHDCA, 131CHDCA, 211CHDCA, 221CHDCA, 231CHDCA, 311CHDCA, 321CHDCA, 331CHDCA and used for further analysis and characterization. Percentage composition of C, H and O was determined using EuroEA Elemental Analyser while the chromium content in this sample was estimated volumetrically using $K_2S_2O_8$ (excess), 0.1 N $K_2Cr_2O_7$ solution and 0.1 N Mohr's salt solution. Empirical formula of these complexes was then deduced. The recorded details have been summarised in Table 1.

Table 1- Preliminary characteristics of the products of cis-4-cyclohexene-1,2-dicarboxylic acid with TBC

Sample	Label	Solvent	Substrate/ Oxidant Molar ratio	MW Irradiation time in sec, 160W	Yield in g	Colour	Empirical Formula
1.	111CHDCA	THF	1.28g/1.0 g;1:1	35s	3.49g	Brown	$Cr_2C_{10}H_{16}O_{13}$
2.	121 CHDCA	THF	1.28g/0.5 g;2:1	50s	2.26g	Dark Brown	$CrC_{10}H_{14}O_{11}$
3.	131CHDCA	THF	1.28g/0.335g;3:1	70s	1.65g	Dark Brown	$Cr_2C_{16}H_{21}O_{12}$
4.	211CHDCA	1,4-dioxane	1.28g/1.0 g;1:1	60s	3.78g	Reddish Brown	$Cr_2C_8H_{16}O_{11}$
5.	221CHDCA	1,4-dioxane	1.28g/0.5 g;2:1	75s	3.76g	Reddish Brown	$Cr_2C_{10}H_{16}O_{13}$
6.	231CHDCA	1,4-dioxane	1.28g/0.335g;3:1	75s	4.16g	Reddish Brown	$CrC_9H_{14}O_{11}$
7.	311CHDCA	CH_2Cl_2	1.28g/1.0g;1:1	50s	1.22g	Light Brown	$CrC_{10}H_{14}O_{13}$
8.	321CHDCA	CH_2Cl_2	1.28g/0.5 g; 2:1	60s	1.70g	Light Brown	$CrC_{10}H_{16}O_{13}$
9.	331CHDCA	CH_2Cl_2	1.28g/0.335g; 3:1	60s	0.99g	Brown	$CrC_{13}H_{16}O_{11}$

RESULT & DISCUSSION

- The nine complexes obtained after the oxidation of cis-4-cyclohexane-1,2-dicarboxylic acid with TBC in different solvents had different physical properties, like colour, solubility, etc.
- The reactions performed in 1,4-dioxane was found to be endothermic, while those performed in other solvents were exothermic. Comparing the reaction time and corresponding yield of products, it can be concluded that 1,4-dioxane serves as the most efficient solvent and the compound sample 111CHDCA, 211CHDCA , 221CHDCA and 231CHDCA was most efficiently prepared sample of cis-4-cyclohexene-1,2-dicarboxylic acid with low time of formation and good yield.
- The substrate oxidant ratio also affects the nature and characteristics of the products formed. With increase in molar concentration of the oxidant for each reaction set intensification of the colour of the products was observed and this may be indicative of the increase percentage of chromium in the sample. This was confirmed from the elemental analysis of the samples as evident from the empirical formula of the compound. It may be due to different oxidation states of chromium in different products.

- The extent of oxidation of the substrate increases as the ratio of the oxidant increases.
- In case of 111CHDCA and 211CHDCA, when the ratio of oxidant is maximum, the most stable oxidant state i.e., III is observed in the form of $Cr_{2}O_{3}$. In other cases oxidation states of chromium are less than III.
- The oxidation is more efficient in 1,4-dioxane than in THF and dichloromethane as supported by the presence of less oxidized product in 121CHDCA, 311CHDCA, 321CHDCA and 331CHDCA.

ACKNOWLEDGEMENTS

The authors are thankful to Gandhigram Rural Institute, Gandhigram for DTA-TGA analysis and CDRI, Lucknow for providing useful data of elemental analysis and FTIR spectrum.

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RUJOST, ISSN: 2319-4227, Vol.7, No.1, January, 2022, pp. 109-112
A Biaanual Peer Reviewed Referred Ranchi University Journal of Science & Technology

Geology

Morphological study of leaf of *Nipaniophyllum* from Mandro in Rajmahal hills and determination of its age.

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Abstract: The Rajmahal hills include rich fossilized localities of Upper Gondwana sequences in India. The record of fossil plants helps to understand the long process of plant evolution, type of vegetation, climate and environment of prehistoric life. The fossil evidence from Rajmahal hills of Jharkhand has helped to explain the origin of major classes of plants and the location of these fossils, including both their temporal (age) and their spatial (geographical) arrangement, can be used to determine past climates. The plant fossils play a key role in biostratigraphy- based on fossils, rock units can be arranged in stratigraphic order. The present study has taken into account the morphology of the plant fossil in order to identify the genus and accordingly assign the age of the rock.

Keywords: Pentoxyleae, *Nipaniophyllum*, Morphology, Upper Gondwana, Mandro

INTRODUCTION

The group of Pentoxyleae is a group of gymnosperms that shows unusual combining features of Pteridosperms, Cycadales, Bennettitales and Coniferales. They have various parts: the stem genus *Pentoxylon* and ovulate cone *Carnoconites*[1], leaf genus *Nipaniophyllum*[2], and micro-sporangiate organs *Sahnia*[3]. Sahni reconstructed the Pentoxylon plant (Fig. 1) and proposed the name Pentoxyleae for the group. The anatomy of Pentoxyleae leaves, then known as *Taeniopteris spatulata* was later studied by, Prof Sahni and renamed as *Nipaniophyllum raoi*[2]. Early fossils of *Nipaniophyllum raoi* were found mainly in the fossiliferous localities of Nipania, Amarjola, and Sonajori of Rajmahal hills of Jharkhand.[4] This plant-bearing formation in Jharkhand is known as the Rajmahal Series of the Upper Gondwana Division. It has yielded a rich flora consisting mainly of impressions of cycads, conifers, and ferns, which was first described by Oldham and Morris and later by Feistmantel and others.[2] For a description of the geology of the Rajmahal hills, reference may be made to the early work of Ball (Fig. 2), where the stratigraphical position of the different plant-bearing beds within the volcanic sequence is indicated. The present paper deals with the morphological study of *Nipaniophyllum* leaf found in the Mandro area in the Sahibganj district of Jharkhand. The identification of the genus is based on morphological characters. Upon identification of the fossil plant, it is correlated with similar fossils of different areas and the age of the rock is determined.

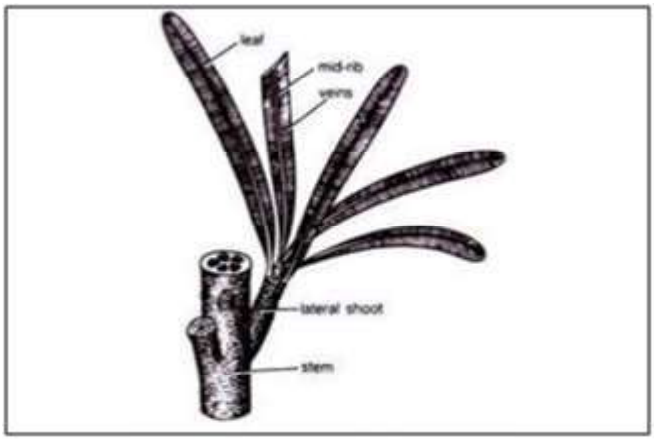

Fig.1: Pentoxylon sahni. Reconstruction of stem and leaves (*Nipaniophyllum raoi*) (After Sahni, 1948)[2]

Location and Geology: The fossil sample has been found in an open field about 1 km South-East of a hill named Gormipahar in Mandro block of Sahibganj district, Jharkhand, India. The fossil sample has been collected from Latitude 25°7'49.62"N and Longitude 87°30'55.24"E.

The whole area near about appears to be mainly composed of medium-grained sandstone. The hill is about 78 metres high, covered with fairly moderate vegetation, chiefly composed of small trees and shrubs. The outcrops show a general north-west dip.

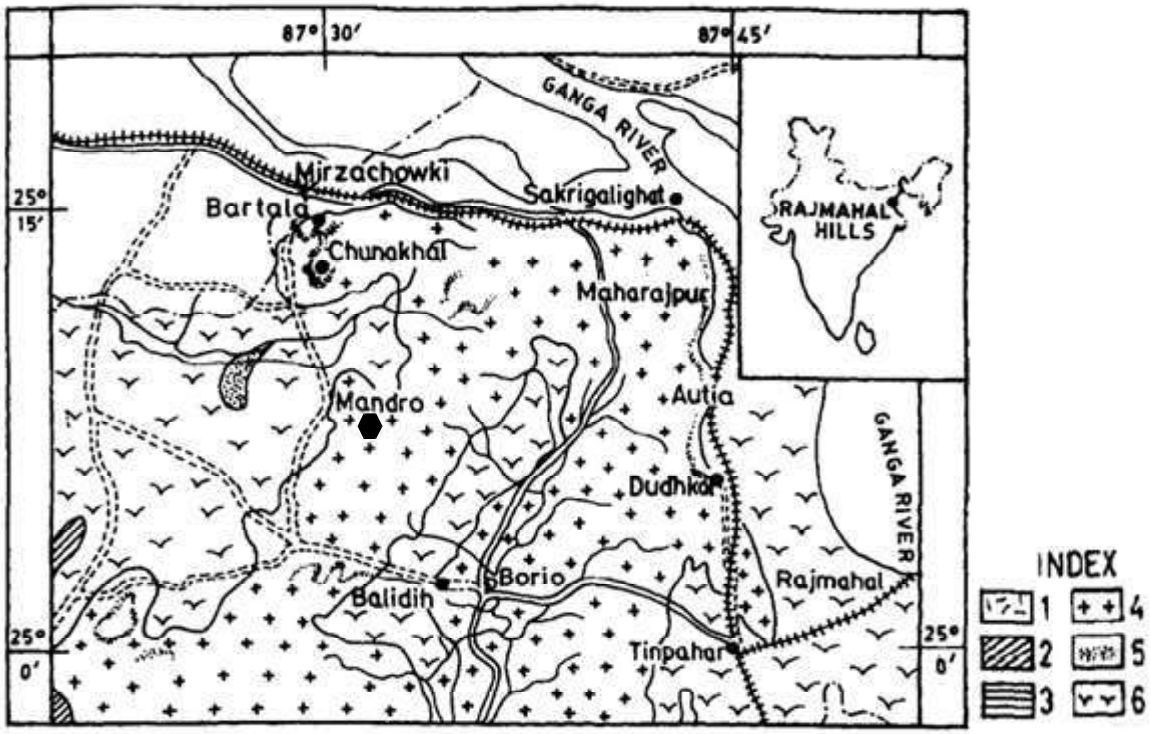

Fig. 2: The geological map of the Rajmahal hills showing some fossil localities (After Ball, 1877; Sen Gupta, 1988). 1: Chotanagpur granite gneiss ; 2:Barakar formation ; 3: Dubrajpur formation ; 4: Rajmahal formation (traps) ; 5: Rajmahal formation (intertrappean) ; 6: Alluvium[5]. (Hexagonal dot represents the location of sample)

The Rajmahal basin displays well-developed Upper Gondwana sediments. The basin comprises the Rajmahal traps (lava flows) and associated inter-trappean beds. The district of Sahibganj has various exposed areas of intertrappean beds which contain the Upper Gondwana plant fossils. The fossiliferous section is characterized by pale white to reddish shale and grayish-white, medium-grained sandstone.

MATERIALS & METHODS

The plant fossil material was collected from the succession of Rajmahal Formation, exposed as a small hillock at about 1 km towards the East of the Gormipahar in Mandro (25°7'62''N, 87°30'24''E), near Borio, Sahibganj District, Jharkhand, India. The leaves of *Nipaniophyllum* have been collected as an impression in shale. The fossil has been found on the outcrop in an open field in the locality of Mandro which is a part Rajmahal series. The Rajmahal series mainly comprises freshwater shales interbedded with a considerable series of lava flows, associated with some volcanic ash. The fossil rock was first dusted off using a brush to remove some of the mud and then it was washed under gentle running tap water and was air-dried. The fossil was then observed under Gippon inc Japan Stereo-microscope for morphological study of the leaves imprint.

RESULT

Description and Comparison:

5 Leaves with sizes less than 11cm long and 1cm wide appear to arise from a point that has been destroyed in the rock. Lanceolate leaves are predominantly entire with a smooth straight margin. The margin terminates with an acute apex. The petiole is not known. Midrib prominent, from 1 to 1.5 mm wide with a median groove. Parallel, lateral veins that are closely spaced arise from mid-rib.

The angle of emergence of the lateral veins varies from 60° to 90° to the midrib. The venation is dense, the number of veins varies from 2 to 5 per mm.

Class	:	Pentoxylopsida (Pant,1959)
Order	:	Pentoxylales
Family	:	Pentoxylaceae
Genus	:	*Nipaniophyllum* (Sahni,1948)
Horizon	:	Rajmahal formation, Upper Gondwana[6]
Age	:	Upper Jurassic-Lower Cretaceous[6]

It is a simple leaf with a distinct midrib from which the lateral veins arise at right angles and divide at all levels. Sahni instituted the genus *Nipaniophyllum* for the petrified leaves found in association with Pentoxylon in the Nipania chert[7]. Pentoxylon leaf is identified as *Nipaniophyllum* associated with the taeniopteroid leaves[8] which have similar morphological characters.The venation pattern of the specimen described here is best comparable with *Nipaniophyllum raoi* (Sahni, 1948)[2] earlier known as *Taeniopteris spatulata* McClelland (1943). The apex is acute and not acuminate as described by Rao (1943a, p. 337) in the specimen. The lateral veins remain straight and form a right angle to the midrib. The margin of the lamina is almost straight and tapering gradually as in Fig.3.

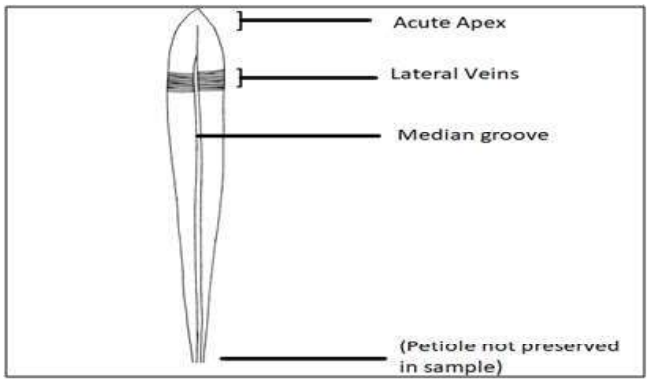

Fig. 3: Reconstruction of *Nipaniophyllum* leaf found at Mandro.

DISCUSSION

The group of Pentoxyleae consists of various organ genera as given (Fig.1). The leaves impression found in the shale of locality of Mandro shows similarity to *Nipaniophyllum raoi*. The morphological characters of the leaves are very similar to the *Nipaniophyllum* studied by Sahni. The leaves of *Nipaniophyllum raoi* which resemble *Taeniopteris spatulata* in external morphology show wide variations in shape and size. The present studied fossil though shows similarity in various aspects with *Nipaniophyllum raoi*, differs in having broader lamina and mid-rib and thus may belong to different species of *N. raoi.*[2] The Pentoxyleae originally collected from Nipania do not occur at many places in the Rajmahal Hills. *Nipaniophyllum raoi* has been mostly reported and studied from Nipania, Amarjola and Sonajori localities of the Rajmahal hills. *Carnoconites rajmahalensis* (Wieland) have been reported from Mandro which is a seed-bearing organ of Pentoxylon plant.[9] The presence of the reproductive organ of the Pentoxylon plant in the area opens up the possibility of finding certain other parts of the plant associated with it. In the present study, the presence of the leaf genus of the Pentoxylon plant, known as *Nipaniophyllum* is supported by *Carnoconites* found earlier in the area.

According to Drinnan & Chambers, the age range of the Pentoxylales group is uncertain.[10] Pentoxyleae belongs to the Jurassic-Cretaceous age but the age of *Nipaniophyllum raoi* has been debated over the years. "In general composition, the fossil flora of Nipania seems to compare with the fossil flora of Jabalpur and Kota stages of Upper Gondwanas and is, therefore, to be believed from the upper-most strata of Rajmahal stage".[11] *Nipaniophyllum raoi* has also been described from the Lower–Middle Jurassic Talbragar Fish Beds in New South Wales[12] and from the Middle Jurassic Walloon Coal Measures in Queensland.[13]

CONCLUSION

Based on morphological characters the leaf impression suggests leaves of Pentoxylale named *Nipaniophyllum*. To work on species-level more detailed study needs to be done, as for now the species of the fossil is not known. Though *Nipaniophyllum* has been reported confined to Nipania and Sonajori localities, the study of the seed-bearing organ of Pentoxyleae-*Carnoconites* from Mandro[9] supports the presence of *Nipaniophyllum* leaf impression which has been reported and studied for the first time from this area. Parts of Pentoxylales have been encountered in a few localities in New Zealand, Australia and India that are of Jurassic-Early Cretaceous age. Early Cretaceous age is suggested for the strata bearing Pentoxylales in Antarctica. Sahni originally described the Pentoxyleae as a group of extinct

gymnosperms from Jurassic-Cretaceous sediments in the Rajmahal Hills, India. *Nipaniophyllum* from Mandro indicates a great similarity between the floras of Antarctica, Australia (Victorian, Talbragar) and New Zealand during the Jurassic-Early Cretaceous. Pentoxylales is considered a characteristic group of the southernmost region of Gondwana during the Jurassic-Early Cretaceous times. Based on correlation with these areas, the age of the studied *Nipaniophyllum* fossil ranges from Upper Jurassic to Lower Cretaceous.

ACKNOWLEDGEMENT

The author is thankful to CSIR for providing financial assistance through NET-JRF.

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RUJOST, ISSN: 2319-4227, Vol.7, No.1, January, 2022, pp. 113-118
A Biaanual Peer Reviewed Referred Ranchi University Journal of Science & Technology

Geology

Pegmatite deposits of Bihar Mica Belt (BMB) in Koderma district with special reference to rare mineral potentiality

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Abstract: Bihar Mica Belt is world famous for Ruby mica but it is lesser known that the same pegmatite which is host for ruby mica can be a good source of Rare minerals. As the molten granite crystallize, the melt that remains becomes enriched in water and rare element concentrate in the melt because rare ions do not participate in the crystallization of common rock forming minerals. In the presence of remaining water, minerals crystallise slowly at high temperature and pressure grows in enormous sizes, forming rocks known as pegmatite.

Keywords: BMB, Koderma, Pegmatite, rare mineral potentiality

INTRODUCTION

The Bihar Mica Belt (BMB), in Eastern India a belt of metasediments of Archean age which runs in WSW to ENE direction through Nawadah, Gaya, Hazaribagh, Giridih and Munger districts of Bihar, and it is around ~150 km long and ~20 km wide.[1] Northeastern extremity of the Satpura orogenic belt is mark by this belt.[2,3] The presence of conglomerate at the contact of metasediments with Chotanagpur gneissic complex (CGC) indicate the BMB metasediments deposition on the Chotanagpur gneissic complex (CGC).[4,5] The migmatitic gneisses of granite-granodiorite- tonalite composition occurring on the northern and southern fringes of the metasedimentary belt, considered as belonging to the CGC by Ghose (1983)[2] and were included within the BMB by Saha *et a1.* (1987)[1].

GEOLOGY OF AREA

BMB shows a number of isolated granitic plutons with domical to phacolithic form occur within the suite. The diameters of the plutons vary from 2 to 5 km. Their small sub-circular shapes and confinement within a linear metasedimentary belt are important sites for geological investigation. Metasediments shows pluton intrusion, and the regional folds provide emplacement of migmatite at its core and limb.[1,6] These granite bodies grouped under a single magmatic suite on the basis of factors like tectonic setting, metamorphic grade of the country rocks, petrological and geochemical similarities.[1]

The Archean meta-sedimentary rock cover Bihar Mica Belt (BMB) which comprising rock type like muscovite schist, biotite schist and muscovite-biotite schist carrying sillimanite,garnet and staurolite, with micaceous quartzite, hornblend schist, calc-silicate granulite and hornblende gneiss. Well foliated and puckered and exhibit metamorphic grade of almandine-amphibolite facies rocks deposited in geosynclinal basin. Extensively intrusion of post-Dharwarian plutons of medium to coarsed-grained, massive, leucocratic biotite granites of pink and light grey colour, containing subordinate hornblend and often large phenocrysts of pink feldspar is present. Very frequent intrusion of meta-dolerite, garnetiferous–orth-amphibolites and actinolite-tremoliteschist and of pegmatites are observerd.[7]

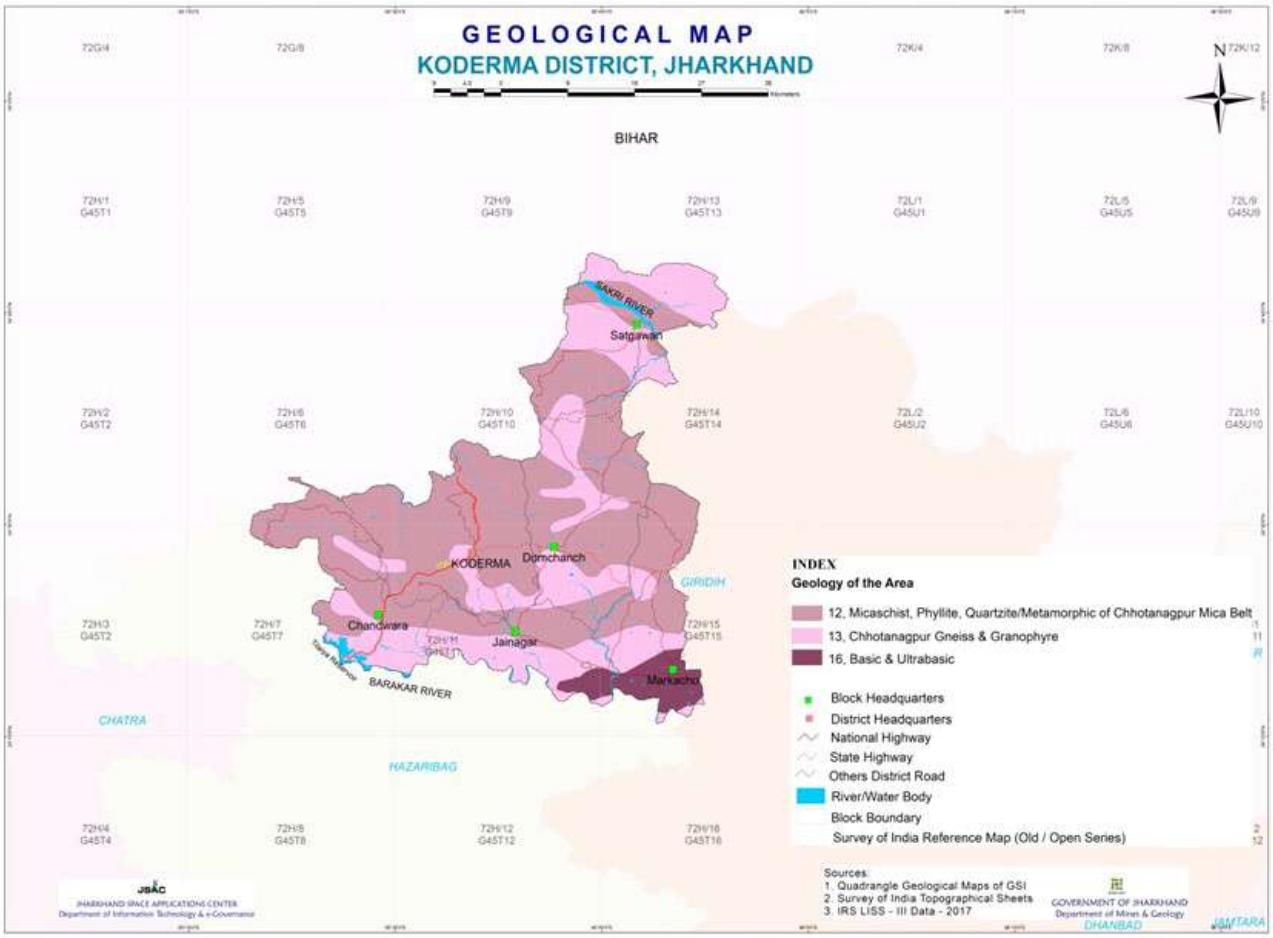

Fig. 1- Geological Map of Koderma District. (Source-Dept. of Mines and Geology-Govt. of Jharkhand)

Table 1- Geological succession of Bihar Mica Belt (after Ramachandran *et al.* 1994)[8]

AGE	ROCK TYPE
Recent	Alluvium
Permo-carboniferous	Gondwana sediments
----------Unconformity----------	
Proterozoic	Dolerite dyke Rapakavi granite and Pegmatites Biotite augen gneiss, coarse porphyritic granite gneiss and Pegmatite Medium grained massive granite and pegmatites Amphilbolites and anorthosites Massive quartzite with phyllite and slatyinterclations Sillimanite- muscovite schist, calc-silicate rock and Hornblend schist Schistose quartzite and quartz-mica schist Hornblend-schist, , Garnetiferous biotite schist Migmatites and composite gneisses
---------- Unconformity----------	
Archean	Chotanagpur granite gneiss

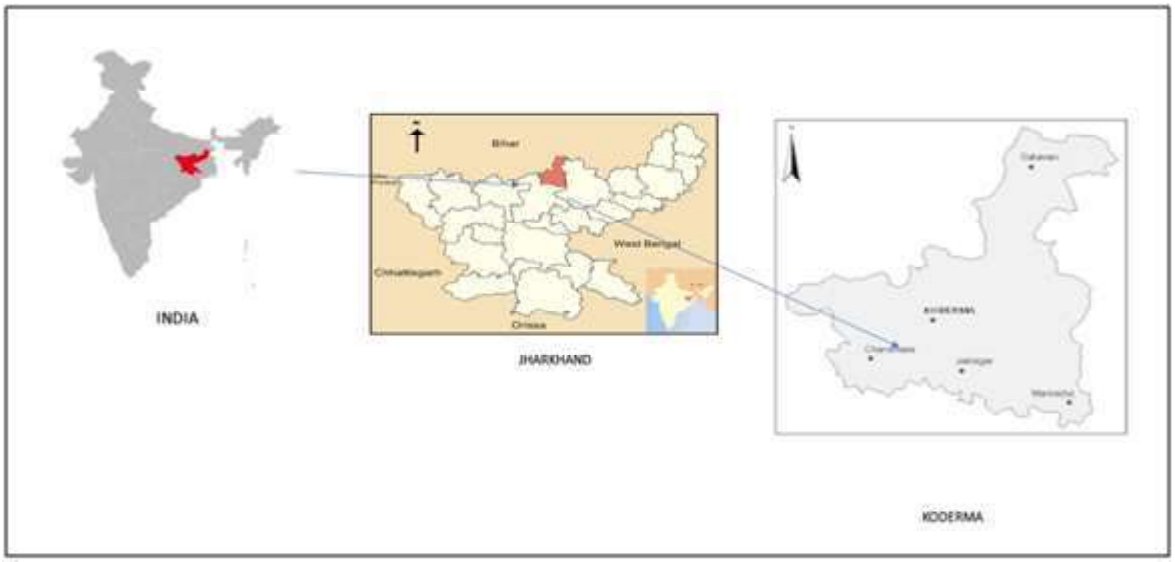

Fig. 2- Location map of study area (Source-www.mapsofindia.com)

Source Rock for Rare Minerals-Pegmatite

The pegmatite formed by slow crystallization at high temperature and pressure at depth and exhibities large interlocking crystals i.e. coarse grained minerals.

Koderma mica belt which is the part of BMB, it emplaced along joints, fractures, foliation, bedding, shear planes, noses and limbs of folds etc.; the massive pegmatite intrusion appears to have been localized in superposed fold. With the country rock they have generally concordant relation, but are occasionally discordant to it also. The dip varies from vertical to recumbent.[9]

Pegmatite association-

In the earlier studies by Saha *et al.* 1968; Saha,1986 stated that three groups of pegmatites have been described within the BMB metamorphites viz. (a) deformed simple pegmatites, rich in potash feldspar and carrying occasional small sized flakes of muscovite and rarely beryl, (b) undeformed complex pegmatiles, rich in oligoclase and often with books of muscovite, and (c) undeformed pegmatites, rich in potash feldspar and often cleavelandite and containing various rare minerals, radioactive minerals, columbite-tantalite, beryl, lithium and tin mineral.[10]

Fig. 3- Representative field photograph of Pegmatite intrusion in Dhab (Koderma district)

Rare Minerals prospecting-

In the early stages of crystallization, the ions that form high temperature minerals are depleted from the melt. Rare ions that do not participate in the crystallization of common rock-forming minerals as incompetent element and become concentrated in the melt and in the excluded water. These ions can form the rare minerals that are often found in pegmatites. Examples are small ions such as lithium and beryllium that form spodumene and beryl; or large ions such astantalum and niobium that form minerals such as tantalite and niobite. Rare elements concentrated in large crystals make pegmatite a potential source of valuable ore.[9]

Pegmatite is the host rock for many rare mineral deposits. These minerals can be commercial sources of: Beryllium, bismuth, boron, cesium, lithium, molybdenum, niobium, tantalum, tin, titanium, tungsten, and many other elements.

STRUCTURE

Three phase of superimposed deformation are encountered in the BMB. The tight isoclinal folds on E-W axes formed during the early two phases of deformation have caused interference patterns of type-1 i.e. domes and basin structure and type-3 i.e; hook-shaped folds. The third phase of deformation was weak and less extensive. Early two phase of deformation control granite emplacement in this area. Third deformation phase is responsible for late tectonic granite and some of them host lithium mineralisaion. BMB and Betul belt of CITZ (Central Indian Tectonic Zone) are correlated.[9]

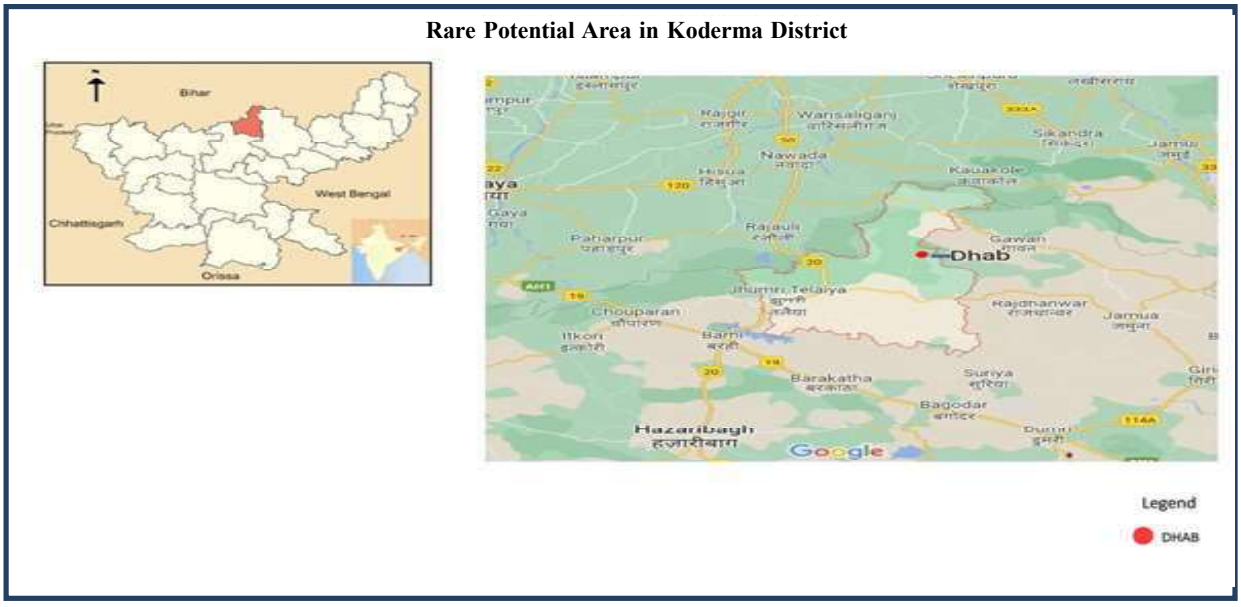

Fig. 3- Rare Minerals Potential Area inKoderma

GORIYADIH (DHAB)-

(Longitude 85º3¹33º N and Latitude 85º43¹43º E and SOI toposheet no.72H/10)

This study area is located 26 km away from Koderma town in Domchanch -Satgawa road. The area is situated in highly dense Sal forest. It is reachable by small vehicle. The pegmatite present in this area show large interlocking of tourmaline, quartz, feldspar and muscovite. In general pegmatite form long and narrow veins and mostly these lenticular veins pinch out and swell. In this region pegmatite show near vertical to flat dip angle with the host rock. Which indicates its structurally driven emplacement and condition of their formation.

METHEDOLOGY

The methodology comprises detail literature survey, field investigations, megascopic hand specimen study and GIS analysis. Preliminary field investigation carries out based on available previous geological and topographic map of area. Sample and data were collected from working mine and surrounding. Based on available topological, geological, mineralogical and GIS data a Rare Minerals potential area marked on the map of the Koderma district.

SCOPE FOR FURTHER WORK-

The aim of the present review is not to provide the best solution for the petrogenetic evolution of the BMB

plutons, but to point out the existing gap sin knowledge and, which can be solved with new ideas and modern data. Some of are as follows:

a) In eastern Indian shield the BMB plutons are among the first report of post-orogenic granite reported. Partial melting of granodiorite-tonalite component of CGC derive these post orogenic BMB plutons with subordinate involvement of the BMB metasediments intheirgenesis.[10] Existing geochemical data of BMB pluton are far less than sufficient to argument over geochemical evolution.

b) During the evolution of pegmatite the early crystallization in which the ions that form high-temperature minerals are depleted from the melt. Rare ions that do not participate in the crystallization of common rock-forming minerals become concentrated in the melt and in the excluded water. These ions can form the rare minerals that are often found in pegmatites. Examples are small ions such as lithium a beryllium that form spodumene and beryl; or large ions such astantalum that form minerals such as tantalite. For Rare elements concentration these late crystallise pegmatite must be targeted for a potential source of valuable ore.

Although there are lots of research and development efforts going on but there are no significant breakthroughs yet in the direction of Rare Minerals. Because of these reasons, the Rare Minerals demand increased manifold and currently vigorous exploration efforts are going on to find out new Rare Minerals ore deposits in this area.

DISCUSSION

Bihar mica belt, which mark the north eastern extremity of the Satpura orogenic belt and show tectonic significance in form of fold, fault, shear plane. It is significant from the studied Bhola (1968)[9] that the area is (i.e; pegmatite) rich with Rare Minerals, atomic minerals like beryl, uranium minerals, columbite-tantalite, lithium and some gem minerals like tourmaline, quartz, microcline, plagioclase feldspar (moonstone), topaz and garnet. Preliminary field survey data show large interlocking crystals of pegmatite which is result from the slow crystallization at high temperature and pressure at depth.

CONCLUSION

The area have good potential for mica, gem minerals, atomic minerals. For the Rare Minerals prospecting, pegmatite need to encounter which are localized close to fringe of granite plutons which is very much seen in Dhab area of Koderma. This pegmatite has to formed on early stages of crystallization, in which the ions that form high-temperature minerals are depleted from the melt that results into rare ion concentration. The study area has a great potential for Rare Minerals which required investigation and intense geochemical study in the interest of country strategic future.

ACKNOWLEDGEMENT

The authors show their gratitude towards faculty members of University Department of Geology, Ranchi University, Ranchi & University Department of Geology, Dr. Shyama Prasad Mukherjee University, Ranchi for their enormous support. The authors give special thanks to the anonymous local support in Dhab region for field guidance. The authors also thank the reviewer for improving the manuscript.

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RUJOST, ISSN: 2319-4227, Vol.7, No.1, January, 2022, pp. 119-126
A Biaanual Peer Reviewed Referred Ranchi University Journal of Science & Technology

Geology

Analytical Techniques for Gem Diagnostics

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Abstract: Gemstones are crystalline minerals having a definite chemical composition that occurs in the earth's crust naturally by inorganic processes as well as organic processes. These are adored for their beauty, durability, and rarity and are treasured for various reasons like decorative ornaments, religious symbols, medicinal property as well as for displaying wealth and status. With the advancement of technology, numerous man-made or synthetic gems are available in the market. Various traditional and laboratory equipments are available to distinguish between naturally occurring gemstones like diamond, ruby, sapphire, emerald, organic gemstones like pearl, coral, amber, etc., and man-made/ synthetic gemstones to study their properties. Some types of equipment could be considered portable and some are placed in the laboratory for lab analysis. This paper discusses the portable, traditional, and advanced laboratory equipments needed for Gem Diagnostics.

Keywords: Gemstones, Portable /traditional equipments, Advanced Lab equipments, Gem Diagnostics.

INTRODUCTION

Gemstones are beautifully crystallized rare minerals that occur in favorable sites in the earth's crust. These are sufficiently beautiful, durable, rare, and large enough to be cut into salable stones. Gemstones have been treasured throughout history for a variety of reasons like beautiful decorative ornaments, religious symbols, barter, medicinal, display wealth, status, power, and for investment purposes. Gemstones are minerals that occur as crystalline grains in rocks. These are the chemical elements that are formed in nature by the inorganic processes possessing a unique internally arranged atomic structure. The crystal structure of a mineral appears as an organized geometric pattern formed by certain atoms attracting each other. These grains compete with the neighboring ones for very limited space and here time plays an important role in the crystal growth. Slower cooling time results in the formation of larger crystals into open spaces like in fractures and hollows in the rocks. Minerals are distinguished by their characteristic external crystal shapes based on their atomic structure which helps to identify and distinguish one gem mineral from another. The earth's crust contains an array of gemstones from various depths and locations. The human population has long been fascinated by gemstones. A high rate of growth has been observed in the gemological sciences in the last five to ten years. Identifying gemstone's origin was first implemented in the 1980s and has become increasingly important to the gemstone trade since then. More than half a century has passed since the first gemological instruments were produced. A microscope and basic gemological equipment have been the norm for most gemologists for many decades. Nevertheless, modern and advanced technologies have been introduced like UV-Vis spectrophotometry, FTIR spectrometry, LA-ICP-MS, and ED-XRF. Today in order to judge the quality of gemstones, it is essential to have sophisticated laboratory equipment. The equipment means a lot of expensive and cumbersome for some and for others, it may mean only a few instruments that are carried in a shirt pocket. Gemological testing equipment is vital to a gemologist. Knowing every instrument's capability in depth is essential. Various gemological testing equipment is discussed below:

1. Portable and traditional instruments for field identification.

I. Loupe
II. Gemological microscope
III. Refractometer
IV. Dichroscope
V. Polariscope
VI. Spectroscope
VII. Chelsea filter

Lab and advanced instruments for gem identification.

I. Gemological microscope
II. X-ray diffraction (XRD)
III. X-ray spectroscopy (XRF)
IV. Raman spectroscopy
V. LA-ICP-MS
VI. LIBS
VII. EMPA
VIII. SIMS
IX. FTIR
X. SEM-EDS

Figure 1- Tourmaline rough crystals, Afghanistan.

Figure 2- Amber, an organic gemstone

Portable and traditional instruments for Gemstone Diagnostics in field:

I. Loupes:

Loupes are the familiar friend of a gemologist. It is portable, versatile, and the most frequently used gemological equipment. It requires a good source of light and a pair of tweezers. It is a magnification device used to see small details more closely and is worn close to the eye. It is handled free and consists of a focusing lens contained in an opaque cylindrical cone. Different types of loupes are available in the market. 10X or 20X loupe and also darkfield loupes are present. Loupes give us magnified views of inclusions and surface features including surface cracks, flaws, scratches, faceted edges, and cutting quality of gems helping in the identification of gemstones. The 10X loupe is also called "Triplet loupe" as it consists of three lens elements with a ten-power magnification. It has an inch focal rangeand minimizes distortion around the edge of the viewing area. Gemstone here must be one inch away from the loupe and the loupe should be one inch away from the eye. It is the standard tool used by gemologists to identify gemstones. The darkfield loupe is a bit complicated loupe and requires the holding practice of a gem in one hand and a flashlight with a loupe in another hand. The distance between the loupe and eye should be 3-5 cm. In this dark field loupe, light is directed through the sides of the gemstone against a dark background. Internal characteristics like inclusions, flaws, clarity, and uniformity can also be detected using the loupe (fig. 3a).

II. Refractometer:

It is an extremely useful identification tool. A refractometer can be used to conclude the optical nature of a gem and obtain its Refractive Index (RI), which is the degree of refraction occurring within a gemstone.It consists of an eyepiece, a polarizing filter attachment, a glass stage for gem placement, and a gap at the back of the equipment for the light source to enter the refractometer. For obtaining RI, sodium light should be used which has a wavelength of 589nm. RI fluid also called contact fluid is used in the refractometer which has an RI of 1.79 -1.89. A refractometer works best when used with a monochromatic sodium light source. According to Read (2013)[1], the refractometer uses the phenomena of critical angle to measure the RI of a gemstone. For the gems of higher RI than the fluid RI

gives a negative result. With the aid of a refractometer optical nature of the gemstone can be determined. The optical nature of the gemstone is expressed by the no. of shadow edges present in a gem while viewing through the refractometer. Gemstones can be singly refractive as well as doubly refractive in nature. With the help of a refractometer, the RI of a gemstone can be concluded and birefringence can be calculated which is the value between two RI. (fig. 3b).

III. Dichroscope:

Dichroscopes are simple tools working on the principle of pleochroism. It helps us to see the pleochroic colors of doubly refracting gemstones which split transmitted light into two separate rays that vibrate in different directions. Rays in different directions absorb light differently, which results in different colored appearances. This phenomenon is called Pleochroism. With the help of this tool, many synthetic and imitation gemstones can be identified. It can be used for both rough and cut gemstones. Only the colored gemstones are tested with the help of a dichroscope. Gemstones with similar colors that are singly refractive are distinguished from those that are doubly refractive with the aid of dichroscope. According to Read (1983)[2] there are various dichroscopes available: London Dichroscope consists of two polarizing filters with their vibration direction 90° to each other. A Calcite Dichroscope is made up of rhomb calcite, which is an Iceland spar, placed in a metal tube containing an eyepiece, a lens, and a square aperture at the one end. Rayner Dichroscope is a compact instrument with a calcite rhomb and it measures 50x14mm. Similarly, Gem Dichroscope makes use of calcite rhombs and measures 38x16mm. It is also a bit smaller than Rayner Dichroscope (fig. 3c).

IV. Polariscope:

A polariscope is a quick and useful tool that helps us to identify the optical nature of a gemstone and conclude whether it is singly or doubly refractive. It also helps us to identify isotropic and anisotropic gemstones respectively. It can be used in rough, cut, set, and loose gemstones. Limitations of polariscope are that the gemstone must have some transparency and the setting doesn't inhibit us from viewing any light traveling through the stone. A polariscope is made up of two polarizing filters.

There are two types of polariscope: A portable polariscope- It is portable and requires an additional light source for viewing the internal refraction. In this, the filters are fixed at 90° to one another. A bench polariscope- It is not portable and is fixed in a place with a built-in light source that plugs into the mains. In this, the top filter is rotatable and must be turned to be fixed at 90°. According to Gubelin and Koivula (1986)[3] Polarizing filters are useful for spotting the "tata-mi" and "tabby extinction" patterns in synthetic spinel which are used to imitate many gems like aquamarine. Two crossed polaroid discs are oriented at right angles to each other. A light source is located at the bottom of the instrument and it passes through them. In the polariscope, the gemstone should be placed between the two discs. Lower polaroid discs are fixed and referred to as polarizers, while upper ones rotate and are referred to as analyzers. Furthermore, a polariscope can also be used to determine a gemstone's pleochroism and optic figure (uniaxial/biaxial) (fig. 3d).

V. Spectroscope:

Spectroscopes are another important, easily portable instrument used to identify gemstones. It measures the wavelength absorbed by the gemstones further producing a spectrum. When light passes through a material to be tested, the spectroscope separates it into its component spectrum. The gemstone will reveal its characteristic appearance if it absorbs specific types of light. Spectroscopes show vertical black lines in the spectrum for the wavelengths absorbed by the stone. In addition to rough gemstones, cut gemstones can also be examined with spectroscopes. It can identify gemstones by identifying their absorption lines due to their chemical composition. A strong light source is essential to see the subtle spectral characteristics of most stones. There are two types of spectroscopes available:

1. The prism spectroscope
2. The diffraction-grating spectroscope

The prism spectroscope works on the principle of diffraction. The light enters through a narrow-slit present in the device and then gets dispersed through a series of prisms. The diffraction-grating spectroscope is smaller and more portable which offers a uniform distribution of visible colors, making it easier to see absorption lines in the red region (fig. 3e).

VI. Chelsea color filter:

Chelsea color filter is developed to distinguish between genuine emeralds and their paste and doublet simulants. These contain a combination of gelatine filters that allows

light only to pass through wavelengths of deep red and yellow-green. The combination was chosen because of the peculiar spectral response of emerald, which permits light to transpose in the deep red and get absorbed in the yellow-green. It is also known as an emerald filter or color filter. A strong light source and close proximity to the eyes are required for the Chelsea filter to work. Emerald simulants can be identified by this filter (fig. 3f).

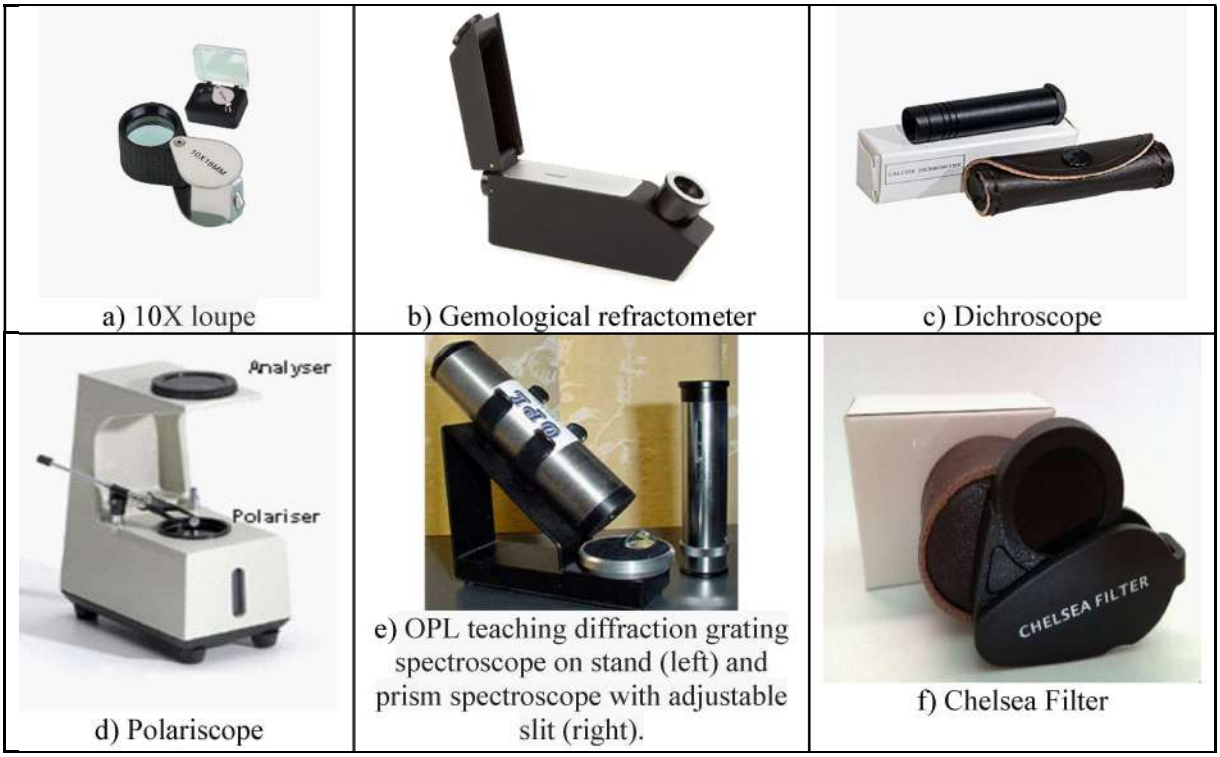


Figure 3: Portable Gemstone Diagnostic instruments.

Lab and advanced instruments for gem identification.

I. Gemological Microscope:

It is one of the most important and widely used bench-held gem testing equipment. A gemological microscope is a binocular microscope working on the zoom principle fitted with light field illumination. It has a great zooming quality from 10x to 90x magnification, overhead illumination, adjustable iris, and a stone holder. The stereoscope provides a 3D magnified view of the inclusions and the dark field illumination highlights the inclusions of gemstone in dark background. This equipment helps in distinguishing between a natural and synthetic stones. According to Read (2013)[1], the maximum magnification used for gemological purposes is 60x to 80x but the widely used range is between 15x to 30x. Matlin and Bonanno (1997, 2008)[4,5] propound different light sources to study the gemstone which includes Darkfield, Bright light, diffused bright field, Overall illumination, Pinpoint illumination by closing the iris, horizontal fiber optic through the side of the gemstone present. (fig. 4a)

II. X-Ray Diffraction (XRD):

XRD (X-Ray diffraction) is the most important tool used in the field of mineralogy and gemology which helps to understand the internal structure of the sample. It is used to precisely identify a mineral or compound's crystallographic structure by comparing diffraction data with a known database of a mineral or compound and gives a fingerprint characterization. According to Jenkins and Snyder (1996)[6] the size and shape of the unit cell for any gemstone can be determined using XRD (fig. 4b)

III. X-Ray Spectroscopy:

Gemstones undergo multiple treatments for enhancing their quality and beauty. With the advanced technology, confirming a gemstone to be natural or synthetic had become difficult. Nowadays people prefer non-destructive and less time-consuming methods for gemstone identification. X-ray spectroscopy is one such

technology that is non-destructive and time efficient which helps in differentiating between natural and synthetic gemstones (fig. 4c). There are two types of X-ray spectroscopy:

1. WD-XRF (Wavelength Dispersive X-Ray Fluorescence)
2. ED-XRF (Energy-Dispersive X-Ray Fluorescence

These instruments are rapid, non-destructive, and give a qualitative chemical analysis of the sample with a large flat polished surface.

X-Ray:

X-Rays help to determine the crystal structure of the solid at the atomic level and are used to analyze the chemical composition of minerals and rocks. The X-ray spectrum can be obtained by bombarding metal targets such as Cu, Mo, and Rh with electrons emitted from a hot tungsten filament. This method is also helpful for the identification of minerals and to further understanding its unit cell dimensions.

Wavelength Dispersive X-Ray Fluorescence (WD-XRF):

WD-XRF uses analyzing crystals to diffract different X-ray wavelengths and numerous detectors are placed at different angles which measures the number of diffracted rays at each angle. It is used for elemental analysis where all the elements are excited simultaneously. The advantage of WD-XRF spectroscopy is that the system is of high resolution and has a minimum spectral overlap which gives an accurate result.

Energy-Dispersive X-Ray Fluorescence (ED-XRF):

ED-XRF is a simple and non-destructive analysis method used for gemstone testing. It is used to determine the elements present in the gem mineral. With the help of ED-XRF natural and man-made gemstones can be identified. This technique does not damage the gem sample. Low power 50w ED-XRF and secondary target with polarization is used which yields excellent performance for elemental analysis. Indirect excitation and polarization give excellent low background tools which ensure that no damage occurs to the gemstone during analysis. The sample to be analyzed should have an almost planar and smooth surface.

IV. Raman Spectroscopy:

Raman Spectroscopy is a non-destructive rapid identification method that gives a confirmatory result for the identification of unknown polyatomic species occurring in any phase *viz*. solid, liquid or gas. Raman spectroscopy has a fundamental relevance in gemstone study. With the help of this method, gem authenticity can be determined. It can identify inclusions in either its solid, liquid, or gaseous stage and also helps in the study of gem fissures, mineral crystal type, crystal defects, vacancies, and substitutions. Analyzing inclusions in the gem determines the geographic origin. Raman spectroscopy is now a well-established method for the identification of crystalline and amorphous phases constituting ancient artifacts.[7-12] (fig. 4d)

V. Laser Ablation-Inductively Coupled Plasma Mass Spectrometry (LA-ICPMS):

LA-ICPMS (Laser Ablation-Inductively Coupled Plasma Mass Spectrometry) is an advanced instrument that gives us the result of quantity of major, minor, and trace present in a sample. A beam of laser light is focused on the surface of the sample so that the tiny particles are removed from the surface by the use of flowing gas into a high-temperature plasma torch where they are broken into smaller pieces of individual atoms and are further identified by the detector. With the help of photo micrography closeup images of inclusion features within the gem can be captured which gives the information to determine the condition of how the gems formed and also about its geographic origin. This technique is used with minimal destruction to the sample. The potential use of this method in gemology was first reported by Günther & Kane (1999a, 1999b)[13-14] (fig. 4e). Dempster (1918)[15], Aston (1919)[16], and Stephens (1946)[17] developed this technique which is a highly sensitive method capable of analyzing chemical elements in a sample and the presence of small organic molecules. Mass spectrometry is used for all three phases solid, liquid, and gases.

VI. Laser-Induced Breakdown Spectrometer (LIBS):

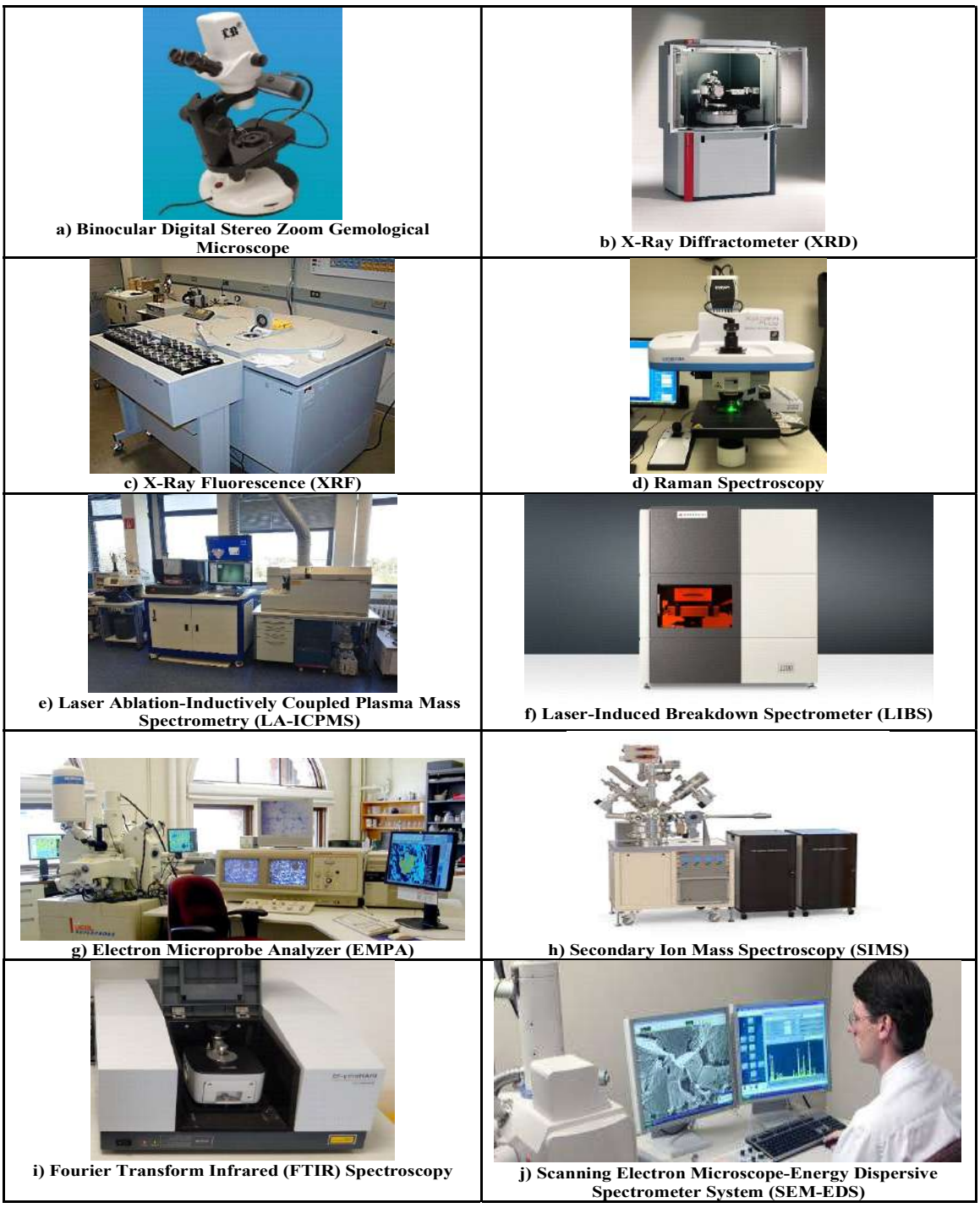
LIBS (Laser Induced Breakdown Spectrometer) is a low-cost and easily operative instrument used for the detection of major, minor, and trace elements.[18] A single pulse high energy laser beam is used to vaporize a microscopic surface area of a sample at a high temperature which breaks down the sputtered particles into atoms, ions, and electrons. Optical emission spectra are generated which are converted to qualitative and semi-qualitative chemical data. Accurate data cannot be obtained with the help of the LIBS method.[19,20] This technique is better used for detecting beryllium diffused ruby and sapphire. (fig. 4f)

VII. Electron Microprobe Analyzer (EMPA):

EMPA is another well-established method used in the field of gemology.[21] In this method, a high-energy focused beam of electrons is used to generate -rays that reveal the elements in a sample. It is a non-destructive fully quantitative method used to analyze the elements ranging from beryllium to uranium (fig. 4g).

VIII. Secondary Ion Mass Spectroscopy (SIMS):

SIMS is a destructive analytical technique that provides highly accurate chemical analysis of a wide range of solid materials without causing damage to the material itself.[22] It is a sophisticated and high lost instrument used in the field of gemology. The sample is bombarded with primary ions such as oxygen/argon, resulting in the



a) Binocular Digital Stereo Zoom Gemological Microscope

b) X-Ray Diffractometer (XRD)

c) X-Ray Fluorescence (XRF)

d) Raman Spectroscopy

e) Laser Ablation-Inductively Coupled Plasma Mass Spectrometry (LA-ICPMS)

f) Laser-Induced Breakdown Spectrometer (LIBS)

g) Electron Microprobe Analyzer (EMPA)

h) Secondary Ion Mass Spectroscopy (SIMS)

i) Fourier Transform Infrared (FTIR) Spectroscopy

j) Scanning Electron Microscope-Energy Dispersive Spectrometer System (SEM-EDS)

Figure 4: Advanced Lab Gemstone Diagnostic instruments.

sputtering of a very small portion of the surface using an electrical field. After being ionized and filtered by an electrostatic and magnetic field, the atoms are then sent to a mass spectrometer for isotopic analysis. Sensitivities as low as parts per billion (ppb) or parts per million (ng/m) can be obtained using this technique for all elements from hydrogen to uranium (fig. 4h).

IX. Fourier Transform Infrared (FTIR) Spectroscopy:

The FTIR technique measures how the sample affects the infrared light scattered or reflected by it. Through this technique, the method was made much faster, more sensitive, and simpler, allowing it to be widely used for identifying almost any material in a matter of seconds. In this technique by using a dispersive prism or graft, the laser beam was separated into different frequencies. Each frequency light comes into contact with the sample (one at a time), and often traverses it, or maybe gets reflected by it. Detectors measure energy at different frequencies coming from samples, resulting in spectra. Raman spectroscopy and FTIR spectroscopy have similar applications today. However, there is one very important difference between the two: FTIR spectroscopy reveals the chemical composition of a sample, whereas Raman provides only one fingerprint that must be compared to a library to be identified. This method can be used to obtain precise quantitative information about the substances present in a mixture or solution for a variety of purposes, including identifying the types of substances and quantifying their amounts present (fig. 4i).

X. Scanning Electron Microscope-Energy Dispersive Spectrometer System (SEM-EDS):

It is one of the principal tools in understanding gem minerals. SEM-EDS also includes electronic components and a computer that enable it to automatically perform complex calculations and display the results. In all practical cases, SEM-EDS can analyze gemological specimen chemical and texturally without damaging or altering the gems. It is an excellent analytical tool for gem research due to its non-destructive nature. A beam of electrons striking a sample induces the sample to return electrons. This is the principle of a scanning electron microscope. These returning electrons show variations in intensity and distribution as a result of the specimen's surface contours or composition (fig. 4j).

CONCLUSION

Various aspects of gemology are always evolving, from identifying the treatments of gemstones and synthetics to grading the cut quality of a faceted stone. Instruments development helped gemologists to identify various known and unknown gemstones occurring in nature. Scientific and traditional instruments are the tools that help in raveling the mysteries of gems. These tools are helpful in identifying the various elements and characteristics present in a gem. As synthesis and treatment technology is evolving simultaneously, these instruments prove to be helpful in distinguishing between the lab-grown and natural gemstones.

ACKNOWLEDGEMENT

The authors would like to acknowledge the Gemological Laboratory at the University Department of Geology, Ranchi University for having the practical approaches of the instruments used. The authors are also thankful to the anonymous reviewers for their insightful criticism, which has greatly improved the manuscript.

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RUJOST, ISSN: 2319-4227, Vol.7, No.1, January, 2022, pp. 127-132
A Biaanual Peer Reviewed Referred Ranchi University Journal of Science & Technology

Geology

Correlation of Petrographic and Proximate analysis data of coals of Raham Block, North Karanpura Coalfield, Chatra District, Jharkhand, India

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Abstract: Coal petrography is an advanced stage of coal sample analysis. Other analyzing techniques include chemical analysis. Proximate analysis is one of them. Raham block is located on the northern limb of north Karanpura basin were strata dips towards south. The geologic features are in continuity with the adjoining area. Raham block is located in Tandwa sub - division of Chatra district of Jharkhand state. The coal seam present today in Raham Block is a result of deposition of plant matter millions of years ago. The research paper has been put forward for correlation of the petrographic and proximate analysis data of coals. Coal Petrography relates to the study of different microscopic organic constituents of coal to understand the Maceral composition and the maturity of coal. Macerals can be distinguished under the microscope on the basis of their colour, shape, size and texture. The source material, colour or level of reflectivity and nature of formation of the macerals imparts them their distinguishing feature. The Proximate analysis data has been acquired by following Indian Standard by analyzing the parameters like moisture, volatile matter, ash and fixed carbon content by difference.

Keywords: North Karanpura, Raham Block, Coal, Petrography, Proximate Analysis

INTRODUCTION

Raham block of North Karanpura coalfield is located in Tandwa sub – division of Chatra district of Jharkhand state.[1] The geologic features are in continuity with the adjoining area of North Karanpura coalfield.[2] The Barakar and Barren measure formation occupy major portion of the Raham block. The petrographic characteristics depends on the conditions operating in the swamps as well as in source area during the formation viz. Water level, subsidence of swamps, pH level, redox potential is responsible for such type of condition in peat formation of the vegetal matter. Present paper has dealt with the representative samples taken from central part of the Raham Block, North Karanpura Coalfield of Jharkhand, India to correlate the data and infer conclusion in relation to different parameters of proximate analysis and Petrographic data.

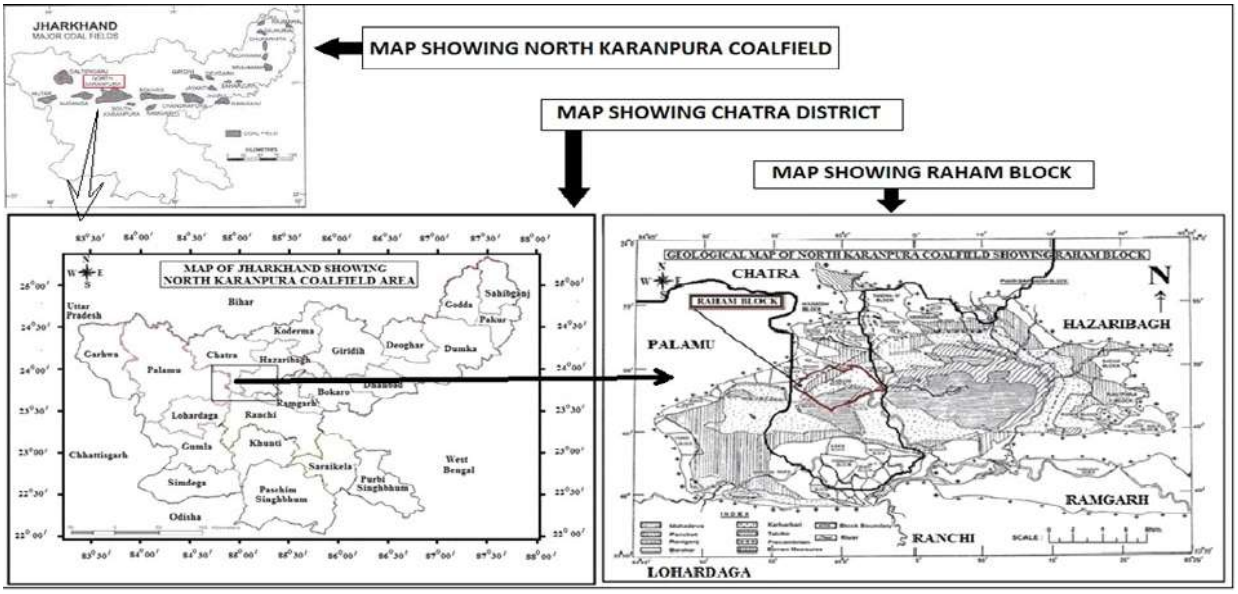
Location of Raham Block

Raham block of North Karanpura coalfield is located in Tandwa sub-division of Chatra district of Jharkhand state. It is bounded by Latitudes 23°47'38'' N and 23°50'22'' N & Longitudes 84°57'38'' E and 85°02'02'' E. (Map 1)

Series of the Upper Gondwana Division. It has yielded a rich flora consisting mainly of impressions of cycads, conifers, and ferns, which was first described by Oldham and Morris and later by Feistmantel and others.[2] For a description of the geology of the Rajmahal Hills, reference may be made to the early work of Ball (Fig. 2), where the stratigraphical position of the different plant-bearing beds.

Geology of Raham Block

The Barakar and Barren measure formation occupy major portion of the Raham block. Four faults are interpreted to be present in the Raham block. Raham block is located on the northern limb of the North Karanpura basin were strata dip towards south. The geologic features are in continuity with the adjoining area of North Karanpura coalfield. Four faults are interpreted to be present in the Raham block.[3] Raham block is located on the northern



Map 1: Location of Raham Block in North Karanpura Coalfield, Jharkhand, India

limb of the North Karanpura basin were strata dip towards south. Barakar, Barren Measure and Raniganj formations of lower Gondwana series occur in the block in the chronological order of younger above older are exposed on the surface.

MATERIALS & METHODS

The half reserve borehole samples were collected by the courtesy of Central Institute of Mining and Fuel Research, Ranchi and analysis was done. More than 100 samples were studied for this purpose and selected 14 numbers of coal samples were selected for Petrographic study.[4] The data was acquired by using Petrographic Microscope- LEICA DM 4500P, by the courtesy of Central Mine Planning and Design Institute, Ranchi. The Indian Standard was followed for analysis of coal samples. Sampling was done as per Indian Standard IS: 436 (Part I)- 1976 and Reduction of Gross Samples was done as per Indian Standard IS: 436 (Part I)- 1964.[5] Chemical analysis i.e., Proximate analysis was done as per Indian Standard IS: 1350 (Part I)- 1984.[6] Petrographic Analysis of Coal was done by (IS: 9127- Part II (2014), Part III (2002), Part IV (2010).[7]

RESULTS & DISCUSSIONS

Table 1: Showing Proximate and Petrographic Analysis Data

Sample Detail	Description				Proximate Analysis Data				Petrographic Analysis Data			
	Bore Location	Depth (m)	Thick (m)	Seam	M %	A %	VM %	FC %	Vitrinite	Liptinite	Inertinite	Visible Mineral Matter
A1	Central Part of the Raham Block	338.07	1.88	IV	3.9	39.1	22.8	34.2	31.8	8.2	37.0	23.0
A2					3.8	41.4	20.6	34.2	30.8	10.4	37.2	21.6
A3					3.9	41.2	20.9	34.0	30.5	10.7	37.4	21.4
A4					3.9	41.1	21.0	34.0	29.9	9.8	37.1	23.2
A5		343.2	6.49	III	3.4	35.2	23.7	37.7	22.7	11.4	30.7	35.2
A6					3.4	41.0	20.9	34.7	30.6	10.9	37.5	21.0
A7					3.4	40.2	20.8	35.6	30.1	10.4	37.7	21.8
A8					3.4	39.7	21.3	35.6	28.9	9.9	36.9	24.3
A9		383.62	2.89	I (T)	2.6	43.1	21.6	32.7	18.6	8.5	43.2	29.7
A10					2.6	47.9	19.8	29.7	30.4	10.8	37.3	21.5
A11		393.35	6.9	I (M)	2.8	40.8	21.9	34.5	21.8	10.6	32.8	34.8
A12					2.8	54.6	7.6	35.0	29.7	10.2	37.6	22.5
A13		411.3	5.13	I (B)	2.4	38.7	19.6	39.3	30.9	10.9	37.6	20.6
A14					2.6	43.2	18.4	35.8	30.7	8.4	37.1	23.8

The data acquired in the Table 1 above shows proximate analysis data indicating range of Moisture content from 2.4% to 3.9%, Ash content from 35.2% to 54.6%, Volatile matter from 7.6% to 23.7% and Fixed Carbon from 29.7% to 39.3%. The Petrographic analysis data range of Vitrinite content from 18.6% to 31.8%, Liptinite content from 8.2% to 11.4%, Inertinite content from 32.8% to 43.2% and Visible Mineral Matter content[8] from 20.6% to 35.2%.

The above acquired data are being correlated to show their relation (Fig 1)

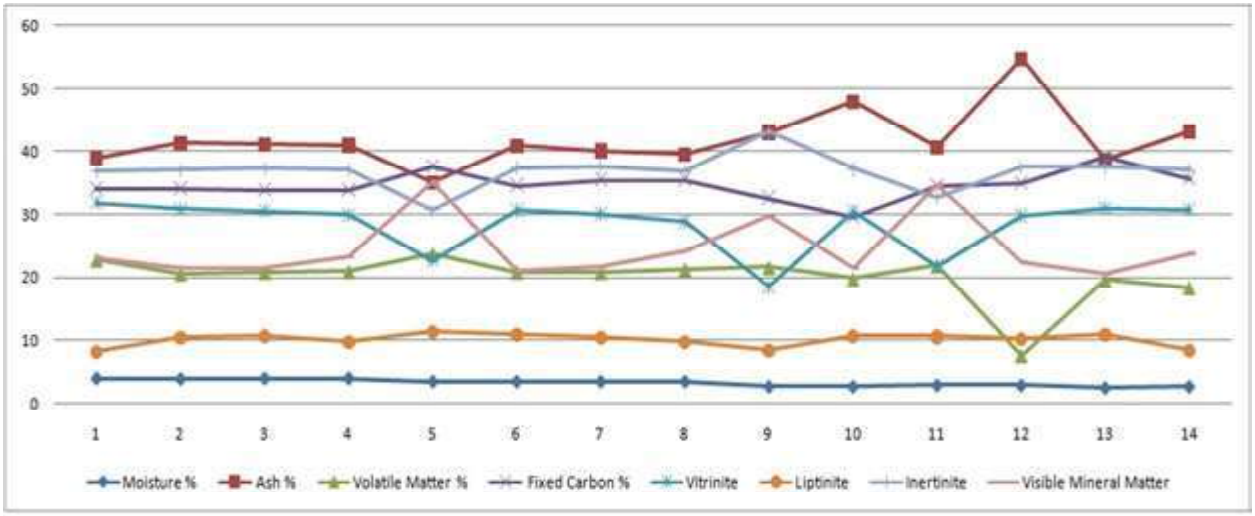


Fig. 1- Showing Proximate and Petrographic data collectively

Correlation of Moisture percentage with Vitrinite, Liptinite and Inertinite content

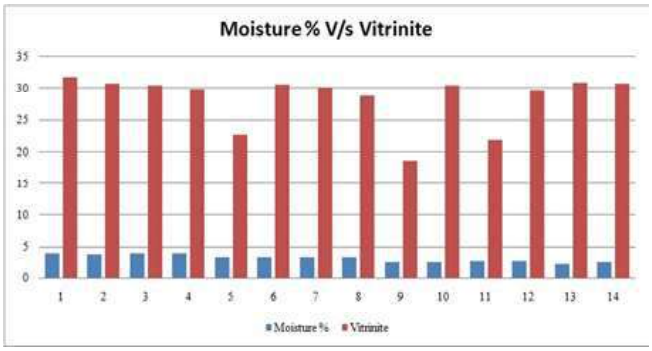


Fig. 2- Correlation of Moisture percentage with Vitrinite content

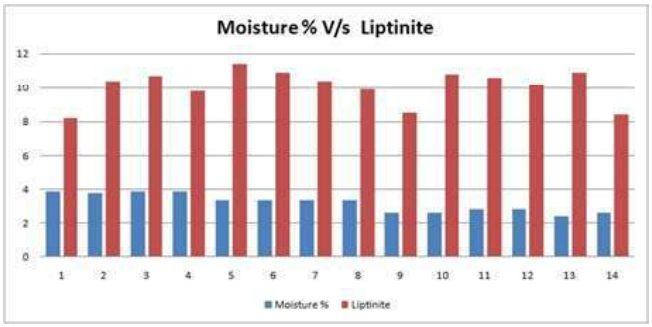


Fig. 3- Correlation of Moisture percentage with Liptinite content

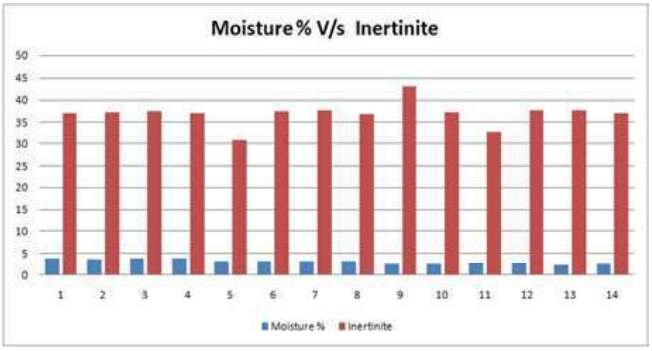


Fig. 4- Correlation of Moisture percentage with Inertinite content

Correlation of Ash percentage with Vitrinite, Liptinite and Inertinite content

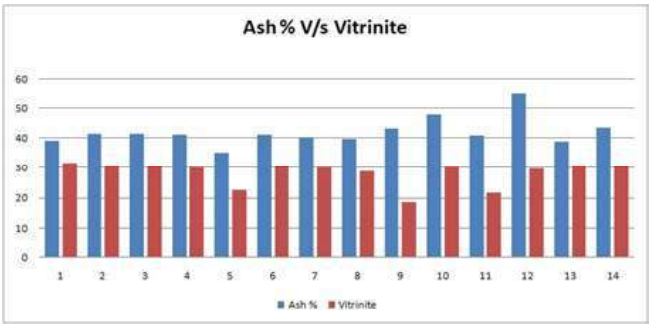


Fig. 5- Correlation of Ash percentage with Vitrinite content

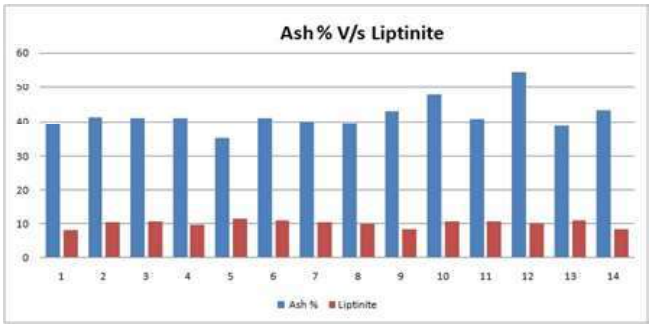


Fig. 6- Correlation of Ash percentage with Liptinite content

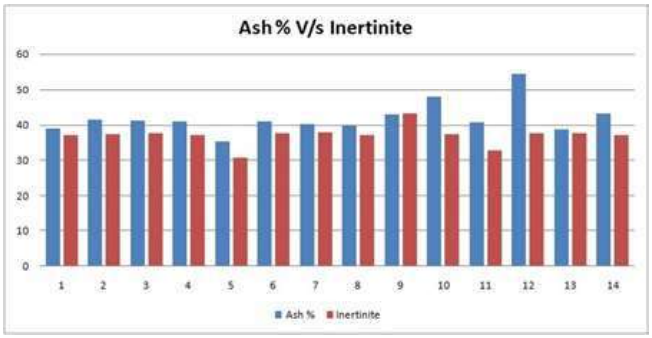


Fig. 7- Correlation of Ash percentage with Inertinite content

Correlation of Volatile Matter percentage with Vitrinite, Liptinite and Inertinite content

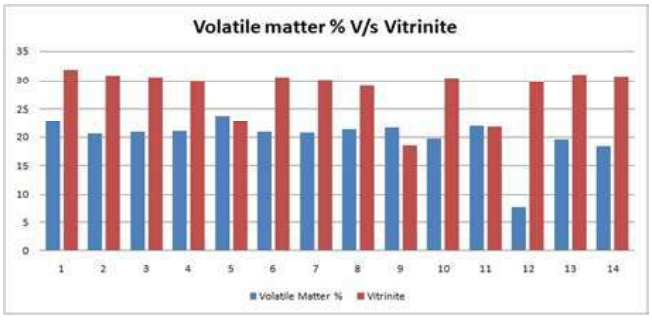


Fig. 8- Correlation of Volatile Matter percentage with Vitrinite content

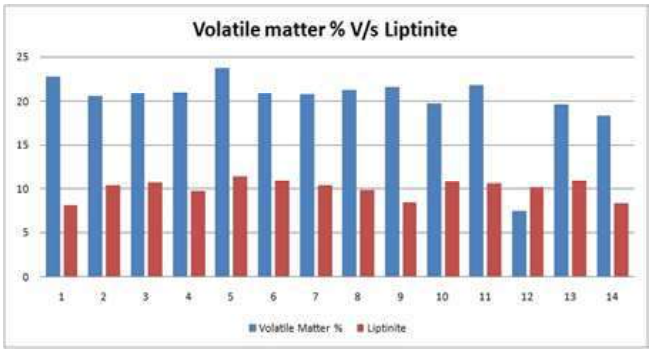


Fig. 9- Correlation of Volatile Matter percentage with Liptinite content

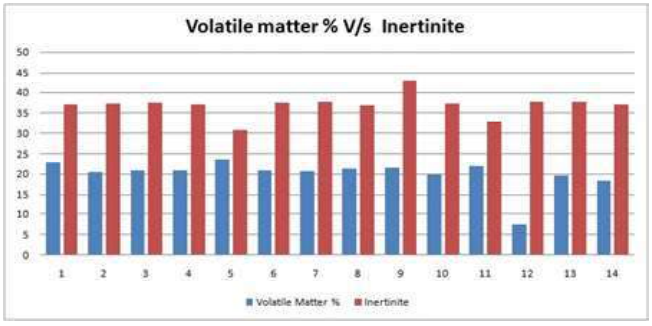


Fig. 10- Correlation of Volatile Matter percentage with Inertinite content

Correlation of Fixed Carbon percentage with Vitrinite, Liptinite and Inertinite content

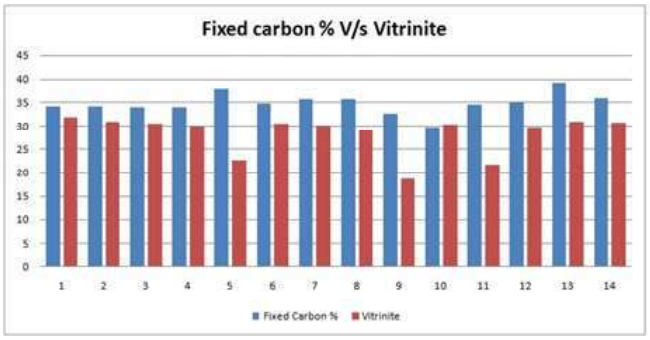


Fig. 11- Correlation of Fixed Carbon percentage with Vitrinite content

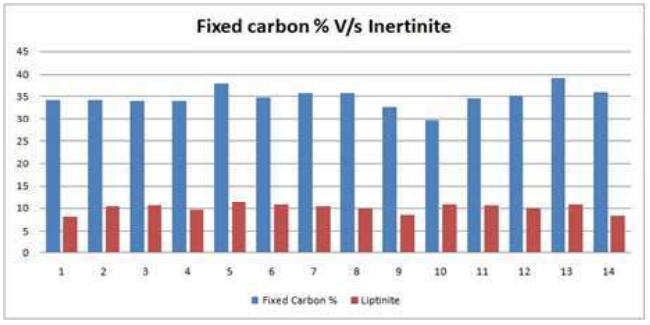


Fig. 12- Correlation of Fixed Carbon percentage with Liptinite content

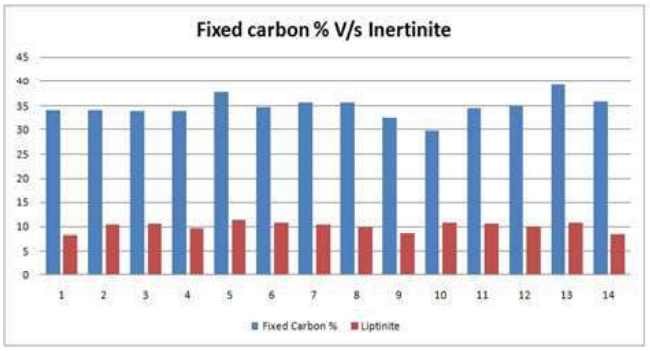


Fig. 13- Correlation of Fixed Carbon percentage with Inertinite content

Inference

Raham block has not felt the thrust of any mining activity since it is still a virgin block. The Authors have used proximate analysis data and petrographic data for correlation using the basic parameters.

Table 2- The correlations of chemical analysis data with petrographic data clearly depict a relation as under

Fig No.	Parameters	Parameters	Inference	Comment
2	Moisture %	Vitrinite%	Direct Relation	Due to high Oxygen content from moisture.
3		Liptinite%	No Relation	-
4		Inertinite%	Indirect Relation	Due to less Carbon content.
5	Ash %	Vitrinite%	Direct Relation	Due to more unburnt Carbon (cellulose/lignin) content
6		Liptinite%	No Relation	-
7		Inertinite%	No Relation	-
8	Volatile Matter %	Vitrinite%	No Relation	-
9		Liptinite%	Indirect Relation	Due to waxy/resinous content
10		Inertinite%	Indirect Relation	Due to less Carbon content.
11	Fixed Carbon %	Vitrinite%	No Relation	-
12		Liptinite%	Direct Relation	Due to more carbon content
13		Inertinite%	No Relation	-

CONCLUSION

The Raham block coal has been taken as representative samples for correlation of proximate data and petrographic data.

It is being concluded that there is direct relation as per Hilt's Law of Moisture % (Due to high Oxygen content from moisture) and Ash % (Due to more unburnt Carbon (cellulose/lignin) content) with Vitrinite content and Fixed Carbon (Due to more carbon content) with Liptinite content.

Parameters have indirect relation as per Hilt's Law of Moisture % (Due to less Carbon content) with Inertinite content, Volatile Matter % (Due to waxy/resinous content) and Liptinite (Due to less Carbon content) with Inertinite content.

There is no relation of Moisture% with Liptinite content, Ash% with Liptinite and Inertinite content Volatile Matter % with Vitrinite and Fixed Carbon % with Vitrinite and Inertinite content.

ACKNOWLEDGEMENT

Authors are very thankful to Central Mine Planning and Design Institute Limited, Ranchi, Jharkhand for assisting in gathering much of exploratory facts about the Raham block. Authors are highly thankful to CSIR - Central Institute of Mining and Fuel Research, Ranchi, Jharkhand for providing infrastructure and technical help in achieving the desired findings.

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RUJOST, ISSN: 2319-4227, Vol.7, No.1, January, 2022, pp. 133-140
A Biaanual Peer Reviewed Referred Ranchi University Journal of Science & Technology

Geology

Macropetrographical and depositional conditions of Coal Seams of Dhori Area, East Bokaro Coalfield, Jharkhand, India

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Abstract: Macropetrographic analysis based on lithotype content have been made on coal samples from Karo Special Seam III, Karo Group of Seam (VI, VII and VIII), Bermo Seam and Kargali Seam. The Karo Group, Bermo and Kargali Seams of East Bokaro Coalfield are important seams and so have been chosen for this study. Macropetrographic characteristics describing the content of Lithotypes suggest variation in the content of Vitrain, Clarain, Durain and Fusain. The study area chosen is Dhori Area, East Bokaro Coalfield. The system proposed by Diessel in 1965 has been applied for lithotype description of seams for the present study. The lithotypes are present in variable amounts in the coal seams of Dhori Area. The lithotype content of Karo Special Seam III are Vitrain (45%, 40%, 13% and 2%). The Karo Group Seams (VI, VII and VIII) have Vitrain (38%,36% and 29%), Clarain (47%,49% and 53%), Durain (11%, 12% and 14%) and Fusain (4%, 3% and 4%). Bermo Seam contain Vitrain (18%), Clarain (62%), Durain (16%) and Fusain (4%). The Lithotype content of the seams of the area were further used to interpret the depositional environment of the coal seams. The presence of Vitrain and Clarain in dominant amount suggest wet terrestrial forest moor setting for majority the coal seams of the area.

Keywords: Lithotypes, Coal, East Bokaro Coalfield, Bituminous, Deposition, Dhori

INTRODUCTION

Coal has been the most used mineral since decades. Coal is a hard, brittle and combustible meta-sedimentary rock consisting predominantly of carbon. East Bokaro Coalfield is a part of Bokaro Coalfield located east of longitude 85°42'. Earlier works on East Bokaro Coals were been caried out by various authors.[1-7] The current study deals with macropetrographic study of the coals of area which includes the interpretation and analysis of lithotypes present in coal.

Geological Setting

The East Bokaro Coalfield (EBC) is a part of Great Damodar Valley Coal Basin in Eastern India and is the eastern half of main Bokaro Basin. It is east west trending elongated basin within Damodar-Koel river valley basins. The coal field contains a continuous succession from Talchir to Supra-Panchet Formations (Table 1).[8] The EBC is traversed by a number of faults, resulting in the formation of trough and horst structures.

The study area Dhori is located in the eastern part of the EBC that lies within the latitude 23°45'56" to 25°47'05" and longitude 85°39' to 86°01' and is covered by the survey of India Toposheet No. 73I/1 (Fig. 1). The total area of the block is about 6 sq. km. The coal horizons are designated by the local name of the places in the area such as Kargali, Bermo and Karo group of Seams. The Precambrian forms the basement of the basin of disposition and is exposed in the north of the Dhori Block. The contact between Gondwanaland and Precambrian are unfaulted. The Karharbari Formation is characterised by predominance of coarse-grained sandstone, conglomerate indicating shallow water deposition. Karharbari Formation contains Karo Special Seam III and is the attractive both from thickness and quality point of view. Barakar group of seams is characterised by a dominance of arenaceous facies and occurrence of thick coal seams *viz*. Karo Group of seams, Bermo and Kargali. The sandstones are dirty-

white to buff in color medium to coarse grained, hard and compact. Due to compactness of the sandstone, it forms prominent ridges in the north western and central part of the block. Two mica peridotite dykes have been encountered in the old working of Kargali seam. The generalised stratigraphic succession of East Bokaro Coalfields is shown in Table 1.[9]

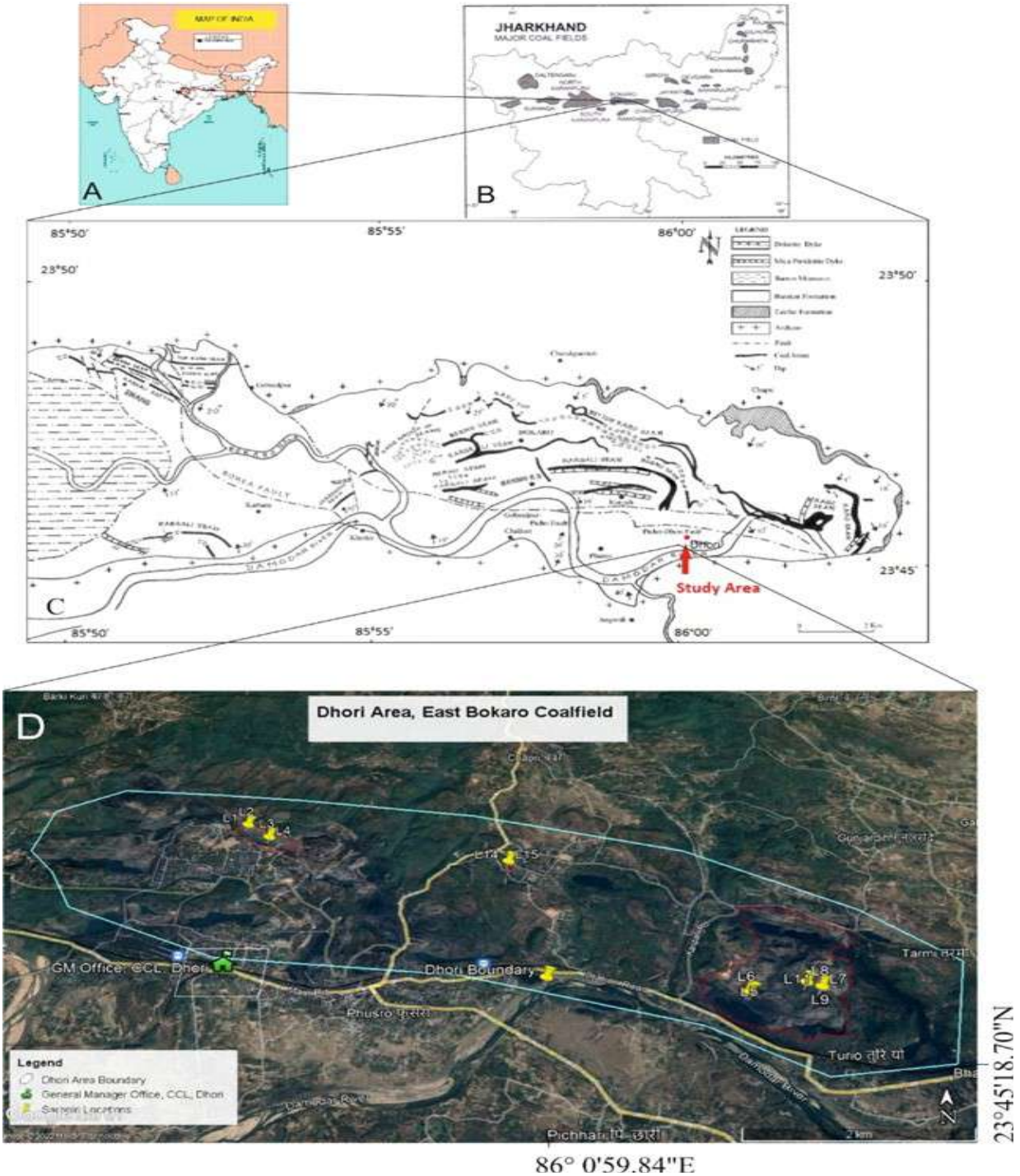

Fig. 1- A). Location of Damodar basin in Map of India. B). Location of East Bokaro coalfield on the Map of Jharkhand. C). Geological Map of East Bokaro Coalfield with location of study area Dhori (modified after[9]). D). Map showing the sampling location on the study area.

Table 1- The generalised stratigraphic succession of East Bokaro Coalfields[8]

Supergroup	Group	Age	Formation	Lithology
Gondwana		Lower Cretaceous	Intrusives	Lamprophyre and dolerite dykes and sills
	Upper Gondwana	Upper Triassic	Supra-Panchet	Coarse grained, ferruginous sandstone, pebbly sandstone and red clay (600m)
		Unconformity		
	Lower Gondwana	Upper Permian	Raniganj	Medium to coarse grained calcareous sandstone, fine grained greenish sandstone, grey shale, carbonaceous shale and thin coal seams (600m)
		Middle Permian	Barren Measures	Flaggy, fine grained ferruginous sandstone, micaceous sandy shale and black shale with sideritic bands(500m)
		Lower Permian	Barakar	Coarse grained, arkosic sandstone, fine grained laminated sandstone, grey shale, carbonaceous shale and coal seams (900m)
		Upper Carboniferous	Talchir	Tillite, greenish sandstones, needle shale, varves and rhythmites.
		Unconformity		
		Precambrian	Basement	Granite, gneiss, amphibolite and mica schist.

MATERIALS & METHODS

Collection of Samples

A total of fifteen samples have been collected for Macropetrographic study of the Coal Seams of Dhori Area from three different mines of Dhori Area following Indian Standard.[10] The three different mines are Amlo Open Cast Mine, New Selected Dhori Mine (Kalyani Colliery), and Dhori Khas. Coal samples represented two samples from Karo Special Seam III, nine samples from Karo Group of Seams, two samples from Bermo Seam Inclined (BSI) Patch, and two samples from Kargali seam, (Table 2).

Macropetrographic coal seam characteristics

The Macropetrographic study included the study of the physical properties and Lithotype characteristics of the coals of Dhori Area. The system proposed by Diessel, (1965)[11] for lithotype description of seams was applied, which defines dull coal, banded coal, bright coal and fibrous coal according to the macroscopic appearance of the seam intervals. The term Lithotype was proposed by Seylor (1954)[12], these are macroscopically identifiable units or bands with a minimum of 3 to 10mm in thickness in bituminous humic coals.[13-15] Four types of lithotypes in bituminous coals were described on basis of their physical dissimilarities such as colour, lustre (bright versus dull) texture and type of stratification., namely Vitrain, Clarain Durain and Fusain. Vitrain and clarain are together the brights or bright Bands of the seam.[14] The Lithotypes content were calculated following the procedures.[14] Brilliant glossy, jet black lithotype with a minimum thickness of 3mm were considered for Vitrain calculation. Another lustrous component of coal similar to Vitrain is Clarain. It is thinly banded lithotype with alternate laminations of bright (vitrain) and dull bands (durain and fusain). The cumulative band thickness of 3mm for clarain were used for calculation. Durain is generally less than 3mm in thickness. Fusain as a rule forms minor fraction of a coal seam, about 2-5%. Various Macropetrographic studies show that the brighter lithotypes are rich in vitrinite, whereas the duller lithotypes show greater contents of inertinite or mineral matter or both.[11,16-19]

Table 2- Listing of Dhori Area Coal samples analysed in this study.

Site Name	Location No.	Sample Type	Sample No.	Latitude	Longitude	Seam Name
Amlo (Open Cast Mine)	L1	ROM Sample	AMLIS1	23°47'16.06"N	85°59'2.85"E	Kargali Top Seam
	L2	ROM Sample	AML1S2	23°47'15.76"N	85°59'3.11"E	Kargali Bottom Seam
New Selected Dhori (Open Cast Mine)	L3	Channel Sample	AML2S1	23°47'8.48"N	85°59'12.56"E	Bermo Seam Inclined (BSI Patch)
	L4	Channel Sample	AML2S2	23°47'8.35"N	85°59'12.86"E	Bermo Seam Inclined (BSI Patch)
	L5	Stock Sample	NSDL1S1	23°45'49.23"N	86° 2'9.29"E	Karo Group Seam VI-VIII
	L6	Stock Sample	NSDL1S2	23°45'49.52"N	86° 2'8.40"E	Karo Group Seam VI-VIII
	L7	Channel Sample	NSDL2S3	23°45'51.87"N	86° 2'34.01"E	Karo Group Seam VI
	L8	Channel Sample	NSDL2S4	23°45'51.97"N	86° 2'34.01"E	Karo Group Seam VI
	L9	Channel Sample	NSDL2S5	23°45'51.87"N	86° 2'34.01"E	Karo Group Seam VII
	L10	Channel Sample	NSDL2S6	23°45'51.97"N	86° 2'34.01"E	Karo Group Seam VII
	L11	Channel Sample	NSDL2S7	23°45'51.87"N	86° 2'34.01"E	Karo Group Seam VIII
	L12	Channel Sample	NSDL2S8	23°45'51.97"N	86° 2'34.01"E	Karo Group Seam VIII
	L13	Channel Sample	NSDL2S9	23°45'53.88"N	86° 2'29.22"E	Karo Group Seam Combined VI-VIII
Dhori Khas (Underground Mine)	L14	Wagon Sample	DKHS1	23°46'54.26"N	86° 0'47.78"E	Karo Special Seam III
	L15	Wagon Sample	DKHS2	23°46'54.26"N	86° 0'47.78"E	Karo Special Seam III

The variations in the distribution of lithotype in seam profiles have been used for the interpretation of relative wetness changes within the precursor mires, with durain represent the wettest condition, and fusain the driest.[20] Geological models of peat accumulation have been developed by the interpretation of compositional characteristics of lithotypes, with brighter lithotype suggesting peat accumulation in a wet forest moor environment, banded dull lithotype suggesting a dryer terrestrial forest moor setting, and the dull lithotypes suggesting open moor conditions during peat accumulation.[21]

RESULTS & DISCUSSIONS

Macropetrographic Coal Seam characteristics

Karo Special Seam III. The Karo Special Seam III is the only workable seam and was sampled from underground mine at Dhori Khas. Considering the exceptionally good quality and coking Properties of this seam among the Karo Group, it has been named as 'Karo Special'. The seam can be traced along Dhori Chapri Area in the east.[8] The Thickness of the Karo Special seam III ranges from 0.75m to 3.74m and the majority is free from dirt bands. The color of the coal here is greyish black to

black in color. The streak ranges from dark brown to black. The lusture of the coal is vitreous to metallic with low specific gravity. The Hardness ranges from 2.5 to 3. Majority of the coal found here are brittle and friable. Lithotype content is variable, Vitrain occurs as well marked narrow bands and splits readily in the fingers in small cube like segments Fig 2. The limiting layers between Vitrain and Clarain or Durain is sharply marked. The Clarian of Karo Special Seam III has silky lusture. Durain content of the seam show variable thickness with irregular and rough surface which is characteristically lustreless, as distinct from the glassy surface of vitrain. The lithotype fusain occurs as patches and wedges. Fusain of the seam is soft often soils fingers and present in only minor amount. Lithotype distribution based on the classification proposed by Diessel in 1965, (Table 3) shows the predominance of Bright Bands with an average value of 45 % for Vitrain and 40% for Clarain (Fig. 2). The dull band Durain are less common averaging about 13%. Fusain constitute about 2% of the coal (Fig. 3).

Karo Group of Seams (VI, VII and VII). The Karo Group of Seams (VIII, VII, VI) combined forms the thickest horizon of the block. The thickness ranges from 34.05 to 54.67 m. In the eastern part of the Dhori Area, the Seam Nos. VIII, VII and VI occur almost as a composite seam with a thin parting of 0.86m to 2.02m thickness of carbonaceous shale/grey shale between Seam no. VIII and Seam Nos. VI and VII. It has been observed that the thickness is more in the eastern part. On the basis of some persistent carbonaceous shale/grey shale bands it is possible to recognise Seam No. VIII as a separate horizon. The seam Karo VI is a thin inferior seam of the Karo group. The seam is highly interbanded with shaly coal/ carbonaceous shale.. The seam Karo VII is interbanded like most of the seams of the Karo Group and the dirt bands increase from west to east. The thickness of the Seam No. VIII varies from 15.21m to 21.59m. Due to inclusion of intervening parting between Seam Nos. VIII and VII and VI the percentage of Dirt bands increase for combined Seam Nos. VIII, VII and VI. The Seam No. VIII has a lower percentage of Dirt bands compared to Seam Nos. VII and VI. The coals of Karo Seam (VI, VII and VII) is greyish black to black in color. The streak ranges from Dark brown to black. The lusture of the coal is vitreous with medium specific gravity, The Hardness ranges from 2.5 to 3. Lithotype content is variable, Vitrain occurs as well marked narrow bands and splits readily in the fingers in small cube like segments. The limiting layers between vitrain and clarain or durain is sharply marked. The Clarian of Karo Special Seam III has silky lusture. Vitrain and clarain are together the brights or bright Bands of the seam. Durain content of the seam show variable thickness with irregular and rough surface which is characteristically lustreless, as distinct from the glassy surface of vitrain. The lithotype fusain occurs as patches and wedges. Fusain of the seam is soft often soils fingers and present in only minor amount. Majority of the coal found here are brittle and friable. Lithotype distribution based on the classification proposed by Diessel in 1965 (Table 3), shows the predominance of Bright Bands with an average value Karo Group Seam VI, VII and VIII of 38%, 36% and 29% for Vitrain and 47%, 49% and 53% for Clarain (Fig. 2). The dull band Durain are less common averaging about 11%, 12% and 14%. Fusain constitute about 4%,3% and 4% of the coal (Fig. 3).

Bermo Seam. Bermo Seam lies in the eastern part of the coal field in Dhori colliery area. This is one of the most important thick seams of East The thickness ranges from 9.07 to 11.58m and the dirt bands are mostly concentrated in top 5m of the seam. The coals of Bermo Seam are greyish black to black in color. The streak ranges from Dark brown to black. The lustre of the coal is vitreous with medium specific gravity. The Hardness ranges from 2.5 to 3. Majority of the coal found here are brittle and friable. Lithotype content is variable, Vitrain occurs as well marked narrow bands and splits readily in the fingers in small cube like segments. The limiting layes between vitrain and clarain or durain is sharply marked. The Clarian of Karo Special Seam III has silky lusture, Vitrain and clarain are together the brights or bright Bands of the seam. Durain content of the seam show variable thickness with irregular and rough surface which is characteristically lustureless, as distinct from the glassy surface of vitrain. The lithotype fusain occurs as patches and wedges. Fusain of the seam is soft often soils fingers and present in only minor amount. Lithotype distribution based on the classification proposed by Diessel in 1965 (Table 3) shows the predominance of Bright Bands with an average value of 18 % for Vitrain and 62% for Clarain (Fig. 2). The dull band Durain are less common averaging about 16%, Fusain constitute about 4% of the coal (Fig. 3).

Kargali Seam. The easternmost of the Kargali Seam lies in Dhori area. The seam occurs as a composite horizon in the west and splits into two sections viz. Kargali Top and Bottom in the south and east. The seam has a crescent shaped outcrop pattern, The thickness of the Kargali seam is 25.05m and where the seam occurs in two splits, Kargali Top seam varies in thickness from 10.67m to 14.49m and Kargali Bottom seam varies from 10.15m to 11.48m. The parting between the Kargali Top and Bottom seams gradually increases towards south. The coals of Kargali Seam are greyish black to blackish in color. The streak ranges from Dark brown to black. The lustre of the coal is vitreous with medium to high specific gravity. The Hardness ranges from 2.6 to 2.9. Majority of the coal found here are brittle and friable. Lithotype content is variable, Vitrain occurs as well marked narrow bands and splits readily in the fingers in small cube like segments. The limiting layers between vitrain and clarain or Durain is sharply marked. The clarian of Karo Special Seam III has silky lusture, Vitrain and clarain are together the brights or bright Bands of the seam. Durain content of the seam show variable thickness with irregular and rough surface which is characteristically lustreless, as distinct from the glassy surface of vitrain. The lithotype fusain occurs as patches and wedges. Fusain of the seam is soft often soils fingers and present in only minor amount. Lithotype distribution based on the classification proposed by Diessel (1965)[11] (Table 3), shows the predominance of Bright Bands with an average value of 43 % for Vitrain and 36% for Clarain (Fig. 2). The dull band Durain are less common averaging about 18%, Fusain constitute about 3% of the coal (Fig. 3).

Depositional Environment Considerations

Geological models of peat accumulation have been developed by the interpretation of compositional characteristics of lithotypes,[19] the brighter lithotype (Vitrain and Clarain) suggest peat accumulation in a wet forest moor environment, banded dull type lithotype (Durain and Fusain) suggest open moor conditions during peat accumulation.

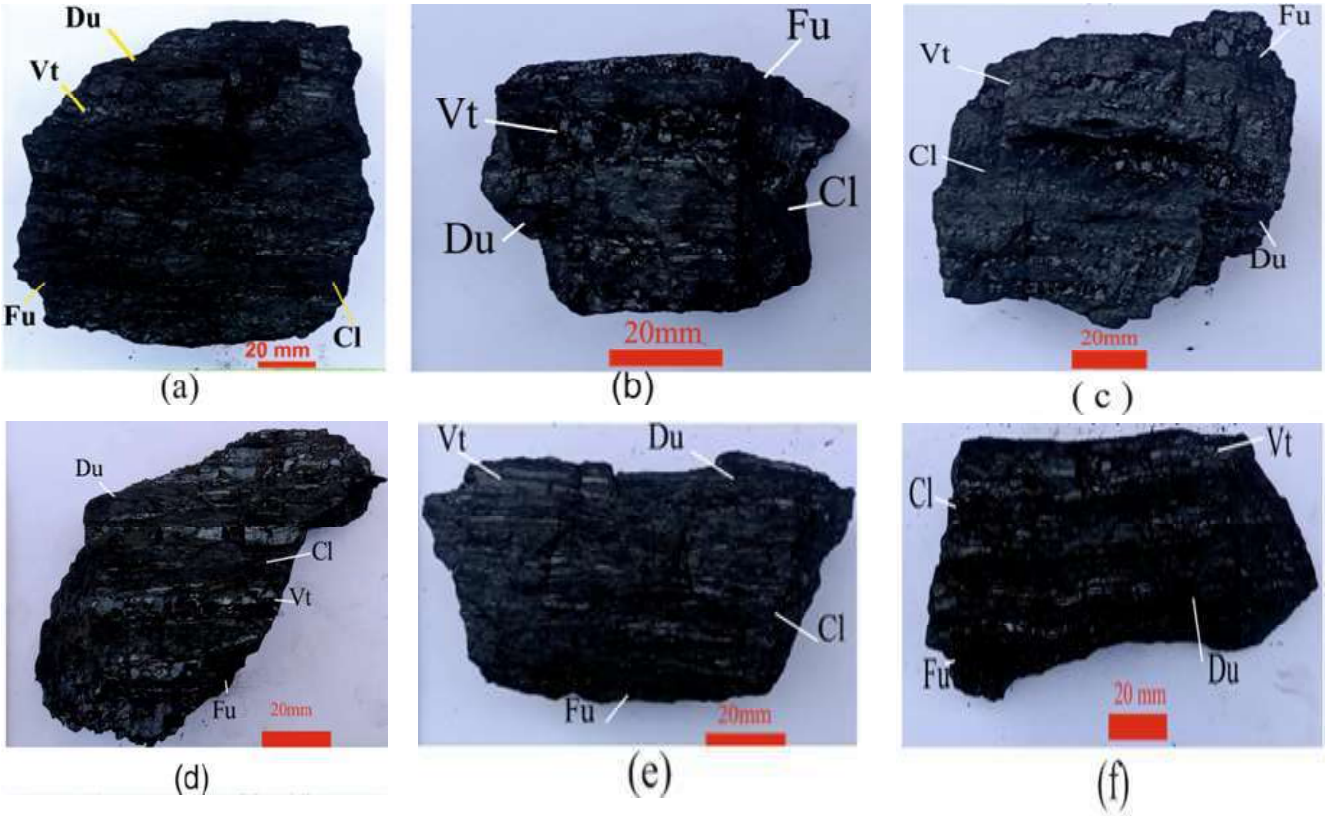

Fig. 2- Photomacrographs of Lithotypes showing the abundance of Vitrain (Vt), Clarain (Cl), Durain (Du) and Fusain (Fu) from the coals a). Karo Special Seam III, b). Karo Group Seam VI, c). Karo Group Seam VII, d). Kargali Seam, e). Bermo Seam and f). Karo Group Seam VIII under visible light.

Table 3- Lithotype distribution in coal seams of Dhori Area.

Mine	Seam	Seam Thickness (m)	Lithotype (%)			
			Vitrain (Vt)	Clarain (Cl)	Durain (Du)	Fusain (Fu)
Amlo (OCM)	Kargali	11.37-14.49	43	36	18	3
	Bermo	9.7-11.58	18	62	16	4
Kalyani (OCM)	Karo Seam VIII	15.21-21.59	29	53	14	4
	Karo Seam VII	1.62-14.73	36	49	12	3
	Karo Seam VI	7.95-3.27	38	47	11	4
Dhori Khas	Karo Special Seam III	0.75 to 3.74	45	40	13	2

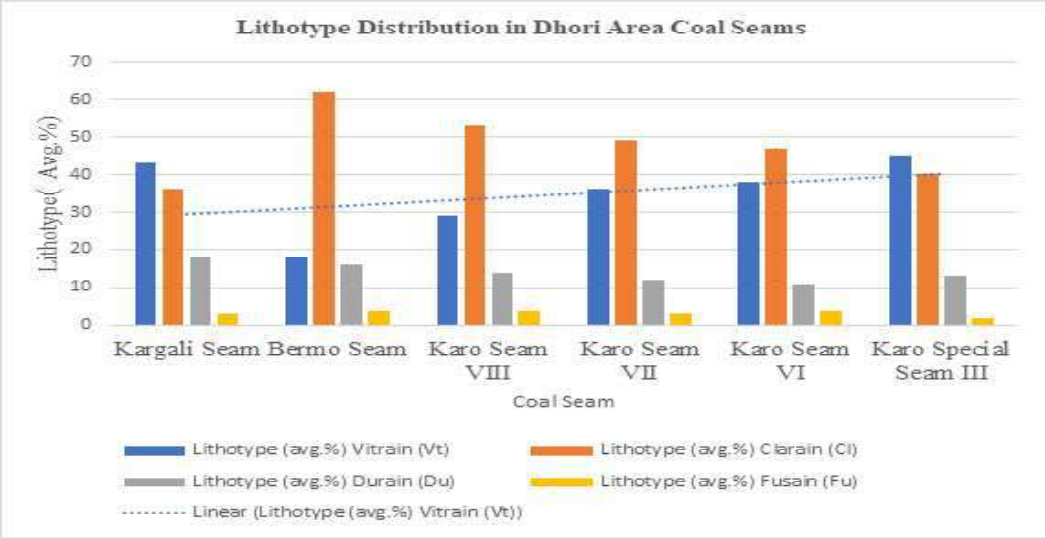

Fig. 3- Diagram showing relation between Lithotype % with Vitrain, Clarain, Durain and Fusain content

CONCLUSIONS

The study indicate that Lithotype distribution is variable in the seams of Dhori Area. Karo Special Seam III Thickness range from 0.75 to 3.74m (mean 2.244m). Karo Group Seam VI, VII, VIII ranges from 7.95-3.27 (mean 5.61m), 1.62-14.73 (mean 8.175m) and 15.21-21.59 (mean 18.4m). Bermo Seam thickness ranges from 9.7-11.58 (mean10.64). Kargali Seam ranges from 11.37-14.49 (mean 12.93). The coal seams of Barakar formation show a distinct improvement in quality from bottom to top. Medium to high proportion of impure coal (coal shale and shaley coal), carbonaceous shale and partings is characteristical for all seams which often reduces the thickness of the coal seams. Coal Lithotypes are variable with banded coal predominant in the Karo Special Seam III Fig 3. Vitrain and Clarain are present is average amount in all the seams. Durain and Fusain content are lesser than the other lithotypes. The present study deals with lithotype profiles of the seams as their Macropetrographic characteristics, and identifies brighter lithotypes as seams of higher quality and dull lithotypes as seams of lower quality. The depositional environment based on the content of Lithotypes (Vitrain, Clarain, Durain and Fusain) suggest that the peat accumulation for the formation of Coal seams of Dhori area were in wet forest moor environment.

ACKNOWLEDGEMENT

The authors are indebted to the editor and the learned reviewers for their kind suggestions and discussions which has helped in upgrading the quality of the paper. The authors would like to express gratitude to the HRD, CCL Dharbhanga House, Ranchi, General Manager, Dhori Area and the staff members for Geological field work and sampling processes. The authors wish to thank Head,

Department of Geology, Ranchi University, Ranchi and my colleagues for supporting the work that went into producing this paper.

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RUJOST, ISSN: 2319-4227, Vol.7, No.1, January, 2022, pp. 141- 144
A Biaanual Peer Reviewed Referred Ranchi University Journal of Science & Technology

Mathematics

Analysis and Application of Queuing Theory to Minimize the Waiting Time of Customer at Bill Paying Counter of Big Bazaar

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Abstract: Waiting lines or queues are a common phenomenon in life, especially in the province of organizations that are for profit making, such as petrol filling stations, supermarkets stores, clinics, hospitals, motor park, manufacturing firms etc. An interesting aspect of queuing process resides in the measures of its systems performance, especially in terms of average service rate, systems utilization and the costs implied for a given capacity level. This paper examines efficient queue management in Big - Bazaar. as one of the case studies in which expected gains from queuing system is to review the efficiency of the models in terms of utilization and waiting length, by increasing the number of queues for customers will not reduce waiting time, when servers are too busy. In others words, we shall to estimate the waiting time and length of queues in this paper. We shall also estimate that at a given time how many servers will be required so that the total waiting time is optimized.

Keywords: Queuing Theory, Bill Paying Counter

INTRODUCTION

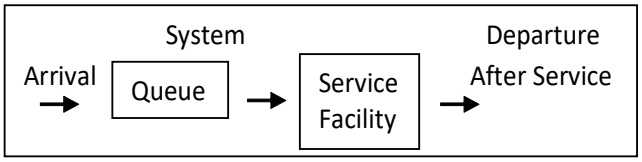
Delays and queuing problems are most common features not only in our daily life situations such as at a bank or postal office at a ticketing office in public transportation or in a traffic jam but also in more technical environments such as in manufacturing, computer networking and telecommunications. They play an essential role for business process reengineering purposes in administrative trace "Queuing models provide the analyst with a powerful tool for designing and evaluating the performance of queuing system."

whenever customers arrive at a service facility, some of them have to wait before they receive the desired service, which means that the customers have to wait for his/her turn, when customers arrive at a service facility with several queues, each with one server live checkout counter, the customers choose a queue of a server according to some mechanism live shortest queue. This paper is the analysis of queuing theory and for empirical study of the sales checkout service unit of a Big-Bazaar.

The main purpose of this paper is to review the application of queuing theory and to evaluate the parameters involved in the service unit for the sales checkout operation in the Big-Bazaar. Therefore, a mathematical model is developed to analyze the performance of the checking out service unit. Two important results need to be known from the data collected in the Big Bazaar, one of them is the 'Service Rate' provided to the customers during the checking out process and the other is the time gaps between the arrival times of each customer per hour. In order to get an overall perspective of the customers quality of service, the questionnaires which indicate the result in percentages are also used for evaluation purposes.

PROPOSED METHODOLOGY AND DISCUSSION

M/M/1 queuing model:



Queuing model means that the arrival and service time are exponentially distributed in poison Process, for the analysis of transaction counter to verify M/M/1 queuing model in which the notation used are specified as:-

λ : The mean customers arrival rate

μ : The mean service rate

ρ : λ/μ = Utilization factor

Probability of zero customers in the queue $P_0 = 1-\rho$

The probability of having n customers in the queue $Pn = P_0\rho^n$

We estimate $E(L_S)$ i.e. average number of customers in the system.

Which Can Be Computed As

Mathematically $E(Ls) = \sum_{n=0}^{\infty} np_n = \sum_{n=0}^{\infty} n.(1-\rho)\rho^n$

$= (1-\rho).\rho \sum_{n=1}^{\infty} n.\rho^{n-1}$

$= \rho(1-\rho).[1+2\rho+3\rho^2+ .....]$

$= \rho(1-\rho).(1-\rho)^{-2}$

Thus, $E(L_S) = \frac{\rho}{1-\rho}$

By Substitute the value we have

$E(L_S) = \frac{\lambda}{\mu-\lambda}$

We Estimate E (Lq) i.e. expected queue length which is average number of customers in the queue or average length of waiting line. May be Computed as

Mathematically

$E(Lq) = \sum_{n=0}^{\infty}(n-1)p_n$

[Which means (n-1) units in the queue excluding one in service]

$= \sum_{n=1}^{\infty} n\,p_n - \sum_{n=1}^{\infty} p_n$

$= E(Ls) - \left[\sum_{n=0}^{\infty} p_n - p_0\right]$

$= E(Ls) - [1-(1-\rho)]$

$= E(Ls) - 1 + (1-\rho)$

$= \frac{\rho}{1-\rho} - 1 + 1 - \rho$

$= \frac{\rho^2}{1-\rho} = \frac{\lambda^2}{\mu(\mu-\lambda)}$, $[\rho = \frac{\lambda}{\mu}]$

*** We further Compute expected waiting time in the queue expressed Mathematically as**

$E(Wq) = \frac{L_q}{\lambda} = \frac{\rho}{\mu-\lambda} = \frac{\lambda}{\mu(\mu-\lambda)}$

$E(Ws) = \frac{L_s}{\lambda} = \frac{1}{\mu-\lambda}$

Model (M/M/S): (∞/FCFS)

This is the queuing model with Poisson arrival, Poisson service, if S>1 more service channels, we consider service rate at each channel identically, (l_1) the service discipline is first come first serve. In this model the length of the waiting line will depend on the number of occupied channels.

If n<s then there will be no customer waiting in the queue as all of them will be served, and so in this case S-n service channels will remain idle, and the rate of service $\mu_n = n\mu$

If n=s then all the service channels will be busy and the rate of service $\mu_n = s\mu$

If n>s then all the service channels will be busy, while n-s customers will be waiting in queue and the rate of service $\mu_n = s\mu$

The mean customers arrival rate = λ

The mean service rate = μ

utilization factor $\rho = \lambda/s\mu$

Probability of zero customers in the system

$P_0 = \frac{1}{\left[\sum_{n=0}^{s-1}\frac{(s\rho)^n}{n!} + \frac{(s\rho)^s}{s!(1-\rho)}\right]}$

Probability of having n customers in the system

$P_n = \frac{(n\rho)^n}{n!}P_0$, when $1 \le n \le s$

$P_n = \frac{s^s.\rho^n}{s!}.P_0$, $n \ge s$

Average number of customers in the queue E(Lq)

if n>s, then n-s customers will be waiting in queue

Therefore,

$E(Lq) = \sum_{n=s}^{\infty}(n-s)Pn$

$\sum_{n=s}^{\infty}(n-s)\frac{s^s.\rho^n}{s!}P_0$

$\frac{(s\rho)^n}{s!}P_0\sum_{n=s}^{\infty}(n-s)\rho^{n-S}$

$= Ps[0+\rho+2\rho^2+3\rho^3+ .....]$ Since $Ps = \frac{(s\rho)^s}{s!}.p_0$

$= \rho\,Ps(1-\rho)^{-2}$

Therefore $E(Lq) = \frac{\rho Ps}{(1-\rho)^2}$

Average number of customers in the system $E(L_S)$

We have,

$E(W_S) = E(W_2) + \frac{1}{\mu}$

$\lambda E(W_S) = \lambda E(Wq) + \frac{\lambda}{\mu}$

Or $E(Ls) = E(Lq) + \frac{\lambda}{\mu}$

Or $E(Ls) = \frac{\rho Ps}{(1-\rho)^2} + S\rho$

To find $E(W_S)$

We have $E(W_S) = \frac{1}{\lambda} E(L_S)$

$= \frac{\rho Ps}{\lambda(1-\rho)^2} + \frac{S\rho}{\lambda}$

To find $E(Wq) = \frac{1}{\lambda} E(Lq)$

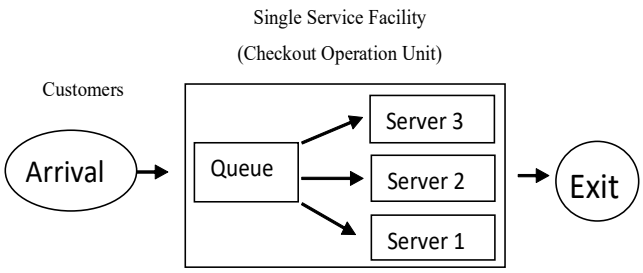
$= \frac{\rho Ps}{\lambda(1-\rho)^2}$

ASSUMPTIONS

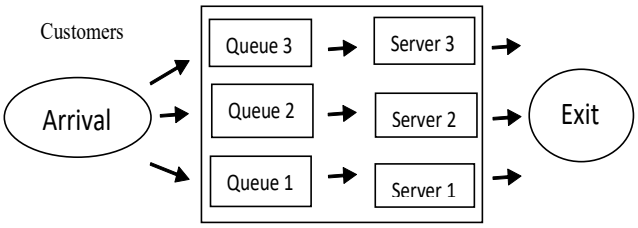
Data for this analysis be collected from Big-Bazaar. The method employed during data collection were direct observation, personal interview and questionnaire administering by the researcher. Data were collected for 1 weak. The following assumption were made for queue system which is to be analyzed for a week.

1. Arrivals follow a Poisson probability distribution at an average rate of λ customers per unit of time.
2. The queue discipline is First - come, First - served (FCFS) basis and is no priority classification for any specific arrival.
3. Service times are distributed exponentially, with an average of μ customers per unit of time.
4. There is no limit to the number of the queue but for this analysis, we consider them queue at time
5. The service providers are working at their full capacity.
6. The average arrival rate is greater than the average service rate.
7. Servers line represent employees of the Big-Bazaar.

Comparison of Single Queue - Multi Server Verses Multi Queue Multi Server: -



1. Queuing model with single- queue and multiple parallel servers.



2. Queuing Model with Multiple Queue and Multiple Server

Figure 1 describes model with single Queue and Multiple Parallel Servers. Which can be described with M/M/S queuing system.

Figure 2 describes Model with Multiple Queue and Multiple Parallel Servers Which can be described with M/M/1 Queuing Systems.

Queuing system

Since we have S number of checkout stands in the Big-Bazaar, and the customers arrival rate is λ and the service rate of each checkout is μ. Thus, we estimated the value of Lq, Ls, Ws and Wq in both cases and compare all the parameter for each case after consideration is applicable will be.

If there is only one queue then the under M/M/S system. We will estimate Lq, Ls, Wq and Ws. If there are S queues in the system the queuing system considered as S isolated M/M/1 queuing system, in this case we will use single server queuing model to estimate Lq, Ls, Wq and Ws and the customers arrival rate becomes λ/s for M/M/I model.

The following data is collected from Big-Bazaar, Ranchi, which has 3 checkout counters. The data has been divided into 3. slots morning, afternoon and evening.

ANALYSIS OF DATA

Present Model: -

Here, the customers join the queue by themselves. The data were collected for s=3 checkout counters. So, there are three queues and the customer select their own queue. In this case customers in any queue may switch to shorter queue.

The following calculation are made using the performance measures of M/M/1 queuing model having 3 isolated checkout counters.

Counter 1.

Time Slot	Arrival Rate λ	Service Rate μ	Ls	Lq	Ws	Wq
9am to 1pm	15	20	3	2.25	12 min	9 min
1pm to 5pm	10	20	1	0.5	6 min	3 min
5pm to 9pm	16.67	20	5	4.1667	18 min	15 min

Counter 2.

Time Slot	Arrival Rate λ	Service Rate μ	Ls	Lq	Ws	Wq
9am to 1pm	13.33	20	2	1.33	9 min	6 min
1pm to 5pm	6.67	20	0.5	0.1667	4.5 min	1.5 min
5pm to 9pm	16.67	20	5	4.1667	18 min	15 min

Counter 3.

Time Slot	Arrival Rate λ	Service Rate μ	Ls	Lq	Ws	Wq
9am to 1pm	11.67	20	1.40	0.8	7.20 min	5.76 min
1pm to 5pm	3.33	20	0.2	0.03	3.60 min	0.10 min
5pm to 9pm	16.66	20	3.5	4.14	18 min	15 min

Proposed Model:

There are 3 checkout counters but only one queue and a display shown at the over head of customer which provided indication to the next customer for selection as soon as one counter gets vacant, displaying machine have announcement or beep sound to allow next customer to join the queue.

The calculation is made using the performance measure of M/M/S queuing model having s=3 checkout counters.

Time Slot	Arrival Rate λ	Service Rate μ	Ls	Lq	Ws	Wq
9am to 1pm	40	20	2.8889	0.8889	4.33 min	1.33 min
1pm to 5pm	20	20	1.0455	0.0455	3.14 min	0.14 min
5pm to 9pm	50	20	6.0112	3.5112	7.21 min	4.21 min

CONCLUSION

We observe that Single Queue Multi Server is better in comparison to Multi Queue Multi Server. The waiting time of customers in the queue is reduced almost 3 times in comparison to the previous model.

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RUJOST, ISSN: 2319-4227, Vol.7, No.1, January, 2022, pp. 145-148

A Biaanual Peer Reviewed Referred Ranchi University Journal of Science & Technology

Mathematics

Trade Off between order quantity and credit period for seasonal commodity under price break

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Abstract: To attract new buyers and to avoid lasting price competition, a seller frequently offers its buyers a permissible delay in payment. However, the policy of granting a permissible delay in payment is associated with an additional dimension of default risk to the seller. The default risk in incurring in sales revenue is incorporated in objective of profit maximization. In case of seasonal or perishable items, the demand is considered to be generally high in the beginning of the season and the inventory level is also not high, so a seller may not offer a discount initially, but at the end of the season due to high production level and high inventory level, the seller prefer to provide discount to maximise profit and minimise inventory carrying cost. The necessary and sufficient conditions to obtain the seller's optimal decision about setting the permissible credit period, purchase quantity under price break is discussed in this paper.

Keywords: Inventory, Trade-credit, Risk optimisation

INTRODUCTION

In competitive business environment, it is important to increase or retain the customer credential, for that sufficient inventory item is to be required. When the inventory is inadequate, the demand of the customer is unfulfilled, which may result in the loss of goodwill and henceforth loss of the business.

On the other hand, if the demand rate increases, then, we have to look into the inadequate inventory, which shouldn't be kept idle in warehouse. In that respect, some discount may be offered by the sellers to encourage large purchase orders.

For seasonal items, the demand of the product is generally high in the beginning of the season, so the seller may not offer any discount initially, whereas, at the end of the season, the seller would prefer to provide the discount keeping in mind the production or the items procured in inventory. Our objective from seller's point of view is to maximise profit within the purview of the offering discount for perishable items and minimise inventory carrying cost. Sellers may offer even higher discount for perishable items depending upon the seasonal variation.

Every buyer has a tendency to prefer more purchase or bulk purchase on discounted price. The discount offered by sellers may be in the form of quantity or in terms of price.

Many researchers have suggested different inventory models like "An inventory model with price break: Fuzzy Approach" by Syed and Aziz (2010)[1], "A Simple procedure for price break models" by Goyal (2007)[2], "An Intuitionistic Approach for Price Breaks in EOQ from Buyer's Perspective" by Kaur and Deb (2015)[3], "Formulation of Optimal Economic Order Quantity under Different Inventory Models" by Mallick (2013)[4], "Analysis of An Inventory Model For Optimum Ordering Policy And Economic Purchasing Strategy" by Umamaheswari *et al.* (2015)[5], "An Inventory Model with Finite Replenishment Rate, Trade Credit Policy and Price-Discount Offer" by Sarkar *et al.* (2013)[6].

In this paper, we discuss inventory problems incorporating price breaks for purchase of items of different lot size.

ASSUMPTIONS

The following assumptions are used:

i. The seller deals with seasonal items.
ii. No discount is given in the beginning of the season.
iii. Higher discount may be offered at the end of the season.
iv. Shortages are not allowed.
v. Lead time is negligible.
vi. Demand rate is considered to be the function of credit period and time involving price break i.e.

$R(M,t) = a_j (1 + b_j t) M^{\beta}$,

where $a_j > 0$

$0 \le b_j < 1$

which is rate of change of demand with respect to time for j^{th} price break where $\beta > 0$ is constant.
vii. The rate of risk for specified credit period M is considered as $F(M) = 1 - M^{-\gamma}$, $\gamma > 0$ is constant.

NOTATIONS

The following notations are used in analysing the problem:

P_j = Price of an item when quantity purchased Q_j lies in between b_{j-1} to b_j, where b_j is the j^{th} price break
R_j = Demand rate for the purchased quantity Q_j
C_j = set up cost per order for j^{th} price break
$I_j(t)$= Inventory level at any instant t, where $0 < t < T$ for jth price break
M = Credit period offered which is a decision variable
T = Cycle time, a decision variable
$\pi(M,t)$= Seller's profit per unit time
SR = Seller's revenue after risk
A = Ordering cost per order

MATHEMATICAL ANALYSIS

Rate of change of inventory

$\frac{d}{dt} I_j = a_j (1 + b_j t) M^{\beta}$ with initial conditions $I_j(T) = 0$

$I_j(t) = a_j M^{\beta} [(T - t) + \frac{bj}{2} (T^2 - t^2)]$

& $I_j(T) = 0$, i.e. Final inventory = 0

Initial inventory $Q_j = I_j(0)$

$= a_j M^{\beta} [T + \frac{bj}{2} T^2]$

$\frac{\partial}{\partial T} Q_j = a_j M^{\beta} (1 + b_j T) = R_j(M, T)$……(1)

Rate of change of inventory

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Initial inventory $Q_j = I_j(0)$

$= a_j M^{\beta} [T + \frac{bj}{2} T^2]$

$\frac{\partial}{\partial T} Q_j = a_j M^{\beta} (1 + b_j T) = R_j(M, T)$……(1)

$C_j(Q_j)$ = Total expected cost for one year for jth price break

$= \frac{Cj\ Rj}{Qj} + \frac{1}{2} I_j P_j Q_j + R_j P_j + \frac{1}{2} C_j I_j$

Purchase cost = $Q_j C_j(Q_j)$

$= Q_j [\frac{Cj\ Rj}{Qj} + \frac{1}{2} I_j P_j Q_j + R_j P_j + \frac{1}{2} C_j I_j]$

$= \{C_j + P_j a_j M^{\beta} (T + \frac{bj}{2} T^2)\} \{a_j M^{\beta} (1 + b_j T)\} + \frac{1}{2} I_j P_j Q_j^2 + \frac{1}{2} C_j I_j Q_j$

Revenue after risk in the J^{th} price break

$SR = P_j \int_0^T R_j(M,t)dt . \{1 - F(M)\}$

$= a_j P_j M^{\beta-\gamma} T [1 + \frac{1}{2} b_j T]$

Holding cost for jth price break

$= h_j \int_0^T I_j(t)dt$

$= h_j a_j M^{\beta} \frac{T2}{6} (3 + 2 b_j T)$

Holding cost depends on the number of units in the inventory. It depends on the purchase order when the purchase order is more, the holding cost is less and when the purchase order is less, the holding cost is more.

Seller's profit per unit time.

$= \pi(M, T)$

$= \frac{1}{T} [$ Revenue after risk – Purchase cost- ordering cost- Holding cost$]$

$= \frac{1}{T} [a_j P_j M^{\beta-\gamma} T(1 + \frac{1}{2} b_j T) - \{C_j + P_j a_j M^{\beta} T (1 + \frac{bj}{2} T)\} \{a_j M^{\beta} (1 + b_j tT)\}$

$- \frac{1}{2} I_j P_j Q_j^2 - \frac{1}{2} C_j I_j Q_j - A - h_j a_j M^{\beta} \frac{T2}{6} (3 + 2 b_j T)]$

As $I_j(T) = 0$, therefore,

$\pi(M, T) = a_j P_j M^{\beta-\gamma} (1 + \frac{1}{2} b_j T) - \{\frac{Cj}{T} + P_j a_j M^{\beta} (1 + \frac{bj}{2} T)\} \{a_j M^{\beta} (1 + b_j T)\}$

$- 0 - 0 - \frac{A}{T} - h_j a_j M^{\beta} \frac{T}{6} (3 + 2 b_j T)$

The necessary condition for credit period and cycle time for maintaining annual profit per unit time, we have,

$\frac{\partial}{\partial M} \pi(M, T) = 0$

$\frac{\partial}{\partial T} \pi(M, T) = 0$

Now, $\frac{\partial}{\partial M} \pi(M, T) = 0$

$a_j P_j \left(1 + \frac{1}{2} b_j T\right)(\beta-\gamma) M^{\beta-\gamma-1} - \frac{a_j C_j}{T}(1 + b_j T)\beta M^{\beta-1} - P_j a_j^2 (1 + b_j T)(1 + \frac{bj}{2} T).$
$2^{\beta} M^{2\beta-1}\} + 0 - h_j a_j \frac{T}{6}(3+2 b_j T)\beta M^{\beta-1} = 0$

$a_j M^{\beta} \frac{1}{6M} [\, 6 P_j \left(1 + \frac{1}{2} b_j T\right)(\beta-\gamma) M^{-\gamma} - 6 \frac{Cj}{T}(1 + b_j T)\beta - 6 P_j a_j (2+ 3b_j T+ b_j T^2)\beta M^{\beta} - h_j T (3+2 b_j T)\beta] = 0$

$As \frac{\partial}{\partial T} \pi (M, T) = 0$, therefore,

$\frac{1}{2} a_j P_j M^{\beta-\gamma} b_j - a_j C_j M^{\beta}(-\frac{1}{T2}) - P_j a_j^2 M^{2\beta}(1 + \frac{bj}{2} T)(1 + b_j T).\frac{b_j}{2} + \frac{A}{T2} - \frac{1}{6} h_j a_j M^{\beta}(3+4b_j T) = 0$

$a_j M^{\beta} \frac{1}{6T2} [\, 3 P_j b_j T^2 M^{-\gamma} + 6 C_j - 3 P_j b_j C (3+2 b_j T) T^2 + \frac{6A}{a_j M^{\beta}} - h_j (3+4b_j T) T^2] = 0$

NUMERICAL EXAMPLE

Application of the model analysis can be validated with an example of a grocery shop dealing with a seasonal product where ordering cost is Rs. 180 per order, unit price of the product is Rs. 18.50 and holding cost is 10% per unit price. For the expected annual sell of 8000 units, the necessary and sufficient condition for maximizing annual profit per unit time for different credit period and cycle time is illustrated as follows:

Here, we have

A = 180 per order

Cj = Rs. 18.50 per unit

h_j = 10 % of Rs. 18.50 i.e. Rs. 1.85

a_j = 8000 units

β = 4,

γ = 2

b_j = 0.1

P_j = Rs. 18.50 per unit

Now, for maximizing seller's annual profit per unit time and the credit period M for different cycle times T may be calculated as follow:

a) For cycle time T= 0.5, we have,
Credit period M = 0.1722
Seller's purchase quantity, Qj= 3.6068
Seller's profit per unit time, π (M, T) = 2876.48

b) For cycle time T= 1.0, we have,
Credit period M = 0.1727
Seller's purchase quantity, Qj= 7.4746
Seller's profit per unit time, π (M, T) = 3220.90

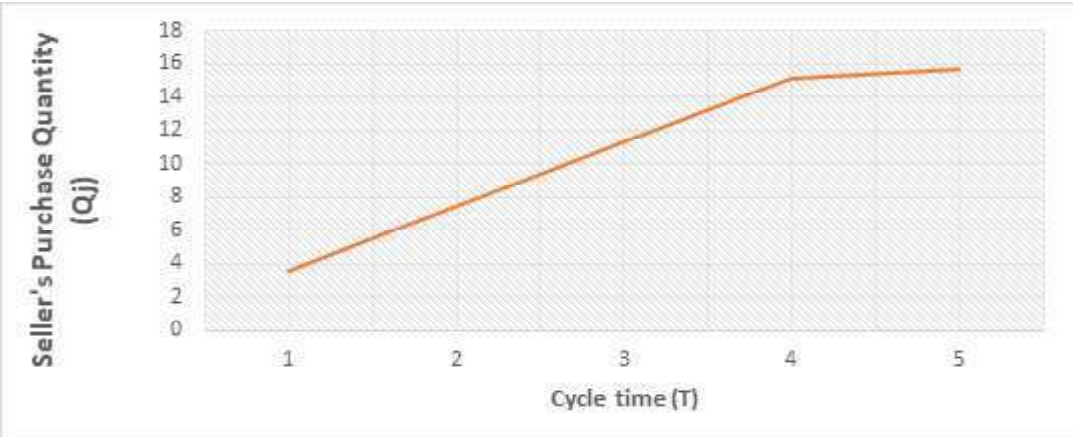
c) For cycle time T= 1.5, we have,
Credit period M = 0.1720
Seller's purchase quantity, Qj= 11.2872
Seller's profit per unit time, π (M, T) = 3355.64

d) For cycle time T= 2.0, we have,
Credit period M = 0.1711
Seller's purchase quantity, Qj= 15.0682
Seller's profit per unit time, π (M, T) = 3437.57

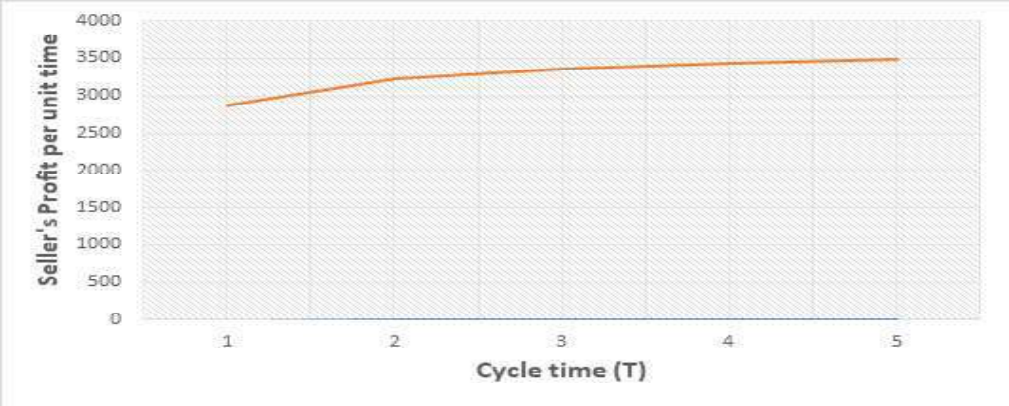
e) For cycle time T= 2.5, we have
Credit period M = 0.1707
Seller's purchase quantity, Qj= 15.70
Seller's profit per unit time, π (M, T) = 3498.13

GRAPHICAL REPRESENTATION

The change in seller's purchase quantity with different time cycle can be represented as follow:



The change in seller's profit per unit time with different time cycle can be represented as follow:



CONCLUSION

On the basis of illustrated examples, we observe that with the increase in cycle time, the Seller's purchase quantity increases sharply and then with the lapses of time gradually shows an asymptotic trend, which co-relate the conceptual meaning from seller's point of view. Similarly, with the increase in time cycle, Seller's profit per unit time also increases with moderate rate at initial cycle but with further enhancement in time cycle it also shows static behaviour and finally stabilizes with time cycle.

Thus, from a seller's point of view, an optimal cycle may be decided at a point where the rate of increase in seller's profit becomes asymptotic.

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RUJOST, ISSN: 2319-4227, Vol.7, No.1, January, 2022, pp. 149-156

A Biaanual Peer Reviewed Referred Ranchi University Journal of Science & Technology

Mathematics

Application of Markov Chain to Compare Trend of Sun Pharma Shares - Before & During COVID - 19 Period

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Abstract: This paper attempts to apply Markov chain model to forecast the behaviour of Sun Pharma share indexed in Bombay Stock exchange. Bombay stock exchange is a trading platform of shares in India. The stock market is attractive platform for investment, it is considered that both foreign and local investors will seize the opportunity and invest in the stock market. An understanding of the stock market trend in terms of predicting price movements is important for investment decisions. Markov Chain model has been widely applied in predicting stock market trend. In many applications, it has been applied in predicting stock index for a group of stock but little has been done for a single stock. The overall objective of this study was to apply Markov Chain to model and forecast trend of Sun Pharma shares Pre COVID and Post COVID. The study was conducted through 4 years historical data of Sun Pharma shares daily closing price. The outbreak of the Covid-19 pandemic started from 2020 in India, in this study there is a comparison of Pre COVID i.e., 1st January, 2018 to 31st December, 2019 and COVID-19 period i.e., 1st January, 2020 to 31st December, 2021 of Sun Pharma share prices. Secondary quantitative data on the daily closing share prices was obtained from Bombay stock exchange website over a period covering 1st January, 2018 to 31st December, 2019 forming a 491 days trading data panel and period covering 1st January 2020 to 31st December 2021 forming a 500 days trading data panel. A Markov chain model was determined based on probability transition matrix and initial state vector. MATLAB software used to determine initial state vector, transition matrix and trend values using moving average. In the long run, irrespective of the current state of share price, the model predicted that the Sun Pharma share prices would increase, decrease and remains constant with a probability of 0.4755, 0.5224 & 0.0020 respectively but to due to sudden outbreak of COVID-19 predicted value shows slight difference in probabilities of increase, decrease and remains constant which is equal to 0.5230, 0.4749 and 0.0020.

Keywords: Markov Chain, Transition Matrix, Initial State Vector, MATLAB, Moving Average

INTRODUCTION

Sun Pharmaceutical Industries Ltd. (Sun Pharma) is the fourth largest specialty generic pharmaceutical company in the world with global revenues of over US$ 4.5 billion. It is supported by more than 40 manufacturing facilities. Industry provides high-quality, affordable medicines. It is trusted by healthcare professionals and patients, to more than 100 countries across the globe. From humble beginnings in 1983, Sun Pharma has grown to become one of the largest generic pharmaceutical companies worldwide in North America, Asia, Europe, Africa, South America, Oceania.

The sudden outbreak of the COVID-19 pandemic is an unmatched shock to the Indian economy. The economy was already in an uncertain state before COVID-19 hit. With the continued country-wide lockdown, global economic downturn and associated disruption of demand and supply chains, the economy is likely to face a protracted period of slowdown. But on the other side, there is a slight increase in share prices of pharmaceutical industries. Due to unprecedented environment of COVID-19, products manufactured by pharmaceutical industries are on great demand. In this paper we describe the state of the analysis of Sun Pharma share prices in the Pre COVID-19 period with COVID-19 periods and assess the potential impact of the pandemic on pharmaceutical industries.

Odhiambo *et al.* (2020)[1] seeks to model the effect of COVID-19 pandemic on Kenyan Gross Domestic Product (GDP) contributors using a Discrete-time Markov Chain Analysis and seeks to find the ultimate effect of the COVID-19 to the top five key sectors of the Kenyan economy that contributes massively to GDP growth by looking at the proportion of the contributors at steady state. Maneejuk *et al.* (2020)[2] analyze the time-varying correlation between COVID-19 shocks (positive and negative) and energy markets (natural gas, gasoil, heating oil, coal, and crude oil) in the time-varying environment, their study adds to the literature by implementing the Markov-switching dynamic copula with Student-t distribution to explore the unexpected COVID-19 pandemic shock effects on energy markets. Leo *et al.* (2021)[3] identifies the effects of India's Gross Domestic Product contributors and economic compensations due to the Covid-19 pandemic using the long-term distribution of a Markov Chain Analysis and seeks to find the effects of the five sectors of the Indian economy due to the COVID-19. Otieno *et al.* (2015)[4] applied Markov Chain to model and forecast trend of Safaricom shares trading in Nairobi Securities Exchange, Kenya.

Transition Matrix

The probability of moving from one state to another state or remaining in the same state during a single time period is called the transition probability.

Mathematically,

$$P_{ij} = \mathrm{P}\left\{Next\ state\ S_j\ at\ t = 1 \middle| Initial\ state\ S_i\ at\ t = 0\right\}$$

Here, i = initial state & j = next state

With the help of transition probability matrix (TPM) we predict the movement of system from one state to the next state.

$$\mathbf{P} = \begin{array}{l}\textbf{Initial}\\ \textbf{state (i)}\\ \textbf{[n = 0]}\end{array} \begin{array}{c} \\ s_1 \\ s_2 \\ s_3 \end{array} \begin{array}{c} \begin{array}{ccc} s_1 & s_2 & s_3 \end{array} \\ \begin{bmatrix} P_{11} & P_{12} & P_{13} \\ P_{21} & P_{22} & P_{23} \\ P_{31} & P_{32} & P_{33} \end{bmatrix} \end{array}$$

$P_{11} = \mathrm{P}\{in\ state\ S_1\ in\ next\ state\ \ at\ t = 1 | in\ state\ S_1\ in\ Initial\ state\ \ at\ t = 0\}$

Or,

$$P_{11} = P[S_1\ at\ time\ t = 1 | S_1\ at\ time\ t = 0]$$
$$P_{12} = P[S_2\ at\ time\ t = 1 | S_1\ at\ time\ t = 0]$$
$$P_{21} = P[S_1\ at\ time\ t = 1 | S_2\ at\ time\ t = 0]$$

[One step transition Probability]

$P_{11}^{(2)} = P[S_1\ at\ time\ t = 2 | S_1\ at\ time\ t = 0]$ [2 – *step transition matrix*]

$$\mathbf{P^{(2)}} = \begin{array}{l}\\ \textbf{state}\\ \textbf{i}\end{array} \begin{array}{c} \textbf{state j} \\ \begin{array}{ccc} s_1 & s_2 & s_3 \end{array} \\ \begin{bmatrix} P_{11}^{(2)} & P_{12}^{(2)} & P_{13}^{(2)} \\ P_{21}^{(2)} & P_{22}^{(2)} & P_{23}^{(2)} \\ P_{31}^{(2)} & P_{32}^{(2)} & P_{33}^{(2)} \end{bmatrix} \end{array} \begin{array}{l} s_1 \\ s_2 \\ s_3 \end{array}$$

$P_{11}^{(n)} = P[S_1\ at\ time\ t = n | S_1\ at\ time\ t = 0]$ [n – *step transition matrix*]

$$\mathbf{P^{(n)}} = \begin{array}{l}\\ \textbf{state}\\ \textbf{i}\end{array} \begin{array}{c} \textbf{state j} \\ \begin{array}{ccc} s_1 & s_2 & s_3 \end{array} \\ \begin{bmatrix} P_{11}^{(n)} & P_{12}^{(n)} & P_{13}^{(n)} \\ P_{21}^{(n)} & P_{22}^{(n)} & P_{23}^{(n)} \\ P_{31}^{(n)} & P_{32}^{(n)} & P_{33}^{(n)} \end{bmatrix} \end{array} \begin{array}{l} s_1 \\ s_2 \\ s_3 \end{array}$$

Assumptions: -

1) Row Sum = 1
2) Each element of TPM is probability and non – negative ($0 \le P_{ij} \le 1$)
3) In a square matrix, Row shows – Initial state & Column shows – Alternate state in next move or state

$$\mathbf{P} = \begin{array}{l}\\ \textbf{state}\\ \textbf{i}\end{array} \begin{array}{c} \textbf{state j} \\ \begin{array}{ccc} s_1 & s_2 & s_3 \end{array} \\ \begin{bmatrix} P_{11} & P_{12} & P_{13} \\ P_{21} & P_{22} & P_{23} \\ P_{31} & P_{32} & P_{33} \end{bmatrix} \end{array} \begin{array}{l} s_1 \\ s_2 \\ s_3 \end{array}$$

Retention (diagonal: P_{11}, P_{22}, P_{33}); Retention & Gain; Retention& Loss

Consider a Markov chain having state space S = {0, 1…} and one step transition probabilities p_{jk} for j, k $\in S$. The Chapman – Kolmogorov equations are given by

$$p_{jk}^{(m+n)} = \sum_{k=0}^{\infty} p_{jt}^{(m)} p_{tk}^{(n)} \quad for\ all\ m, n \ge 0\ and\ all\ i, j = 0, 1, \ldots\ldots$$

As we know that In one step transition probabilities, the probability of X_n is associated with X_{n+1} i.e, the probability of the outcome at the nth step given the outcome at the previous step; p_{jk} gives the probability of unit – step transition from the state j at a trial to the state k at the next following trail. The m – step transition probability matrix is denoted by

$$p_{jk}^{(m)} = \Pr\{\ \{X_{n+m} = k | X_n = j\}$$

$p_{jk}^{(m)}$gives the probability that from the state j at nth trail, the state k is reached at (m+n) th trail in m steps, i.e. the probability of transition from the state j to the state k in exactly m steps. For simplicity the one step transition matrix $p_{jk}^{(1)}$ are denoted by p_{jk}. *Assume*

$$p_{jk}^{(2)} = \Pr\{ \{X_{n+2} = k | X_n = j\}$$

The state k can be reached from the state j in two steps through some intermediate state x. Let us assume a fixed value r, we get

$$Pr\{X_{n+2} = k, X_{n+1} = r \,|X_n = j\} = \Pr\{ \{X_{n+2} = k \,|X_{n+1} = r, X_n = j\} Pr\{X_{n+1} = r|X_n = j\}$$

$$= p_{rk}^{(1)} p_{jr}^{(1)} = p_{jr}\, p_{rk}$$

These intermediate states r can assume values
r = 1,2,……..r

$$p_{jk}^{(2)} = \Pr \{X_{n+2} = k|X_n = j\} = Pr\{X_{n+2} = k, X_{n+1} = r \,|X_n = j\} = \sum_r p_{jr}\, p_{rk}$$

By Mathematical Induction, we have

$$p_{jk}^{(m+1)} = \Pr \{X_{n+m+1} = k|X_n = j\}$$

$$= \sum_r Pr\{X_{n+m+1} = k \,|X_{n+m} = r\} Pr\{X_{n+m} = r|X_n = j\} = \sum_r p_{rk} p_{jr}^{(m)}$$

Similarly, we get $$p_{jk}^{(m+1)} = \sum_r p_{jr}\, p_{rk}^{(m)}$$

Therefore, we can conclude that

$$p_{jk}^{(m+n)} = \sum_{k=0}^{\infty} p_{jr}^{(m)} p_{rk}^{(n)} = \sum_{k=0}^{\infty} p_{rk}^{(m)} p_{jr}^{(n)}$$

This equation is special case of chapman – Kolmogorov equation, which is satisfied by the transition probabilities of a Markov chain.[5]

Thus, we get $p_{jk}^{(m+n)} \geq p_{jr}^{(m)} p_{rk}^{(n)}$ for any r.

Chains Classification

If C is a set of states such that no state outside C can be reached from any state in C, then C is said to be closed. If C is closed and $j \in C \; while \; k \notin C, then \; p_{jk}^{(n)} = 0$ for all n i.e. C is closed if $\sum_{j \in C} p_{ij} = 1 \; for \; every \; i \in C.$ Then the submatrix $P_1 = (p_{ij})$, $i, j \in C,$ is also stochastic and P can be expressed in the canonical form as: $P = \begin{bmatrix} P_1 & 0 \\ R_1 & Q \end{bmatrix}$

A closed set may contain one or more states. If a closed set contains only one state j then state j is said to be absorbing; j is absorbing iff $p_{jj} = 1, p_{jk} = 0, k \neq j.$ In below example states 0 and 4 are absorbing.[5]

A particle performs a random walk with absorbing barriers, as 0 and 4. Whenever it is at any position t (0 < t < 4), it moves to t + 1 with probability P or to (t - 1) with probability q, p + q = 1. But as soon as it reaches 0 or 4 it remains there itself. Let X_n are the different positions of the particle. $\{X_n\}$ is a Markov chain whose unit – step transition probabilities are given by:

$$\Pr \{X_n = t + 1|X_{n-1} = t\} = p$$

$$\Pr \{X_n = t - 1|X_{n-1} = t\} = q$$

$$\Pr \{X_n = 0|X_{n-1} = 0\} = 1$$

$$\Pr \{X_n = 4|X_{n-1} = 4\} = 1$$

Every finite Markov chain contains at least one closed set. If the chain does not contain any proper closed subset other than the state space, then the chain is called irreducible, the transition probability matrix of irreducible chain is an irreducible matrix. Markov chains which are not irreducible are said to be reducible or non – irreducible.[5]

The irreducible matrices may be subdivided into two classes: Primitive (aperiodic) and imprimitive (cyclic or periodic).

Application of the transition Probability Matrix and corresponding analysis of the model with respect to Sun Pharmaceutical Shares

In this study we have taken 2 year Pre – Covid daily closing Sun Pharma share prices from 1st January 2018 to 31st December 2019 and 2 Year Covid period daily closing share price from 1st January 2020 to 31st December 2021 from Bombay stock exchange. Using MATLAB software, computation of Initial state transition matrix and 5 Days Moving average has been done. Daily closing share prices of Sun Pharma has been taken from website of Bombay Stock exchange.

We constructed a transition probability matrix from the past behaviour of the system and this transition probability matrix in conjunction with the probability values of the present state of the system is used to determine the probabilities of the next state.

The transition matrix involves three states. The states are the chance of closing share value increases, decreases and remain unchanged.

This research is based on historical data from 1st January, 2018 to 31st December, 2019 & 1st January, 2020 to 31st December, 2021 of Sun Pharma shares listed in Bombay stock exchange. The transition state i.e., share price movement pattern which could be increase in price followed by another increase or increase in price followed by decrease or increase in price followed by unchanged etc. was observed from the trading days data collected.

	Increase in Share Price (Inc)	Decrease in Share Price (Dec)	Unchanged in Share Price (Unc)
Increase in Share Price (Inc)	11	12	10
Decrease in Share Price (Dec)	21	22	20
Unchanged in Share Price (Unc)	01	02	00

1 denotes increase (Inc)
2 denotes decrease (Dec)
0 denotes unchanged (Unc)

Pre – Covid

From period 01/01/2018 to 31/12/2019, there are 491 trading days. According to daily closing share price from 01/01/2018 to 31/12/2019, the proportion of increase, decrease and unchanged in share price are 232, 256 and 1 respectively. The proportions (probabilities) for share prices that Increase (Inc) Decreases (Dec), Unchanged (U) were (232/491) = 0.4755; (256/491) = 0.5224 and (1/491) = 0.0020 respectively. This gives rise to initial state vector as: *(*0.4755, 0.5224, 0.0020)

	Increase in Share Price (Inc)	Decrease in Share Price (Dec)	Unchanged in Share Price (Unc)
Increase in Share Price (Inc)	107	124	1
Decrease in Share Price (Dec)	125	131	0
Unchanged in Share Price (Unc)	1	0	0

Initial State Matrix: - $[0.4755 \quad 0.5224 \quad 0.0020]$

Transition Matrix $P = \begin{bmatrix} 0.4612 & 0.5345 & 0.0043 \\ 0.4883 & 0.5117 & 0 \\ 1.0000 & 0 & 0 \end{bmatrix}$

Fig. 1 implies that 0.4612 Sun Pharma share prices that increase will still increase; 0.5345 share prices that increase will decrease and 0.0043 will remain unchanged.

Fig. 2 implies that 0.4883 Sun Pharma share prices that decrease will increase; 0.5117 share prices that decrease will decrease and 0 will remain unchanged.

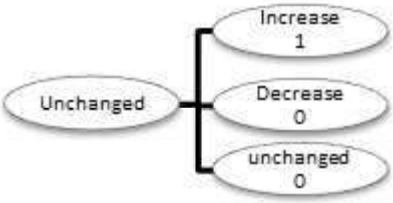


Fig. 3 implies that 1 shares unchanged will increase; 0 share unchanged will decrease and 0 unchanged will unchanged.

The higher order Transition probability matrix of the transition matrix $P^{(n)}$ was calculated to observe the behaviour of the share price using MATLAB Software

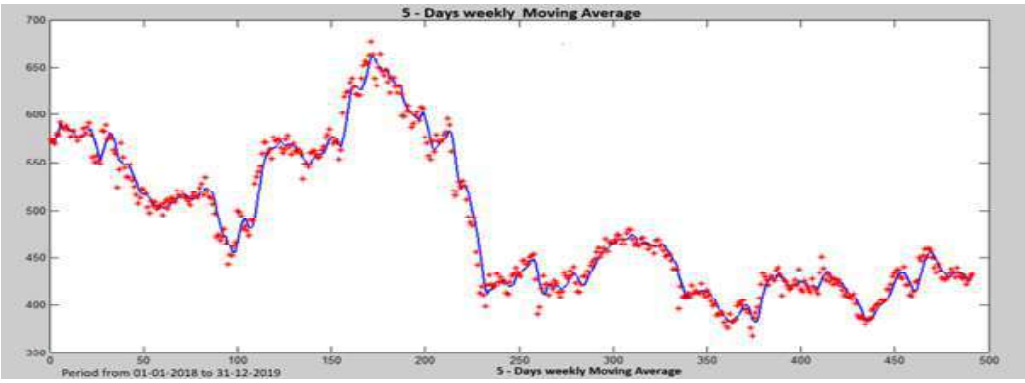
$$P^2 = \begin{bmatrix} 0.4780 & 0.5200 & 0.0020 \\ 0.4751 & 0.5228 & 0.0021 \\ 0.4612 & 0.5345 & 0.0043 \end{bmatrix}$$

$$P^3 = \begin{bmatrix} 0.4764 & 0.5216 & 0.0021 \\ 0.4765 & 0.5215 & 0.0020 \\ 0.4780 & 0.5200 & 0.0020 \end{bmatrix}$$

$$P^6 = \begin{bmatrix} 0.4764 & 0.5215 & 0.0021 \\ 0.4764 & 0.5215 & 0.0021 \\ 0.4764 & 0.5215 & 0.0021 \end{bmatrix}$$

$$P^{15} = \begin{bmatrix} 0.4764 & 0.5215 & 0.0021 \\ 0.4764 & 0.5215 & 0.0021 \\ 0.4764 & 0.5215 & 0.0021 \end{bmatrix}$$

Graphical Representation of trend of Moving Average



The 5 days weekly moving average starts with a bearish trend at a share price of 575. This is followed by a bearish trend up to a Share price of 460 and then engages a bullish trend to a high of 660 share price. The trend then again takes a bearish pattern to as low as 420 then again experience sideway trend to 420. Afterwards trend value ranges from 400 to 450.This shows that on pre – covid period (Data taken from 01/01/2018 to 31/12/2019) demand of pharmaceutical products decreased with a greater extent, as during this period share values rises upto 650 and decreases upto 370.

During Covid

From Period 01/01/2020 to 31/12/2021, there are 500 trading days. According to daily closing share price from 01/01/2020 to 31/12/2021, the proportion of increase, decrease and unchanged in share price are 260, 237 and 1 respectively. The proportions (probabilities) for share prices that Increase (Inc) Decreases (Dec), Unchanged (U) were (260/500) = 0.5230; (237/500) = 0.4749 and (1/500) = 0.0020 respectively. This gives rise to initial state vector as: (0.5230, 0.4749, 0.0020)

	Increase in Share Price (Inc)	Decrease in Share Price (Dec)	Unchanged in Share Price (Unc)
Increase in Share Price (Inc)	126	133	1
Decrease in Share Price (Dec)	134	103	0
Unchanged in Share Price (Unc)	0	1	0

Initial State Matrix: - $[0.5230 \quad 0.4749 \quad 0.0020]$

Transition Matrix: - $\begin{bmatrix} 0.4846 & 0.5115 & 0.0038 \\ 0.5654 & 0.4346 & 0 \\ 0 & 1.0000 & 0 \end{bmatrix}$

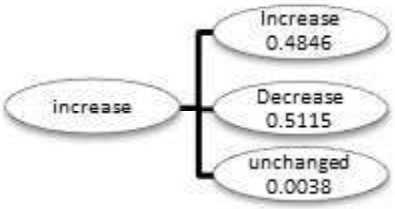


Fig. 1 implies that 0.4846 Sun Pharma share prices that increase will still increase; 0.5115 share prices that increase will decrease and 0.0038 will remain unchanged.

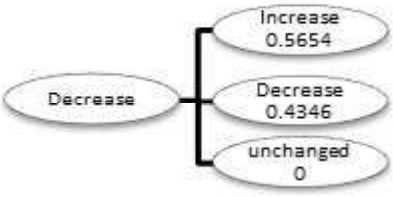


Fig. 2 implies that 0.5654 Sun Pharma share prices that decrease will increase; 0.4346 share prices that decrease will decrease and 0 will remain unchanged.

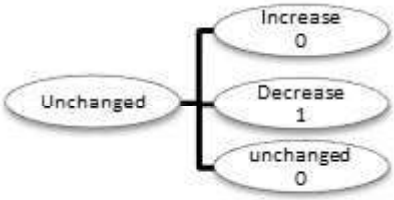


Fig. 3 implies that 0 shares unchanged will increase; 1 share unchanged will decrease and 0 unchanged will unchanged.

The higher order Transition probability matrix of the transition matrix $P^{(n)}$ was calculated to observe the behaviour of the share price using MATLAB Software:-

$$P^2 = \begin{bmatrix} 0.5241 & 0.4741 & 0.0019 \\ 0.5197 & 0.4781 & 0.0022 \\ 0.5654 & 0.4346 & 0 \end{bmatrix}$$

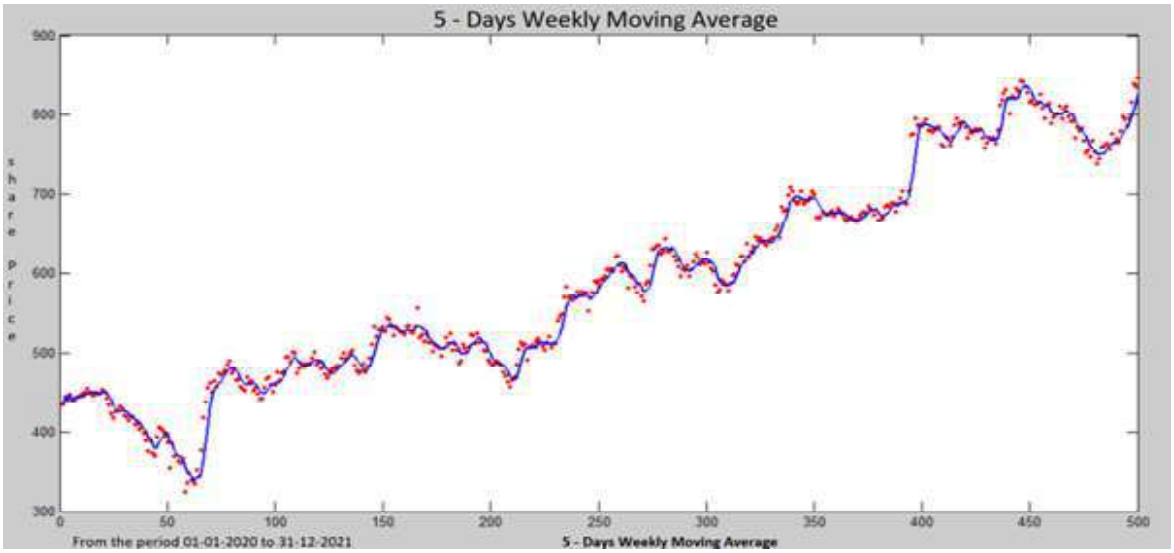
$$P^3 = \begin{bmatrix} 0.5220 & 0.4760 & 0.0020 \\ 0.5222 & 0.4758 & 0.0020 \\ 0.5197 & 0.4781 & 0.0022 \end{bmatrix}$$

$$P^6 = \begin{bmatrix} 0.5221 & 0.4759 & 0.0020 \\ 0.5221 & 0.4759 & 0.0020 \\ 0.5221 & 0.4759 & 0.0020 \end{bmatrix}$$

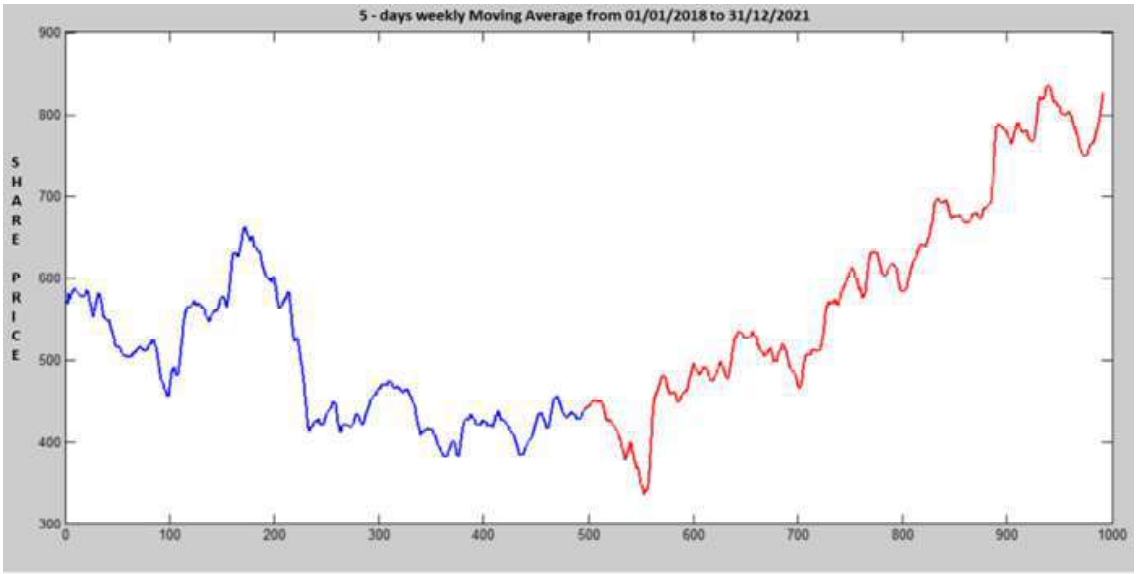
$$P^{15} = \begin{bmatrix} 0.5221 & 0.4759 & 0.0020 \\ 0.5221 & 0.4759 & 0.0020 \\ 0.5221 & 0.4759 & 0.0020 \end{bmatrix}$$

The 5 days weekly moving average starts with a bearish trend at a share price of 320. This is followed by a bullish trend up to a peak of Share price of 470 and then engages a bullish trend to a high of 800 share price. This bearish trend in share values of Sun Pharma is still raising. The gradual increase in share price of Sun Pharma share during the COVID period denotes the demand of pharmaceutical products in India during whole Covid period.

Graphical representation of trend of Moving Average



Graphical Representation of trend of Moving Average graph of Sun Pharmaceuticals from the period 01/01/ 2018 to 31/12/2021



RESULT & CONCLUSION

The objective was to determine the Markov model for forecasting and compare Pre-COVID and during COVID, Sun Pharma share price, it was concluded that on Pre-COVID period derived initial state vector is 0.4755, 0.5224 & 0.0020 and the steady state occurred in transition matrix calculated by taking share price from the 491 trading days after 6 years which equals to 0.4764, 0.5215 & 0.0021 but due to COVID-19 pandemic there is a slight difference in the probabilities. During COVID-19 period, Initial state vector is 0.5230, 0.4749 & 0.0020 and the steady state occurred in transition matrix calculated by taking share price from the 500 trading days after 6 years which equals to 0.5221, 0.4759 & 0.0020 [0.5230, 0.4749, 0.0020]. Additionally, the convergence of transition matrix to a steady state implying ergodicity that is a characteristic of stock market makes the model applicable. Based on the graph of 5 days Moving Average it was concluded that, the daily closing Sun Pharma share price had generally a bullish and bearish trend in Pre-COVID period but during Covid period, it indicates a high growth or increasing trend value of the share price which is a good sign for the investors.

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RUJOST, ISSN: 2319-4227, Vol.7, No.1, January, 2022, pp. 157-160

A Biaanual Peer Reviewed Referred Ranchi University Journal of Science & Technology

Zoology

Effect of pinealectomy on the gonad and body weight of female Indian palm squirrel, *Funambulus pennanti*

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Abstract: Effect of pinealectomy (Px) was studied on the ovary and body weight of Indian palm squirrel, *Funambulus pennanti* during April-May (gonad active phase) and August-September (gonad inactive phase) for a period of forty days. Px animals indicated a significant increase in their body weight as compared to sham-operated (SO) controls during both phases. Px animals also showed significantly higher body weight as compared to normal animals under wild conditions. Px resulted in higher ovary weight in comparison to SO animals and normal animals both during gonad active and inactive phases. The present results suggest that the pineal gland exerts an inhibitory influence on the body weight and ovary weight of *Funambulus pennanti* during both phases of its gonadal cycle.

Keywords: pinealectomy, body weight, ovary, squirrel

INTRODUCTION

It is well documented that the pineal gland plays an important role in the regulation of reproduction in various seasonal breeders.[1-5] Besides reproduction the pineal gland, via its hormone melatonin, affects other seasonal body functions in a number of species. These functions include hibernation and body weight.[6,7] A season dependent response is exhibited by the animals as regards their gonads, body mass, lipid mass, pelage colour and so on.[8-12] It is suggested that these body functions are triggered by environmental photoperiod. The pineal gland comes to play an important role at this point of time. Its hormone, melatonin, is involved in the transduction of the photoperiodic information into a biological signal.[13] There are reports on the changes in body weight in relation to the photoperiod and pineal gland in some animals.[7] However, most of the research has centred on temperate zone animals. Animals living in tropical regions are exposed to entirely different climatic conditions than the temperate zone animals.

In view of the above and considering the fact that there are scanty reports on the pineal control of body weight in relation to gonads in tropical species[14] the present study was conducted in a tropical seasonal breeder, the Indian palm squirrel, *Funambulus pennanti.* The objective of the investigation was to observe the effect of pinealectomy on the ovary and body weight of female *Funambulus pennanti*. The study was aimed at observing the effects during gonad active as well as inactive phase of the ovarian cycle.

MATERIALS & METHODS

The study was performed during April-May (gonad active phase) and August-September (gonad inactive phase) for a period of 40 days. Adult females of *Funambulus pennanti* were obtained in the first week of April and August and acclimatized, for ten days, in a room fully exposed to ambient environmental conditions. The animals were housed in wire net cages and provided with

food (soaked gram seeds) and water *ad libitum*. After acclimatization they were divided randomly into two groups consisting of eight animals each. Animals of Group I were pinealectomized by the technique of Haldar-Misra.[15] Animals of Group II were sham-operated (SO). After forty days of Px a third group was made by obtaining animals from the fields. These animals of Group III were the normal wild animals.

Animals belonging to Group II and Group III were controls for Group I. After 40 days of Px all the animals were weighed individually. Their body weights were recorded. Ovary of one side only was removed by ovariectomy and weighed on a micro electrical balance. After two days of operation all the animals were released back to the fields. Statistical analysis of the data was done by Student's 't'test.[16] The experiment was performed following the guidelines accepted by the Ethics Committee, Ranchi University, Ranchi for investigations on animals.

RESULTS

Results are presented in Tables 1 and 2. During April-May Px resulted in a significant increase ($p<0.001$) in body weight and ovary weight of the animals as compared to their SO controls and normal controls (Table 1). A similar effect of Px was noted on the body weight and ovary weight of the animal during August-September. The body weights and ovary weights of Px animals were found to be significantly higher ($p<0.001$) than their respective SO and normal controls (Table 2).

Table 1- Effect of pinealectomy (Px) for forty days on body weight (g) and ovary weight (mg/100g body weight) of *Funambulus pennanti* during April-May

	Normal	Sham-operated	Px
Body weight	112.40±3.22	114.10±2.62	140.91±3.11*
Ovary weight	3.62±0.01	3.61±0.01	6.23±0.01*

Significance of difference from sham-operated control: * $p<0.001$

Table 2- Effect of pinealectomy (Px) for forty days on body weight (g) and ovary weight (mg/100g body weight) of *Funambulus pennanti* during August-September

	Normal	Sham-operated	Px
Body weight	68.10±2.04	70.62±2.21	104.50±3.26*
Ovary weight	1.10±0.008	1.11±0.009	2.94±0.003*

Significance of difference from sham-operated control: * $p<0.001$

DISCUSSION

It is evident from the present results that pinealectomy in female *Funambulus pennanti,* has a stimulatory effect on its body weight. Pinealectomized animals always presented significantly higher ($p< 0.001$) body weight as compared to the SO controls as well as normal controls under natural conditions. A similar effect of pinealectomy obtained during both phases of the gonadal cycle confirmed the stimulatory action of pineal removal on the body weight of *Funambulus pennanti*. It has been reported that the pineal 5- methoxyindoles regulate seasonal gonadal cycle in male *Funambulus pennanti*[17] but reports relating to pineal-body weight relationship are lacking in tropical species. An attempt was made in the present study, for the first time, to examine the effect of pinealectomy on body weight of female *Funambulus pennanti.* In a number of animals the pineal gland and melatonin regulate the body weight and hibernation.[13] Effect of pineal gland on body weight of Syrian hamsters has been studied during different periods of its gonadal cycle.[18] It has been reported that pinealectomy prevents body weight and white adipose tissue reduction in male meadow voles transferred to short day lengths.[19] Pineal melatonin is related to obesity.[20] Pineal melatonin is capable of influencing body weight changes in hamsters and turkey hens.[21,22]

In the present study also a similar effect of pinealectomy was observed on the body weight of *Funambulus pennanti*. The body weight of the animal showed significant reduction during August-September as compared to April-May. This was evident in animals of all groups i. e. Px, SO and normal wild animals. Therefore, *Funambulus pennanti* clearly exhibited a seasonal variation in its body weight. Pinealectomy counteracted the short-day inhibition of body weight, as observed in August-September, and always resulted in increased body weight. On the basis of this result, it can be suggested that pineal gland inhibits body weight of *Funambulus pennanti*. Conversely, pinealectomy produces a stimulatory effect on body weight of the animal.

As regards ovarian activity, a trend similar to that of body weight was noted for the ovary weight of the animal. Ovarian weight was high during April-May and it declined in August September. Pinealectomy again appeared stimulatory to ovary and always led to a significant increase in its weight. This effect was evident even during the gonad inactive phase (August-September). The inhibitory

influence of pineal gland on body weight and ovary weight of *Funambulus pennanti* is confirmed by the similar response, to pinealectomy, for both these parameters. The inhibitory effect of pineal gland on body weight has been reported in male *Funambulus pennanti*.[23] Studies on human pineal gland have suggested a relationship between the body weight and pineal gland structure.[24]

A significant decrease in body weight from April-May to August-September was accompanied by a corresponding decrease in ovary weight of the animal thereby suggesting that the declining body weight may be related to decrease in the sexual activity of the animal. This was also clearly evident in lower ovarian weight in normal animals. Pinealectomy has been observed to affect body weight in female Syrian hamsters during gonadal regression and recrudescence.[18] In Djungarian hamster, *Phodopus sungorus* pineal gland affects seasonal changes in body weight, which are reported to be mediated via the pituitary.[25]

Results of the present study indicate a stimulatory effect of pineal removal on body weight and ovary weight of *Funambulus pennanti*. An inhibitory influence of pineal gland is clear on its body weight as well as ovary. These results suggest a possibility of the involvement of pineal gland of this animal in seasonal regulation of its body weight. Further research will elucidate the complete role of pineal gland in this mechanism.

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RUJOST, ISSN: 2319-4227, Vol.7, No.1, January, 2022, pp. 161-164
A Biaanual Peer Reviewed Referred Ranchi University Journal of Science & Technology

Zoology

Zinc Oxide nanoparticles as a method to control *Sitophilus oryzae* (L.) on stored wheat grains

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Abstract: *Sitophilus oryzae* (Linnaeus) causes high risk to wheat seeds in storage such as weight loss, reduced nutrition value, less germination of grains. Screening test was conducted to evaluate the effect of Zinc Oxide nanoparticles (ZnO NPs) at different doses 50 ppm, 100 ppm, 150 ppm, 200 ppm and 1000 ppm per 20 g wheat grains along with control on *Sitophilus oryzae* (L.). The results clearly showed that ZnO nanoparticles had adverse effect on the adults of *Sitophilus oryzae*. The results obtained indicated that the insects mortality (%) increased by increasing the level of concentration of ZnO NPs and the period of exposure. Thus, the ZnO NPs are used as an alternative method as pesticides to control the *Sitophilus oryzae* insect pest because ZnO NPs are safe for human and for environment.

Keywords: Zinc Oxide Nanoparticles, *Sitophilus oryzae* (L.), wheat grains, Mortality (%).

INTRODUCTION

Insects are one of the basic problems of stored grains all over the world due to the quantitative losses, they cause.[1] The *Sitophilus oryzae* (L.) commonly known as rice weevil is a major pest of stored wheat grains. Both the adults and larvae feed on whole grains. The insect pest causes great loss in wheat grain storage and affect the food availability for a large number of peoples. The insect causes both the qualitative and quantitative loss to the stored wheat grains.[2] Throughout the world, the main aim of the entomologist is to find out the efficient method to control the stored grain pest. Synthetic chemical pesticides have been used for many years to control stored grain pests.[3] The application of insecticides and fumigants to control storage insect has led to many problems including the development of insecticide resistance, health hazards especially to mammals, and the risk of environment contamination.[4] The chemical insecticides are so much expensive. Reacting to the disadvantages of using traditional chemical pesticides was the need to use a new method of combat, such as nanotechnology.[5]

Nanoparticles has become one of the most promising new technologies in the recent decade.[6] Nanoparticles have shown great role in agriculture including management of pest. Nanoparticles having one or more dimensions in the order of 100 nm or less.[7] In the present study the ZnO nanoparticles were chosen because they are stable and cheap.

They kill the arthropods by removing or adsorbing the epicuticular lipid layers causing excessive water loss through cuticle.[8] The present research has been carried out to investigate the toxic effect of ZnO NPs on stored wheat grain pest especially *Sitophilus oryzae* under laboratory conditions.

MATERIALS & METHODS

Location of experiment

Research was conducted at the Entomology Laboratory of University Department of Zoology, Ranchi University, Ranchi, Jharkhand, India.

Insect used

The adults of *Sitophilus oryzae* were collected under the wheat grains reared in the Entomology laboratory of University Department of Zoology, Ranchi University, Ranchi. Insects were reared under laboratory conditions of 16±2°C, 50±5% relative humidity in continuous darkness. For the experiment new adults were selected.

Zinc Oxide nanoparticles

The colloidal form of ZnO NPs were obtained from authorized Nano Research Lab.

Supplier	:	Nano Research Lab
Appearance	:	Powder
Colour	:	White
Solubility	:	Dispersed in water
pH	:	Neutral

Preparation of wheat grains and treatment with ZnO NPs

250 g of wheat grains were taken, washed with water and dried in sunlight. Then wheat grains were sieved to remove stone, dust, insects. Batches of wheat grains containing 13% moisture were sterilized at 60°C in hot air oven for one hour and left to cool and reabsorb moisture. In three replicates 20 grams of wheat grains were weighed in 150 ml jar for treatments and control.

Different concentrations of Zinc Oxide nanoparticles, that is concentrations 50 ppm, 100 ppm, 150 ppm, 200 ppm and 1000 ppm were prepared and mixed with the wheat grains in required doses. Jars were shaken manually for 2 minutes for equal distribution of ZnO NPs on wheat grains. The treated jars were left for 24 hours before introduce the adults *of Sitophilus oryzae.* After 24 hours of treatment 20 newly emerged adults of *Sitophilus oryzae* were introduced in each batch for infestation.

All batches were kept at 16±2°C and 50±5% R.H. for all treatment and control. Insect Mortality Percentage was recorded after 6, 12, 18, 24, 30, 36 and 42 days post treatment. Adult mortality of *Sitophilus oryzae* was calculated by using the formula described by Devi *et al.*, (2014)[9].

$$\text{Adult Mortality (\%)} = \frac{\text{Number of dead insects}}{\text{Total number of insects}} \times 100$$

RESULT

Table 1 represents the effect of treatment of ZnO NPs with the stored wheat grains. The result obtained in Table 1 indicated that the percentage mortality increases with the increase of concentration and period of exposure. In the higher concentration 1000 ppm adult mortality percent were varied in the range of 5% to 40% from 6 to 42 days post treatment. The highest mortality percent found in treatment 200 ppm, 150 ppm, 100 ppm and 50 ppm were 33.33%, 26.33%, 11.11% and 2% at 42 days post treatment. In control there was 0% mortality obtained.

Fig. 1- Infested wheat grains treated with ZnO NPs

Table 1- Toxicity of ZnO NPs mixed with the media against *Sitophilus oryzae*

Treatments (ZnO NPs,ppm)	Adult mortality (%) Days after treatment (DAT)						
	6	12	18	24	30	36	42
50	0.00	0.00	0.00	0.00	0.00	0.00	5.00
100	0.00	0.00	0.00	0.00	5.00	5.26	11.11
150	0.00	0.00	0.00	10.00	13.33	16.66	26.33
200	0.00	0.00	15.38	16.67	18.75	20.00	33.33
1000	0.00	5.00	15.78	18.18	21.17	23.07	40.00
CONTROL	0.00	0.00	0.00	0.00	0.00	0.00	0.00

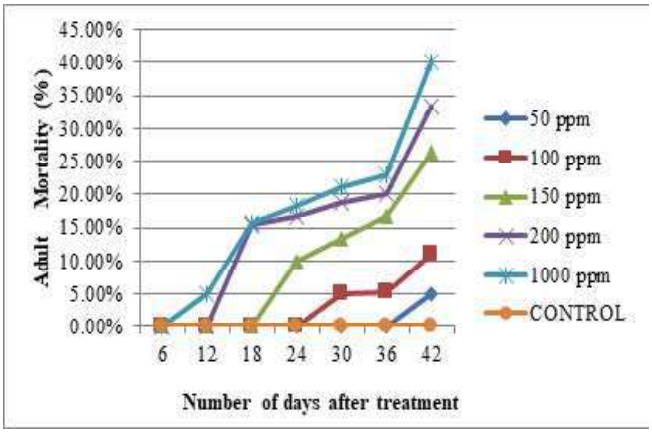

Fig. 2 – Insecticide Bioassay

DISCUSSION

From the data it was evident that the ZnO NPs were effective because of increased insect cuticle contact. Insecticidal efficacy of the dust becomes enhanced if the particles are finely divided.[10] Damage occurs to the insect protective wax coat on the cuticle, by sorption and abrasion.[11] The insects begin to lose water as the water barrier is damaged and due to desiccation they die.[12] Since there is no reviews available on ZnO NPs toxic effect against adult mortality of *Sitophilus oryzae* at low temperature (16±2°C, 50±5% R.H), so reviews of ZnO NPs at 28±2°C, 70±5% R.H and other nanoparticles are presented here. Wille (1923)[13] reported variable duration of life cycle with 45 days in summer but taking as long as five months in cool weather of autumn and winter for completion of one generation. Keratum *et al.,* (2015)[2] noticed that the adult mortality percent ranged between 2 and 46.8% from 3 to 15 days of exposure with concentration % ranged from 0.1 w/w to 0.8 w/w. Rouhani *et al.,* (2012)[14] showed the most mortality effect pertained to 28% ZnO against *Frankliniella occidentalis*. Sabbour (2013)[15] found that aluminium oxide had the highest cumulative mortality 73.3% followed by titanium dioxide reached 59.7% after 7 days against *Sitophilus oryzae.* Debnath *et al.,* (2011)[11] reported, the invitro cellular toxicity in human fibroblast cell line and acute oral toxicity study in mice revealed that similar to the silica nanoparticles (25, 50, 125, 800, 2000, 5000 ppm) nontoxic. ZnO NPs are less toxic than SNPs. So, ZnO NPs can be used to control *Sitophilus oryzae* in stored grains. Nanoparticles can be useful in pest management as insecticides, however more research is required to find out the detailed mechanisms of action of ZnO NPs as nanocides.

CONCLUSION

At the lower temperature the dose 1000 ppm of ZnO NPs caused mortality after 12 days of post treatment of *Sitophilus oryzae* and after that mortality percent increases. From the result it is clear that ZnO NPs can used as an alternative to kill the *Sitophilus oryzae.*

ACKNOWLEDGEMENT

The authors are grateful to Nano Research Lab, Jamshedpur, Jharkhand for the supply of nanoparticle and University Department of Zoology, Ranchi University Ranchi for providing lab facilities.

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RUJOST, ISSN: 2319-4227, Vol.7, No.1, January, 2022, pp. 165-170

A Biaanual Peer Reviewed Referred Ranchi University Journal of Science & Technology

Zoology

In vitro antibacterial activity of chitosan extracted from freshwater crab, *Sartoriana spinigera* (Wood mason, 1871).

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Abstract: *Sartoriana spinigera* is a freshwater crab, found in ponds, fields of Ranchi, Jharkhand. The edible crabs with medicinal value are consumed by the local people of Ranchi. Chitosan is extracted from shell of crab by using chemical method. Chitosan is a straight chain polymer of glucosamine and N-acetyaled glucosamine. In the present study 5 mg/ml concentration of chitosan dissolved in 0.2 % acetic acid, was tested against four strains of bacteria *E. coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853) and *E. faecalis* (ATCC 29912) for its antibacterial efficacy by agar well diffusion method. Data obtained were analyzed using Student's t-test. The result revealed that chitosan showed good antibacterial activity in three bacteria *E. coli, P. aeruginosa* and *S. aureus*. The zone of inhibition observed at chitosan concentration of 5 mg/ml was 19.33±0.57 mm for *E. coli*, 18.66 ±0.70 mm for *S. aureus* and 16.66±0.70 mm for *P. aeruginosa.* In *E. faecalis* no zone of inhibition was observed thus were resistant to chitosan from *S. spinigera*. A positive control, Ofloxacin (5 µg/disc) was used. On comparing with the positive control, it showed significantly higher antibacterial activity than chitosan against *E. coli* ($p<0.001$), *S. aureus* ($p<0.001$) and *P. aeruginosa* ($p<0.001$). Chitosan concentration at 5 mg/ml showed significantly higher antibacterial activity in *E. coli* than *P. aeruginosa* ($p< 0.01$). In *S. aureus* chitosan showed significantly higher antibacterial efficacy than in *P. aeruginosa* ($p<0.05$). There was no significant difference of zone of inhibition between *S. aureus* and *E. coli.*

Keywords: Chitosan, *S. spinigera,* antibacterial activity.

INTRODUCTION

Crab belongs to the largest distributed Phylum Arthropoda among invertebrates under the animal Kingdom. It is well known due to its consumption as food and also as medicine against many health problems. Generally, people consume its meat and throw its shell as waste. In recent times, shell wastes are used as a source of biopolymer, Chitosan. Chitosan is mainly known for usage in food, pharmaceuticals and cosmetics industries. Chitosan is non-toxic, biodegradable, polymer of D-glucosamine, linked by 1,4 -glycosidic bonds, obtained by deacetylation of Chitin, a polysaccharide of exoskeleton of freshwater as well as marine arthropods and molluscs.

Traditional antimicrobials have been used as reliable preservatives to control microbial hazards in the food industry for decades.[1] But these widely accepted compounds are synthetic with harmful side effects. To minimize these effects, there is a need of natural and healthy source of medicines. Due to the negative impact from chemical preservatives, attention has shifted to the use of naturally derived antimicrobial agent to control food borne pathogen.[2] With the increasing claim for food safety and health standards, consumers have been more concerned about the occurrence of chemical residues in the food products,[3] therefore natural antimicrobials are considered better than traditional synthetic antimicrobials. Natural antimicrobials are derived from many animals.[3]

Chitosan have attracted the interest of many researchers, medical, pharmaceutical and industrial fields due to its properties like analgesic, antitumor, antioxidant, haemostatic, hypocholesterolemic, biodegradability and

biocompatibility.[4] Chitosan was shown to have several advantages over other disinfectants, as it possesses a higher antibacterial activity, a broader spectrum of activity, a higher kill rate, and lower toxicity towards mammalian cells.[5,6] In the present study, chitosan is extracted from the shell of one such locally found freshwater crab of Jharkhand, *Sartoriana spinigera* to determine its antibacterial activity.

MATERIALS & METHODS

Collection of animals

Sartoriana spinigera were purchased from local market of Ranchi, Jharkhand. Shells were scraped free of loose tissue from the crab wastes in laboratory, washed thoroughly with tap water to remove impurities. They were dried at 60ºC and pulverized using pestle and mortar for further analysis.

Preparation of chitosan and chitosan solution

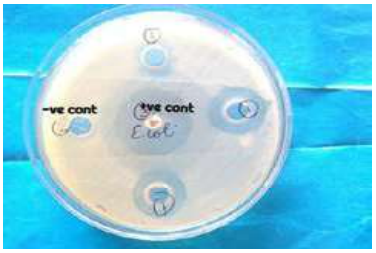
Chitin and chitosan were prepared from *Sartoriana spinigera* shell waste according to Takiguchi (1991 a&b)[7,8] with some modifications[9] for purification of chitosan. The production of chitosan from crustaceans shell generally consists of three basic steps demineralization, deproteinization and deacetylation. In the preparation of chitosan solutions 0.5%(w/v) chitosan were dissolved in 0.2%(v/v) acetic acid solution.

Bacterial Strains

The antibacterial activity of the prepared chitosan from *Sartoriana spinigera* was tested against four strains, *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Enterococcus faecalis* (ATCC 29912) were obtained from the Department of Microbiology, Rajendra Institute of Medical Science, Ranchi, Jharkhand.

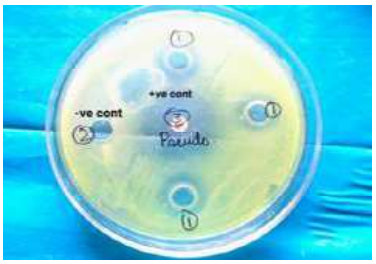
Nutrient broth was prepared and sterilized in an autoclave at 15 lbs pressure for 15 min. Individual species of bacteria were inoculated in the sterile nutrient broth and incubated at 37ºC for 24 hrs. Muller Hilton Agar (MHA, Himedia) medium was prepared, sterilized in an autoclave at 15lbs pressure for 15 min and poured into sterile petridishes and incubated at 37ºC for 24 hrs. The antibacterial activity of the individual bacterial strains was tested using Agar Well diffusion method.[10] Wells of 6 mm diameter were made aseptically in the inoculated plates. Bacterial cultures were emulsified in normal saline and turbidity was matched with 0.5% McFarland turbidity standards. 24 hrs old nutrient broth cultures of test bacteria were aseptically swabbed on sterile MHA plates. Solution of chitosan (5mg/ml) in 0.2% acetic acid and 0.2% acetic acid as negative control was loaded using micropipette, in the respective wells. Ofloxacin disc (5 µg/disc) was placed using a sterile forcep, as positive control. The plates were incubated at 37ºC for 24 hr in upright position. The antibacterial assay was carried out in triplicate. After incubation at 37ºC for 24 hrs, zone of inhibition was measured in millimetres.

OBSERVATIONS & RESULTS

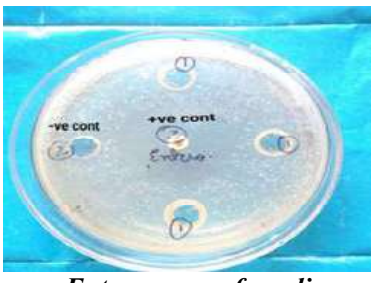


Escherichia coli

Staphylococcus aureus



Pseudomonas aeruginosa



Enterococcus faecalis

Figure 1 : Zone of inhibition obtained by (5 mg/ml) chitosan solution from *Sartoriana spinigera* against four bacteria *E. coli, S. aureus, P. aeruginosa* and *E. faecalis*

Table 1- Comparative sensitivity test of chitosan (5 mg/ml) extracted from *S. spinigera* in bacterial strains

Zone of inhibition (mm)				
Bacterial strains	***E.coli***	***S.aureus***	***P.aeruginosa***	***E.faecalis***
	19.33±0.57	18.66±0.70^{NS}		-
		18.66±0.70*	16.66±0.70	-
	19.33±0.57**		16.66±0.70	-

Values are expressed as mean±SD.*p<0.05 or significant at 5%,**p<0.01 or significant at 1% NS -no significant difference.

Table 2- Antibacterial activity of chitosan (5 mg/ml) of *S. spinigera* and Positive control (5 µg/disc) against four bacterial strains

Bacterial strains	Zone of inhibition (mm)	
	Chitosan (5 mg/ml)	**Positive control (Ofloxacin 5 µg/disc)**
Escherichia coli	19.33±0.57	29.66±0.57***
Pseudomonas aeruginosa	16.66±0.70	24.66±0.57***
Staphylococcus aureus	18.66±0.70	31.66±0.57***
Enterococcus faecalis	-	28.66±6.11

Values are expressed in mean±SD,(-)= no zone of inhibition,*** p<0.001 or significant at 0.1%

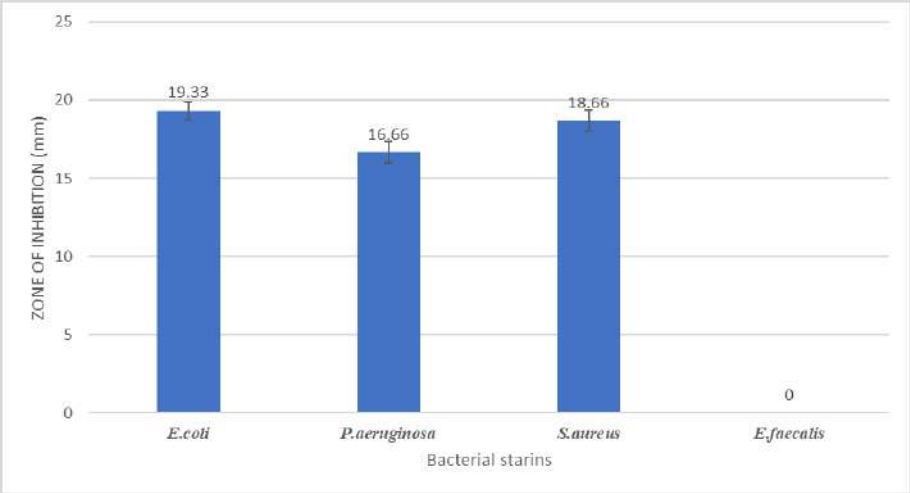


Fig. 2- Comparative sensitivity test of chitosan on bacterial strains

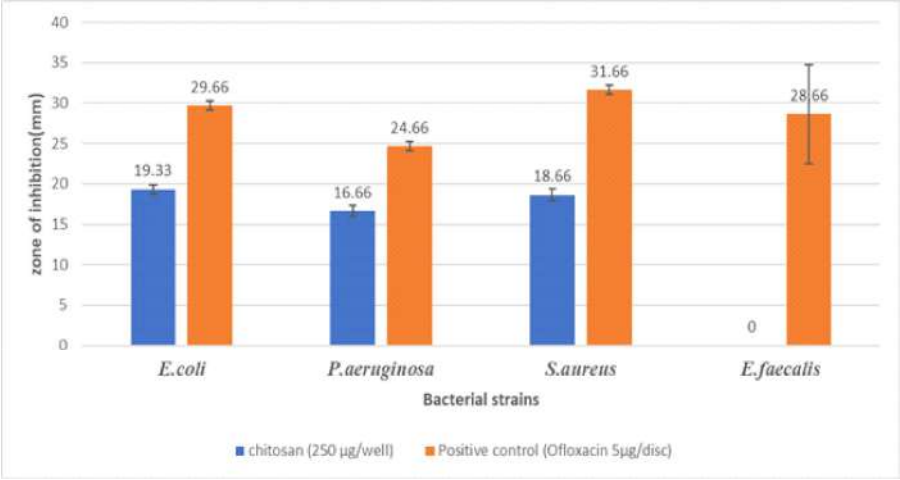


Fig. 3- Antibacterial activity of chitosan (5 mg/ml) of *S. spinigera* and Positive control (5µg/disc) against four bacterial strains.

Table 1 showed that zone of inhibition observed at chitosan concentration of 5 mg/ml was 19.33±0.57 mm for *E. coli*,18.66 ±0.57 mm for *S. aureus* and 16.66±0.70 mm for *P. aeruginosa.* In *E. faecalis* no zone of inhibition was observed. Chitosan showed good antibacterial activity in only three bacteria *E. coli, P. aeruginosa* and *S. aureus.* In case of *E. coli,* chitosan showed significantly higher antibacterial activity than in *P. aeruginosa* ($p< 0.01$) and no significant difference was observed with *S. aureus*. In case of *S. aureus,* chitosan showed significantly higher antibacterial activity than in *P. aeruginosa* ($p<0.05$) *(*Fig.1 & 2).

Table 2 represents a comparative antibacterial activity between chitosan (5 mg/ml) and positive control, Ofloxacin (5 µg/disc). Ofloxacin showed zone of inhibition of 29.66±0.57 mm in *E. coli*, 24.66±1.57 mm in *P. aeruginosa*, 31.33±0.57 mm in *S. aureus* and 28.66±6.11 mm in *E. faecalis*. On comparing with the positive control, Ofloxacin showed significantly higher antibacterial activity than chitosan against *E. coli* ($p<0.001$), *S. aureus* ($p<0.001$) and *P. aeruginosa* ($p<0.001$). (Fig. 1 & 3).

DISCUSSION

In the present study, three bacteria namely *E. coli, P. aeruginosa* and *S. aureus* showed sensitivity and one bacteria, *E. faecalis* showed resistance against chitosan. *E. coli* showed 19.33±0.57 mm zone of inhibition and showed more sensitivity against chitosan (5mg/ml) than *P. aeruginosa* 16.66±0.70 mm at significant level of ($p<0.01$). There was no significant difference in antibacterial activity between *E. coli* and *S. aureus* (18.66±0.70 mm). *S. aureus* showed significantly higher zone of inhibition than *P. aeruginosa* (16.66±0.70 mm) at $p<0.05$. In case of *E. faecalis* at chitosan concentration of 5 mg/ml showed no zone of inhibition. (Table 1, Fig.1&2)

Islam *et al*. (2011)[11], also reported that the antibacterial activity of crab chitosan against *Escherichia coli* showed only 10 mm zone of inhibition in chitosan concentration of 1000 µg/ml. Hmed *et al.* (2017)[2] reported that the effectiveness of crab chitosan as naturally derived antimicrobial agent against *E. coli* showed 49.7±0.31 mm zone of inhibition at 5% concentration by disc diffusion method. The difference might be due to the high concentration of chitosan i.e. 5% concentration of chitosan in case of above mentioned crab by Hmed *et al.* (2017)[2].

Hmed *et al.* (2017)[2] also reported the antibacterial activity of the chitosan from crab shell waste against *P. aeruginosa* which showed (50.4 ±0.71 mm) zone of inhibition at 50 mg/l concentration. The zone of inhibition was significantly higher but in the present case it was only 16.66±0.70 mm against *P. aeruginosa* at concentration of 5.00 mg/ml in *S. spinigera.* Prabu & Natarajan (2012)[12] made study on the antimicrobial activity of chitosan isolated from *Podopthalmus vigil* against *P. aeruginosa* showed no zone of inhibition at any concentration.

According to Hmed *et al.*(2017)[2] antimicrobial effectiveness of the naturally derived chitosan from crab shell waste against *S. aurues* showed 50.4±0.71 mm zone of inhibition in 5% chitosan concentration using disk diffusion assay. Islam *et al.* (2011)[11] reported that the antibacterial activity of chitosan of crab shell from Khulna, Bangladesh against *Staphylococcus aureus* showed 13 mm zone of inhibition at 1000 µg/ml concentration. The present study showed significantly higher zone of inhibition formed by chitosan extracted from *S. spinigera.* Higher chitosan concentration i.e 5 mg/ml may be related to larger zone of inhibition (18.66±70 mm), making chitosan a potent antibacterial agent.

Chitosan extracted from *S. spinigera* showed no zone of inhibition against *E. faecalis*. Thus *E. faecalis* was observed to be resistant to chitosan from *S. spinigera.* Shenoi *et al.* (2016)[12] reported that the antimicrobial efficacy of commercial chitosan against *E. faecalis* showed 15.93±1.53 mm zone of inhibition at 1% concentration. Geethpriya *et al*. (2016)[13] made study that the antibacterial activity of the commercial chitosan solution against *E. faecalis* (ATCC 29212) and *E. faecalis* clinical isolates showed 19.67±0.58 mm zone of inhibition at 12 mg/ml concentration.

According to Jean *et al*. (2011)[14] and Ueno *et al.* (1997)[15], chitosan possesses antimicrobial activity against a number of Gram-negative and Gram-positive bacteria. Chitosan has antibacterial activity against some bacteria as observed in *E. coli, P. aeruginosa* and *S. aureus* in the present study. However some bacteria are resistant against chitosan as *E. faecalis* of present study. The zone of inhibition formed against bacteria are dependent on concentration of chitosan. Antibacterial activity of chitosan is the result of series of reactions, rather than the cause of the reactions. The reactions takes place between molecules of chitosan and cell wall.[16] The bond formed between

positively charged amine group (NH_3^+) of chitosan and teichoic acid of Gram-positive bacteria and lipopoly saccharides of Gram-negative bacteria are responsible for the intracellular leakage and cell death.[17-19]

CONCLUSION

In conclusion, the present investigation revealed that the chitosan from *Sartoniana spinigera* inhibits growth of human pathogenic bacterial strains and have excellent antibacterial activity against *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcua aureus*. Chitosan may be used against bacterial infection.

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RUJOST, ISSN: 2319-4227, Vol.7, No.1, January, 2022, pp. 171-174
A Biaanual Peer Reviewed Referred Ranchi University Journal of Science & Technology

Zoology

The effect of colloidal solution of ZnO nanoparticles on bruchid beetle *Callosobruchus maculatus*: A pest of gram (*Cicer arietinum*).

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Abstract: The treatment of *Cicer arietinum* with ZnO Nanoparticles (NPs) were conducted to know the efficacy of ZnO NPs as insecticide. The study revealed a significant enhancement in the mortality rate of the stored grain insect pest *Callosobruchus maculatus*. The result of this study clearly demonstrated the useful nature of ZnO NPs as seed protecting agent for the control of beetles and also enhance nutritional value of the gram seeds with zinc. Stored grains are lost each year due to beetle attack, so the treatment will increase the durability of stored gram and also the resistance developed by the pests due to chemical method of control would overcome by replacing the method by ZnO NPs treatment. Different concentrations of ZnO NPs were used and mortality rate observed for each concentration. The highest concentration of 200 ppm showed maximum mortality of 100% at the interval of 3 weeks. While the minimum concentration of 50 ppm showed 12.25% and control showed no mortality at the same interval.

Keywords: ZnO NPs, Bruchid beetles, *Callosobruchus maculatus*, Stored gram.

INTRODUCTION

Pulses are considered as an important source of protein for human consumption in many regions of the world. Pulse crops are cultivated over an area of 24 million hectares with production of about 15 million tonnes.

Gram, commonly known as the chickpea is an annual plant of legume family, is widespread in countries with subtropical and tropical climates- India, Turkey, Iran etc.

Among the legume, chickpeas are characterized by high nutritional values, amount of vitamins and other biologically valuable substances which in turn causes high demand for food and feed purpose.[1]

India is the largest producer of pulses in the world, however India is also the largest consumer of pulses in the world due to its increasing population.[2] Due to increase in population, requirement for chickpea also increased and hence yield of gram in the country is quite low, import quantity increased from 4.9lakh tonnes during 2014-2015 to 10.81 lakh tonnes in 2016-17 to fulfill the demand of the population and the export quantity decreased from 3.46 lakh tonnes to 1.37 lakh tonnes in 2016-17.[2]

During storage, the food commodities are attacked by the number of insects pests like *Sitophilus granaries Rhizopertha dominica, Callosobruchus chinensis* and *Callosobruchus maculatus* etc. Among them *C. maculatus* is an important stored grain insect pest distributed in Asia and Africa ranges from tropical to sub-tropical world. It has been reported that the favourite host of *C.maculatus* are cowpea and chickpea but it feeds on other legumes as well.[3] Bruchid beetles, *C. maculatus* (Fabricus) is a major stored product pest responsible for considerable damage in stored pulses and make the pulses unfit for human consumption.[4]

The larvae of *C. maculatus* is totally dependent on the seed of legumes but adults of this pest do not require water and feed but instead spend their limited life on mating and laying eggs at 30°C- 35°C and 70 to 90 % relative

humidity are ideal condition for oviposition and hatching takes place after 8 days of oviposition. Adult emerge within 3-4 weeks under favorable condition.[5] Male and female have average lifespan of 7 days laboratory condition and only few of them survive more than 2 weeks.[6,7]

Traditional synthetic insecticide chemical control is the most commonly used strategy against stored grain insect pests but long term use of these chemicals develop resistance to the pests.[8] Nanoparticles represented a new generation of environmental remediation technologies that could provide cost effective solution to some of the most challenging environmental clean - up problems.[9] Nanomaterials have a lots of role to playing pests control.[10] A variety of metal NPs eg. Ag, Au, Al, Si and Zn and metal oxide based polymers ZnO and TiO_2 etc are being developed for crop pests management.

ZnO has a very less toxic effect and at the same time Zn is the micronutrient. It is a biosafe material that possesses photo oxidizing and photocatalysis impacts on chemical and biological species.[11] NPs distinct properties allows their possible applications in many fields such as biosensors, nanomedicine and bionanotechnology.[12] ZnO NPs are reported by several studies as non toxic to human cells.[13] This aspect necessitated their usage as antibacterial agent, noxious to microorganisms and hold good biocompatibility to human cells.[14]

In this study there is a focus on the mortality rate of the pest of gram, *C.maculatus*, by treating gram seeds with different concentrations of ZnO NPs and allowing the pest to infect in the presence of ZnO NPs and number of insects killed are noted at the interval of 2 days after treatment.

MATERIALS & METHODS

The test beetles were obtained from nearby local market of Lower Chutia, Ranchi (Jharkhand). The beetles were obtained from the infested seeds of gram. The fresh cultures were also prepared as stock cultures by allowing some isolated beetles to infect the sterilized fresh seeds of gram. The average temperature during the period of investigation was 16±2°C and the average relative humidity of 50±5%.

ZnO NPs colloidal solution (1000 ppm) were having the diameter range of 20-30nm was purchased from Nano Research Lab H21, Gopalpur, Jadugoda (E) Singhbhum, Jamshedpur (Jharkhand) 832102, India.

ZnO NPs with three replicates of four different concentrations of 50ppm, 100ppm, 150pp, 200ppm were thoroughly mixed with the sterilized gram seeds (20 grams each) and placed in jar (150ml). Then the mixture was left for 24 hours and then 20 beetles were introduced in each jar. In the interval of 34 days the mortality rate is calculated following the formulae of Devi *et al.*, (2014)[15]

$$\text{Adult Mortality \%} = \frac{\text{Number of dead insects}}{\text{Total number of insects}} \times 100$$

Fig. 1- Picture showing the infested gram pulse with *Callosobruchus maculatus*.

Fig. 2- Picture showing the treated gram seeds with 50 ppm, 100 ppm, 150 ppm and 200 ppm ZnO NPs.

RESULT & DISCUSSION

The result showing the adult mortality rate with different concentrations of ZnO NPs : 50 ppm, 100 ppm, 150 ppm and 200 ppm has been presented in the Table 1. Graph 1, showing the percent mortality v/s four concentrations of ZnO NPs: 50 ppm, 100 ppm, 150 ppm and 200 ppm at the four intervals of 7, 14, 21 and 28 days after treatment (DAT).

The findings are in agreement with the Wazid *et al.* (2018)[16]; Jose *et al.* (2021)[17] who reported that with the increase in concentrations of ZnO NPs and number of days after treatment, there is also an increase in the mortality rate of the beetles.

Mortality of the pulse beetles

In the present investigation, the effect of ZnO NPs is tested on the mortality of pulse beetles at 4 different concentrations of 50 ppm, 100 ppm, 150 ppm and 200 ppm.

It has been observed that with increase in the concentrations and number of days after treatment, the mortality of beetles has also increased. ZnO NPs having a concentration of 200 ppm showed highest mortality of 100% @23 days after treatment (DAT) while 50ppm showed 12.25%, 100 ppm: 35.29%, 150 ppm: 44.44% mortality while the control showed no mortality at the same interval.

In the graph 1, the mortality rates after 7, 14, 21 and 28 days after treatment has been depicted. After 7, 14, 21 and 28 DAT the mortality percent of the beetles were 0%, 5%, 12.25%, 33.33% respectively @50 ppm. For 100ppm it was 0%,14.29%, 35.29% and 50%; for 150ppm -0%, 27.27%, 44.44% and 56.20% and for 200ppm-15%, 42.86%, 100%. The control showed no mortality @28 DAT.

ZnO NPs may be attributed to the damage of the protective wax coat on the cuticle of insects, both by sorption and abrasion so the insects begin to lose water and die due to desiccation.[18]

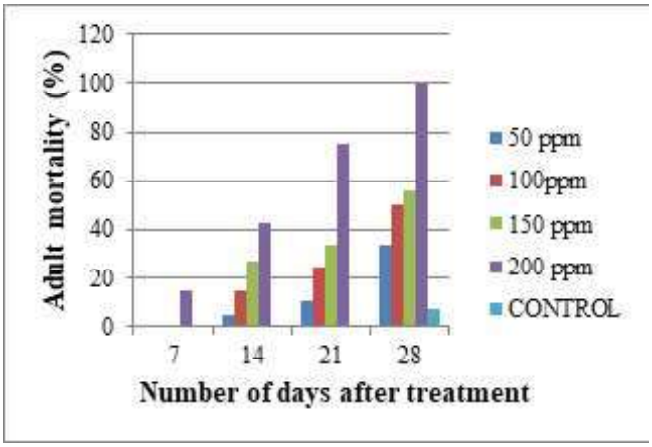
Table 1- Mortality rate @2-34days after treatment (DAT).

Conc. of ZnO NPs in ppm)	(MORTALITY RATE (in %)											
	2DAT	5DAT	8DAT	11DAT	14DAT	17DAT	20DAT	23DAT	26DAT	29DAT	31DAT	34DAT
50	0.00	0.00	0.00	0.00	5.00	5.88	10.53	12.25	33.33	42.86	62.50	100
100	0.00	0.00	6.67	11.76	14.29	15.00	25.00	35.29	50.00	60.00	100	
150	0.00	0.00	15.00	22.22	27.27	28.57	33.33	44.44	56.20	100		
200	0.00	15.00	25.00	33.33	42.86	50.00	75.00	100				
CONTROL	0.00	0	0	0	0	0	0	0	0	0	7.14	7.14

CONCLUSION

The treatment of stored pulses with ZnO NPs would be the effective treatment with respect to the beetle's mortality.

With the increase in the concentration of ZnO NPs, the mortality rate of the beetles also increased. Therefore, ZnO NPs may take over the role of pest control in godowns to increase the durability and thus prevent the heavy economic loss due to the attack of stored grain pests.



Graph 1- Number of days after treatment V/S adult mortality rate

ACKNOWLEDGEMENT

The authors are thankful to the Head of Department and the Dean, Faculty of Science, Ranchi University, Ranchi, Jharkhand, India for providing the facilities during the present investigation.

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RUJOST, ISSN: 2319-4227, Vol.7, No.1, January, 2022, pp. 175-178
A Biaanual Peer Reviewed Referred Ranchi University Journal of Science & Technology

Zoology

Life history, ecology and pest status of *Sahyaddrassus malabaricus* Moore, A polyphagous pest of timber trees in Jharkhand

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Abstract: Forests provide many useful materials to us. Timber wood is chief among them. Timber is a valued natural resource that serves directly as a material for use in construction, paper manufacturing, especially wood products such as furniture and as a fuel source, home construction etc. A healthy tree trunk has highly economic value, but if it is hollow or damaged by insects, its economic value reduces. Among many pests related to damage of timber trees, one of them is *Sahyaddrassus malabaricus*, Moore. The Life History, Ecology and Pest Status of *Sahyaddrassus malabaricus*, Moore were investigated in Jharkhand. It is a stem borer which bores and destroys the tree trunks. It attacks various trees including *Gmelina arborea, Tectona grandis, Terminalia arjuna, Albizzia lebbek* causing economic loss.

Keywords: *Sahyaddrassus malabaricus*, polyphagous Pest , Timber Trees

INTRODUCTION

A forest is a biotic community spread over a large tract of land and composed predominantly of trees, shrubs and woody climbers and often with a closed canopy. Forests provide many useful materials which give people a lot of things to live and make life comfortable. People depend on forest products like timber, firewood, nuts, fruits, seeds, medicinal plants etc., without which human life shall become miserable. Timber wood is chief among them. Timber is a valued natural resource that serves directly as a material for use in construction, paper manufacturing, especially wood products such as furniture and as a fuel source, home construction etc. Forests affect the natural environment by influencing temperature, humidity etc. It also affects the soil composition, structure, chemical property and water contents. It also plays an important role in checking soil erosion and flood conditions. Apart from it forest is a suitable habitat for a number of plants and animal species.

A healthy tree trunk has highly economic value, but if it is hollow or damaged by insects, its economic value reduces. Among many pests related to damage of timber trees, one of them is *Sahyaddrassus malabaricus*, Moore. It is a stem borer which bores and destroys the tree trunks. It attacks various trees including *Gmelina arborea* (Gamhar), *Tectona grandis* (Teak), *Terminalia arjuna* (Arjun) *Shorea robusta* (Sal), *Dalbergia sisso* (Shisam), *Albizzia lebbek* (Siris) causing economic loss.

The present study deals with the preliminary study on *Sahyaddrassus malabaricus*, Moore. synonym (*Phassus malabaricus*, Moore) as a polyphagous pest of some important trees in Jharkhand. A survey was made for continuous three years to study their intensity of damage in the tree trunks. The life history of the pest was studied in laboratory conditions and in natural condition and detailed studies were made.

The larvae cause damage to saplings of various tree species by boring into the stem. Attacked saplings can be easily recognised by the dome- shaped mass of woody particles covering the point of attack. When this cover is removed, a large borer whole can be seen, which extends down along the central core of the stem.

Beeson (1941)[1], Browne (1968)[2], Fletcher (1914)[3], Lefroy (1909)[4], Nair (1982,87)[5,6,] Raja (2018)[7] reported *Sahyaddrassus malabaricus*, Moore from South India. A survey was made continuously during my research period and since then up to recent years the insect is continuously seen attacking the saplings of different trees.

In the present work, studies were made on the biology and ecology of the insect and its pest status.

MATERIALS & METHODS

Samples of insect pests were collected from different protected forest areas under Birsa Agricultural University, Kanke, Ranchi, regularly for three years.

The sites selected were -

1. Rarha Forest Research Station (B.A.U.), Ranchi
2. Kumharia Agroforestry Plantation, (B.A.U.), Ranchi
3. Road side plantations from Kanke to Pithoria, Ranchi.
4. Plantations in Ratu and Morahabadi, Ranchi.

Details of each survey were noted in a performa as per requirement. During collection of pest specimens notes were made to find out the emergence period of the pests and their feeding habit. Insects brought from field were kept in separate containers along with insect card which indicated -

1. Field collection number
2. Plant host
3. Stage of insect collected
4. Date of collection of larva , pupa and adult
5. Date of further changes in life stages (from egg to adult)
6. Breeding period

The host plants selected for study were *Gmelina arborea, Tectona grandis, Terminalia arjuna, Shorea robusta, Dalbergia sisso, Albizzia lebbek, Eucalyptus* etc. Regular observations were made to study its life cycle. Moth traps were set up in infested saplings of the trees. It was very difficult to rear the insect in the laboratory, because most of the larvae not survived in laboratory conditions. Only few larvae survived that helped in studying life history. Simple traps were developed to determine the emergence period of the moth. It consisted of a piece of plastic netting stitched with cloth border and provided with strings on two sides. It was tied around the stem of the infested sapling and the open end held together by staples. Many larvae cut the trap but many adult moths were also captured by this method. Larvae and pupae were also collected by cutting the branches of infected saplings.

OBSERVATIONS

The larva excavates a long cylindrical tunnel similar to the diameter of the larva. The top position of the tunnel is curved and opens to the outside. Normally the larva rests with head towards the tunnel mouth. It moves rather forwards or backwards with equal ease. The mouth of the tunnel is covered by a mat work consisting of coarse saw dust like particles of wood and bark, spun together with silk secreted by the larva. Dried faecal pellets and moulted head capsule are often attached to this mat. Initially small particles mat cover is gradually extended to more cover area. The larva feeds on the callus tissue that develops around the tunnel mouth. Feeding takes place at night under the cover of mat particles. If the mat cover is removed, it is rebuilt within a day. Rebuilding takes place at night, as the larva is active at night. This dome shaped mat cover of bark particle is a conspicuous sign of infestation by the *Sahyaddrassus malabaricus*.

In most cases, the damage consisted simply of localised bark feeding and tunnelling of the central pith, which causes growth retardation. They have the potential of infesting the stem if they are more in number.

LIFE HISTORY

EGGS

Eggs are rounded. They are produced in very large numbers. These are holometabolous. It undergoes complete metamorphosis with the stages of eggs, larva, pupa and adult.

LARVA

The small caterpillars establish themselves by boring into living saplings.

Usually the stems of sapling is inhabited by only one caterpillar which bores into the centre and excavates a long cylindrical tunnel running axially downwards as far as the roots. The tunnel may reach a length more than a foot and about 0.5 cm in diameter. The tunnel is slightly curved over at its top end. The full grown larva is cylindrical and about 3 inches in length and yellow with a black corrugated hemispherical head.it is able to move rapidly up and down the tunnel. A thick plug of interwoven brownish thread is spun to block the way while it is in the pupal stage.

PUPA

The pupa possesses ridges and teeth like asperities on the movable abdominal segments by means of which it is able to wriggle its way up the tunnel and pushed through the mat. When half projecting the pupal skin splits and the moth emerges leaving the empty skin entangled in the mat. The pupal period lasts about three weeks. The length of the pupa is almost equal to the length of the larva and is reddish brown in colour.

ADULT

Mostly the adult moths emerge in April last week to mid of June. The moths are greyish brown in colour with a wing span of about 5 cm. At rest, the moth hangs vertically on leaves or stems suspended by long flattened hairy legs and resembles dry leaf.

Control Measures

The insect is naturally controlled by the predators like birds. Sometimes ants were also found to attack on their tunnel. In the laboratory some larvae died with fungal attack. Contact poison is also effective for killing the larva in tunnel, but in large areas it is not easy to kill all the larvas. Natural control is more effective in control of pest.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Figure showing life cycle of *S. malabaricus* (Moore) 1. Caterpillar bore into stem, 2. Dome shaped mass of woody praticles covering the point of attack, 3. Larva starts pupating, 4. Pupa, 5. A full-grown larva, 6. moth

CONCLUSION

The survey and study of insect pest *Sahyaddrassus malabaricus*, Moore was done in last three years and during my research work. In both cases it was found that the stem borer is infesting the saplings of many forest trees like *Gmelina arborea* (Gamhar), *Tectona grandis* (Teak), *Terminalia arjuna* (Arjun) *Shorea robusta* (Sal), *Dalbergia sisso* (Shisam), *Albizzia lebbek* (Siris), *Eucalyptus*. It also infested guava tree. The insect is distributed throughout Jharkhand and is a polyphagous pest.

The damage caused by *Sahyaddrassus malabaricus*, Moore is not serious. The larva feeds on the bark and callus tissue. Although in most cases the damage is economically negligible. But control measure is necessary to save the plant with any type of infestation by the stem borer.

ACKNOWLEDGEMENT

The author is grateful to Forest Research Institute, Dehradun and Zoological Survey of India, Kolkata for identification of insects and Faculty of Forestry, BAU Kanke, Ranchi for providing research facilities at their institute.

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RUJOST, ISSN: 2319-4227, Vol.7, No.1, January, 2022, pp. 179-184

A Biaanual Peer Reviewed Referred Ranchi University Journal of Science & Technology

Zoology

Study on the food preservative effect of chitosan extracted from mushroom *Termitomyces heimii*

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Abstract: Chitin is the second most abundant natural polysaccharide after cellulose and is present in the crustacean exoskeleton, insects and fungi. Chitosan, the deacetylated chitin derivative, is a more useful and interesting bioactive polymer. Chitosan has attracted significant interest in a broad range of applied biomedical food, and chemical properties. The film forming property makes it a potential source of food preservative. This study aims at extraction of chitosan from mushroom, *Termitomyces heimii* and its use as a food preservative. The results showed that increasing the concentration of chitosan coating enhanced the beneficial effects of chitosan on extended shelf life and maintained quality of fruits and mushroom.

Keywords: Chitin, Chitosan, *Termitomyces heimii,* deacetylation

INTRODUCTION

Freshness of fruits and vegetables is an important criterion that dictates which product a consumer prefers to buy in the market. Supermarkets face challenges to keep the fruits and vegetables fresh and offer consumers better quality products. The fruits and vegetables are bio degradable and prone to microbial attack. Challenges involving natural ripening and the degradation process of the fruits and vegetables, mainly through enzymatic reaction, are an important concern for food industries. The fruits and vegetables are sensitive to decay and perish, due to rapid ripening and softening, which limits their storage, handling and transport potential.[1] Characteristics that lower the products quality, such as browning, off-flavour development and texture breakdown, are commonly seen on microbiologically spoiled food. Therefore, acceptable methods of preservation are top priority in the food industry. Coating the fruits and vegetables with biocompatible nonallergic polymers is a good choice for preservation. Inadequate and costly solutions for food preservation have led scientists to create natural preservatives which are safe, effective, and acceptable. Keeping in mind relatively long-time storage and transportation, use of biologically derived preservatives with compliance to health and safety regulations can bring a great solution for preservation of the fruits and vegetables.[2]

Edible coating, an innovative method of food preservation, produces physical barriers on the surface of the fruits and vegetables that cause moisture and solute to migration, gas exchange, respiration and oxidative reaction rates reduced for extending the shelf-life.[3] Biopolymer coating materials are formulated to carry active ingredients such as antibrowning agents, colorants, flavours, nutrients, spices and antimicrobial compounds to extend product shelf life and reduce the risk of pathogen growth on food surfaces.[4]

Chitin, poly-β-(1-4)-N-acetyl-D-glucosamine, is a cellulose like biopolymer distributed widely in nature, especially in marine invertebrates, insects, fungi and yeasts. Chitin and its deacetylated form, Chitosan, have attracted significant interest in a broad range of applied biomedical, food, and chemical properties. Use of Chitosan in food

industry is readily seen due to its several distinctive biological activities and functional properties. The antimicrobial activity, antioxidant activity and film forming property make it a potential source of food preservative. Since the use of chitosan as a food preservative is environment friendly and also non-toxic, hence its use as a food preservative will be a great help to the food industry.

MATERIAL & METHODS

1. Collection of the mushroom

Termitomyces heimii was purchased from the local market of Ranchi, Jharkhand. The fruit bodies were cut in the laboratory and washed thoroughly to remove mud. They were dried at 50°C and kept.

2. Extraction of Chitosan from mushroom T. heimii by Erdogan, Kaya & Akata, 2017 method:

Chitin production:

Fruit bodies of mushroom samples were washed and dried in oven at 50°C until dried completely. Then the fruit bodies of the mushroom sample were grinded using a 7-speed blender till fine powder was formed. The isolation of mushroom glucans was performed by modifications of the methodology proposed by Carbonero *et al.*, (2012)[5]. Mushroom powder was treated with distilled water at 50°C 24 h (10 ml per g of sample). After centrifugation super natants were used for chitin isolation. 2M hydrochloric acid in the ratio of 1:5 was used for removal of minerals at 60°C 17 h. Then, after a washing with distilled water until a neutral pH, the deproteinization step was performed using 2M sodium hydroxide solution at 85°C 24 hours. Later the samples were treated with the mixture of chloroform, methanol and distilled water (1:2:4) for decolorization. At the end, after washing and bleaching two hours with 1.5 wt % H_2O_2 aqueous solution (30 ml per g of sample), the samples were washed again with distilled water until a neutral pH was reached and dried at 100°C.

Chitosan production:

The production of chitosan was performed by the methodology described by Galed *et al.* (2005)[6]. The chitin powder was refluxed with 60% NaOH (10 ml per 1 g of chitin) at 100°C for 4 hours. After filtration and washing the obtained chitosan sample was dried at 100°C.

3. The Characterization of chitosan:

The Characterization of chitosan was done by following parameters-

A. Solubility test:

Quality of the chitosan produced was checked by a solubility test with 1% Acetic Acid. Chitosan dissolves completely in 1% Acetic Acid. For the estimation of chitosan produced, the sample was taken out of the storage and weighed. Then the sample was put inside a clean beaker and 10 to 20 ml of 1% acetic acid was added to it. The solution was kept in BOD shaker for 30 to 40 minutes. Then the sample was taken out and weighed, carefully.

B. Degree of deacetylation

The chitosan samples extracted from *Termitomyces heimii* were sent to CIF, BIT Mesra, Ranchi, Jharkhand for FT-IR experiment. Infrared spectroscopy was performed for chitosan sample, extracted from *Termitomyces heimii* using the FT-IR 1720X.

Degree of deacetylation of chitosan was measured using a previously reported method of Brugnerotto (2001)[7]. The Degree of deacetylation was calculated using the graph obtained from FTIR Spectroscopy.

Degree of Deacetylation (DDA%)= 100-Degree of acetylation (DA%)

$$\text{Degree of acetylation (DA\%)} = 31.92 \times \frac{A_{1320}}{A_{1420}} - 12.20,$$

Where, A_{1320} and A_{1420} represent the absorbance at 1320 and 1420 cm^{-1} respectively.

Absorbance= 2- log (%T)

4. Study of food Preservative Effect of the chitosan samples extracted:

The use of chitosan is widely investigated as an edible coating, which is defined as the formation of a thin film directly on the surface of the product they are intended to protect.

Edible coatings were prepared by dissolving 0.5g, 1.0g and 1.5g of crab shell chitosan and fungal chitosan respectively in 100ml of DW that contained 0.7ml of acetic acid (pH 4.8) at room temperature.[8]

- Materials:
  1. Fruits with thick outer layer: Cucumber, Tomato
  2. Fresh button mushrooms
  3. Chitosan sample obtained from mushroom.
- Method for preparation of fruit samples:

Five fresh Cucumbers & Tomatoes were taken and washed thoroughly with tap water. Two sets of experiments were devised: set-I using cucumber as test material and set II using Tomato. One cucumber and one tomato were kept as Control at room temperature (25°C). One cucumber

and one tomato were coated with 1% Acetic Acid. Other two cucumbers & tomatoes were coated by 0.5g and 1.5g Chitosan/100ml -1 Tomato, 1 Cucumber and kept at room-temperature for 3 weeks, and One cucumber and one tomato were kept in refrigerator (4°C). For each treatment, three replicates were used.

♦ Method for Preparation of Fresh-cut Mushroom Samples:

Common cultivated button mushrooms were obtained from a commercial market selected for uniformity and size. Any bruised or diseased mushroom was discarded. Mushrooms were washed and sliced to a thickness of 4 mm (six slices per mushroom) with a sharp stainless knife and then dipped for 1 min in the solutions containing 0.5, 1.0, or 1.5g chitosan/100 mL. The slices treated with 0-g chitosan solution were used as control. After being air-dried at room temperature for another 30 min, the slices were placed into thick polyethylene films bag and then stored at 4°C for progressive assessments. The control and chitosan-treated mushroom samples were stored for 15 days at 4°C and analyzed. For each treatment, three replicates were used.

RESULT & DISCUSSION

1. Sample preparation

The results of sample preparation are presented in table 1 and 2. The sample preparation included extraction of chitin and chitosan. For each treatment used (Table 1), the mushroom chitosan, the weight of mushroom taken was 3000g, while the weight of the fruit bodies of the mushroom taken was 950g. The sample preparation included dry weight and the moisture content of the fruit bodies of the mushroom. The percent yield of chitin from dry weight of fruit bodies was 8.84%.

Table 1- Body Weight, wet and dry weight of fruit bodies of *T. heimii*, weight of Chitin and Chitosan from fruit bodies of *T. heimii*

Batch	Weight of mushroom (g)	Wet weight of Fruit bodies (g)	Dry weight of Fruit bodies (g)	Moisture content of Fruit bodies %	Weight of powder of dry Fruit bodies (g)	Weight of Chitin (g)	Weight of Chitin used for chitosan Production (g)	Weight of Chitosan from Chitin (g)
1	1000	300	25.25	91.58	25	2.38	1.50	1.20
2	1000	350	29.47	91.50	29	2.56	1.50	1.10
3	1000	300	26.04	91.32	26	2.20	1.00	0.84
Total	3000	950	80.76	91.49	80	7.14	4.00	3.14
Mean	1000	316.67	26.92	91.49	26.67	2.38	1.33	1.04

Table 2- Percentage Yield of Chitosan

Sample	Weight of Chitin used for chitosan Production (g)	Weight of Chitosan from Chitin (g)	Percent Yield of Chitosan (%)
Fruit bodies of *T. heimii*	4.00	3.14	78.50

Table 3- Concentration of Chitosan in 1% Acetic Acid

Sample	Initial weight of chitosan taken (g)	Volume of 1% Acetic Acid (ml)	Total dissolved weight of chitosan (g)	Concentration of Chitosan in 1% Acetic Acid (grams/ml)
Fruit bodies of *T. heimii*	1.00	45	0.68	0.0151

2. Characterization of the chitosan samples extracted:

A. Solubility test

It was observed that the Concentration of Chitosan from mushroom in 1% Acetic acid was 0.0151 g/ml.

B. Degree of deacetylation

The Degree of deacetylation was calculated using FTIR Spectroscopy and the result after calculations is:

Degree of deacetylation = 84.98%

The degree of deacetylation is the ratio of the number of glucosamine groups to the overall N-acetylglucosamine and glucosamine groups.[9] The factor effects major properties of chitosan such as solubility, viscosity, flexibility.[10]

3. Food Preservative effect

♦ **Mushroom Set:**

It was observed that the control kept at 4°C had started to show a sign of spoilage by the development of dark spots by the fifth day of storage and went smelly after 7 days. The ones coated with 1% Acetic Acid looked fresh for a week, then they were slimy after 2 days, and hence removed from the storage. The experimental sets coated with the fungal chitosan (0.5g/100ml, 1.0g/100ml, 1.5g/100ml) showed no sign of spoilage after 8 days of storage and looked fresh with no spots. On the other sets, no signs of fungal or microbial attack were observed.

♦ **Cucumber Set:**

It was observed that the control started to spoil by fungal infection by the 5th day and by 11th day, it was completely rotten. The control kept in refrigerator stayed fresh for about 12 days and then showed signs of spoilage. The cucumber coated with 1% Acetic acid showed similar spoilage pattern as that of the control at room temperature. The experimental sets coated with 0.5g/100 ml of fungal chitosan, were fresh till 12 days, and then by 17th day, it was completely rotten. The fungal chitosan (1.5g/100ml) showed a good preservative effect by keeping the fruits fresh for about 20 days at room temperature.

♦ **Tomato Set**

It was observed that the control started to spoil by fungal infection by the 5th day and by 7th day, it was completely rotten. The control kept in refrigerator stayed fresh for about 14 days and then showed signs of spoilage. The tomato coated with 1% Acetic acid showed similar spoilage pattern as that of the control at room temperature. The experimental sets coated with 0.5g/100 ml of fungal chitosan were fresh till 14 days, and then by 17th day, they showed some signs of spoilage. Fungal chitosan (1.5g/100ml) showed a good preservative effect by keeping the fruits fresh for about 17-20 days at room temperature.

Table 4- Observation for mushroom set

Set	Day 3	Day 5	Day 7	Day 9	Day 11
Control	Unchanged	Started to spoil, dark spots seen	Smelly, and totally rotten	-	-
1% Acetic Acid	Unchanged	Unchanged	Little slimy	Slimy, and totally rotten	-
Fungal Chitosan - 0.5g/100ml	Unchanged	Unchanged	Unchanged	Unchanged	Dark spots appear
Fungal Chitosan - 1.0g/100ml	Unchanged	Unchanged	Unchanged	Unchanged	Dark spots appear
Fungal Chitosan - 1.5g/100ml	Unchanged	Unchanged	Unchanged	Unchanged	Looks Fresh

Table 5- Observation for cucumber set

Set	Day 3	Day 5	Day 7	Day 11	Day 14	Day 17	Day 21
Control (At room temperature)	Unchanged	Started to spoil	Spoiled by fungus	Totally rotten	-	-	-
1% Acetic Acid	Unchanged	Unchanged	Spoiled	Totally rotten	-	-	-
Fungal Chitosan - 0.5g/100ml	Unchanged	Unchanged	Unchanged	Unchanged	Started Change in color	Shrink skin	Totally Rotten
Fungal Chitosan - 1.5g/100ml	Unchanged	Unchanged	Unchanged	Unchanged	Unchanged	Unchanged	Ripe
At 4℃	Unchanged	Unchanged	Unchanged	Unchanged	Spoiled by fungus	-	-

Table 6- Observation for tomato set

Set	Day 3	Day 5	Day 7	Day 11	Day 14	Day 17	Day 21
Control (At room temperature)	Unchanged	Started to ripe	Ripe and rotten with shrink skin	-	-	-	-
1% Acetic Acid	Unchanged	Started to ripe	Ripe and rotten with shrink skin	-	-	-	-
Fungal Chitosan - 0.5g/100ml	Unchanged	Unchanged	Unchanged	No Spoilage	No Spoilage	Started to ripe	Rotten
Fungal Chitosan - 1.5g/100ml	Unchanged	Unchanged	Unchanged	No Spoilage	No Spoilage	No Spoilage	No Spoilage
At 4℃	Unchanged	Unchanged	Unchanged	Unchanged	Started to ripe	-	-

CONCLUSION

The conclusion from the above study is that the application of chitosan coating (with optimum concentration 1.5%) could be beneficial and can be considered for commercial application in extending shelf life and maintaining quality and to some extent, controlling the decay of fruits and mushroom. In order to use chitosan for preservation of fruits and mushroom, we consider that chitosan coating to control discoloration and decay of fruits and mushroom stored for longer periods (e.g. 10 days for mushroom and 15-20 days for thick walled fruits) could be better.

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RUJOST, ISSN: 2319-4227, Vol.7, No.1, January, 2022, pp. 185-188

A Biaanual Peer Reviewed Referred Ranchi University Journal of Science & Technology

Zoology

Determination of MIC of mucus of freshwater snail *Bellamya bengalensis* (Jousseaume, 1886) on human pathogen

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Abstract: Freshwater edible snail *Bellamya bengalensis* has potential nutritional and medicinal value. Snail mucus has been applied in human medical and cosmetics and mucus exhibits various biological activities, such as antimicrobial, antioxidant, anti-tyrosinase, and antitumoral activities. In present work MIC of mucus of *B. bengalensis* against *E. coli* was studied. Azithromycin drug was used as antibiotic control. Different concentration of mucus of *B. bengalensis* were taken (0.7µl/ml to 90µl/ml). The lowest concentration of mucus that inhibited the visible growth of *E.coli* was 11.25µl/ml was recorded as MIC value of snail's mucus of *Bellamya bengalensis*.

Keywords: *Bellamya bengalensis,* mucus, Azithromycin, MIC, *E.coli* etc

INTRODUCTION

In modern society, Zootherapy constitutes an important alternative among many other known therapies practice worldwide.[1] Therefore people are looking for traditional remedies for treatment of ailments. Snails are good for health and considered a delicacy for connoisseurs all over the world. They are increasingly appreciated to their culinary and nutritional value as they are comprised of nutrients high in essential amino acid and beneficial fatty acids, as well as being low in calories. The latest studies have found that snail meat is one of the positive nutritional aspects of Mediterranean diet and snails are good source of vitamins and minerals. It contains good amounts of calcium, phosphorus, iron, sodium, potassium, magnesium, manganese, zinc and also selenium. Biochemical constituents and minerals assist to supply energy required for the body to carry out physiological function.

The snail slime (mucus) has many functions in the animal, such as adhesive, emollient, moisturizing, lubricant, and defense. Recently, snail mucus has been applied in human medical and cosmetics. Studies have shown that snail mucus exhibits various biological activities, such as antimicrobial, antioxidant, anti-tyrosinase, and antitumoral activities, anti-leukemic, antibacterial, antiviral activities have been reported worldwide.[2] In addition, many compounds have been found in snail mucus, such as allantoin, hyaluronic acid, peptides and proteins.[3] Moreover, it has been reported that different kinds of mucus are released from the different types of secretory glands in a snail, depending on the way it is stimulated.[4]

MATERIAL & METHODS

Determination of MIC of antibacterial agent (Mucus) by Serial-dilution method[5].

Extraction of mucus[6]:

- Snails were thoroughly cleaned with cleaned napkin to remove all the sand and debris on the shell. The mucus was extracted from the snail by removing the shell with a sterile sharp end metal rod and the mucus aseptically squeezed out from the soft body and collected into a beaker. The

extracted mucus was considered 100% concentration and was stored in the refrigerator at 4°C for biochemical and antibacterial analysis.

- Collection of drug and organism: The azithromycin drug was collected from RIMS. *Escherichia coli* ware collected from the patient sample of RIMS from Microbiology department, Ranchi.
- MIC determination procedure: Muller Hinton broth culture of *Escherichia coli* at 37°C was prepared. The culture was adjusted to obtain turbidity comparable to that of the turbidity of Mc. Farland 0.5 Standard. The inoculums were prepared to obtain 105-106 cfu/ml. 90 mg azithromicin drug was dissolved in 1 ml distilled water for ½ an hour10 sterile tubes were taken and were labeled each 1 to 8 and first tube as AC (Antibiotic control) and last one was labeled as GC (Growth control).
- 1ml of Brain heart infusion (BHI) broth was added in each test tube AC to GC. With sterile micro pipette and tips 90µl of mucus was mixed with 1 ml distilled water and added to test tube no 1. Half quantity of solution was added to next tube (tube no-2). This procedure was repeated through next to next up to the tube no-8 except tube no GC. AC tube which contained 1ml BHI broth and 1ml antibiotic azithromycin (which was dissolved in distilled water) was added.
- From tube no 8, 1ml solution was removed and discarded. The last tube GC received no mucus only 1ml BHI broth added and was served as a growth control. First AC labeled test tube was served as antibiotic control. Each tube was inoculated (including growth control except antibiotic control) with one ml of culture of *Escherichia coli.*
- The tubes were incubated at 37°C for 24 hours. The tubes were examined for bacterial growth and MIC was determined. The tubes were examined for visible cloudy and was recorded growth as +ve and visible clearly no growth as -ve. The OD of all tubes was measured in colorimeter at 540nm. OD of distilled water (blank) and antibiotic control was 0 (zero) and 0.031 respectively.

RESULT

Determination of MIC of antibacterial agent (Mucus) by serial-dilution method.

Table 1- Minimum inhibitory concentration (µl/ml) of mucus of *Bellamya bengalensis* against *E. coli* by serial-dilution method at OD=0.052 of Growth control.

Concentration of antibiotic 90mg/ml broth					
No	**Inculation**	**Concentration of mucus µl/ml broth**	**OD**	**% Growth**	**% Inhibition**
AC	Medium+antibiotic		0.031		
1	Medium+mucus+*E.coli*	90	0.032	61.53	38.47
2	Medium+mucus+*E.coli*	45	0.037	71.15	28.85
3	Medium+mucus+*E.coli*	22.5	0.041	78.84	21.16
4	Medium+mucus+*E.coli*	11.25	0.049	94.23	5.77
5	Medium+mucus+*E.coli*	5.6	0.058	111.53	-11.53
6	Medium+mucus+*E.coli*	2.8	0.063	121.15	-21.15
7	Medium+mucus+*E.coli*	1.4	0.070	134.61	-34.61
8	Medium+mucus+*E.coli*	0.7	0.079	151.92	-51.92
GC	Medium+*E.coli*		0.052		

% Inhibition showed growth of bacteria. OD of AC= 0.031 and OD of GC= 0.052.

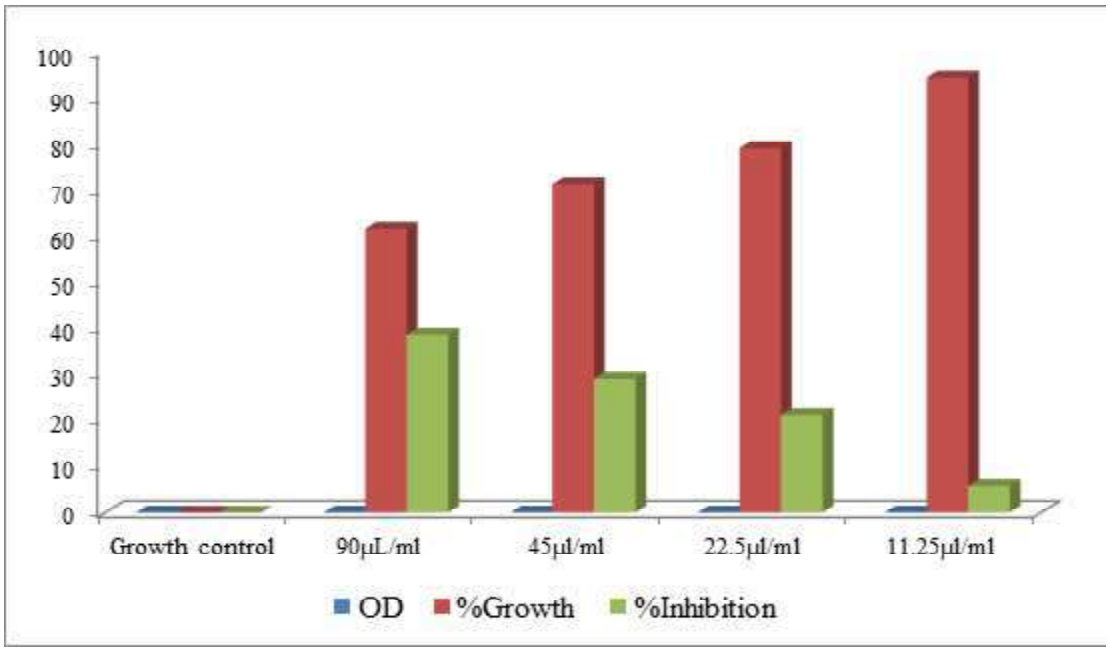

Fig. 1- MIC values for mucus of snail *B.bengalensis* against *Escherichia coli.*

Table-1, fig-1 showed MIC of mucus of *B. bengalensis* against *E.coli*. Different concentration of mucus of *B. bengalensis* were taken (0.7µl/ml to 90µl/ml). The lowest concentration of mucus that inhibited the visible growth of *E.coli* was 11.25µl/ml was recorded as MIC value of snail's mucus of *Bellamya bengalensis*.

DISCUSSION

The aim of this research work was to evaluate the minimum inhibition concentration (MIC) of mucus of snail. MIC of mucus of *B. bengalensis* against *Escherichia coli* was determined using serial-dilution method. Different concentration of mucus of *B. bengalensis* were taken (0.7µl/ml to 90µl/ml). The lowest concentration of mucus that inhibited the visible growth of *E. coli* was 11.25µl/ml that was recorded as the MIC value (table-1, fig-1). Similar study was also made by Harekrishna *et al.* (2017)[7]on comparative study on the antimicrobial activity of freshwater mollusks *Bellamya bengalensis* and marine mollusks *Saccostrea cucullata* and reported that, the MIC value of lyophilized body fluid of *Saccostrea cucullata* exhibit inhibitory activity mostly against bacterial strain *Bacillus subtilis* and fungal strain *Candida albicans* but the value was lower i.e. 4mg/ml than standard antibiotic.

Borquaye[8] made study on antimicrobial and antioxidant properties of the crude peptide extracts of *Galata paradoxa* and *Patella rustica* and reported that the MICs of *Galata paradoxa* and *Patella rustica* towards the various test microorganisms ranged from 20 to 13mg/ml. *Galata paradoxa* extracts recorded highest MIC of 20mg/ml towards *Candida albicans,* with all other microorganisms giving MIC of 17mg/ml. For *Patella rustica,* MIC of 13mg/ml was recorded for *Candida albicans* those of *Enterococcus faecalis* and *Klebsiella pneumoniae* were both 17mg/ml. All other microorganisms have MIC of 20mg/ml. Similar study also made by Kowser[5] according to them MIC values of selected azithromycin capsule against *Staphylococcus aureus* were 0.5mg/ml to 8mg/ml. Nadia (2005)[9] showed that the MIC range of tetracycline, ciprofloxacin and azithromycin for *Staphylococcus aureus* were 0.12mg/ml to 32.0mg /ml.

CONCLUSION

Based on the obtained results, the study confirms that snails *B. bengalensis* could be a source for antibacterial agents they can serve as an alternative to the expensive synthetic antibacterial agents used in bacterial treatment.

ACKNOWLEDGEMENTS

Authors are thankful to Head of Microbiology Department, Rajendra Institute of Medical Sciences, Bariyatu, Ranchi, Jharkhand for providing all lab facilities.

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Submission of Manuscripts : Only original papers are considered for publication. The authors should declare that the manuscript has not been submitted to any another journal for consideration at the same time. Three copies of manuscripts (one original and two photocopies), complete in all aspects including figures and tables, should be submitted to the respective Heads of the Department.

The authors must ensure that the manuscript has been prepared strictly according to the Journal guidelines in all aspects, failing which the manuscripts may not be processed.

Preparation of Manuscripts : The manuscripts should be prepared only in English and should be printed only on A-4 size paper, with double spacing and minimum 2.5 cm margin on all the four sides. First page of manuscripts should be the title page containing full title of the paper, Surname of author(s) and First name(s), Name of the laboratory where the work has been carried out and also the address to which all correspondence concerning the manuscript should be made. E-mail address of the corresponding author should be mentioned in the title page. The title of the manuscript should be brief, specific and amenable to indexing.

A short running title should be given at the end of the title page. The text of the manuscript should run **into Abstract, Introduction, Materials and Methods, Results, Discussion, Conclusion, Acknowledgment** (if any) and **References** or other suitable heading in case of reviews and theoretically oriented paper. Short research communications not exceeding 150 words can also be entertained in running format.

Abstract should be followed by keywords which should not exceed six words. The figures should be drawn on MS-Word sheet with the legends provided on the same sheet. Photographs should be black and white or coloured embedded on a MS-Word sheet.

Tables should be typed on separate sheets bearing a short title, preferably in horizontal firm_ Scanned copies of Table and Photographs will not be entertained.

References should be numbered in superscript serially in the order in which they appear in manuscript References should not include unpublished source materials. The list of References at the end of the text should be in the following format:

1. **Feyissa T., Welander M. & Nagesh L. (2005).** Micropropagation of *Hagenia abyssinica*: a multipurpose tree. *Plant Cell and Tissue Culture*. **80(2):** 119-127
2. **Rathore P., Suthar R. & Purohit S. D. (2008).** Micropropagation of *Terminalia bellerica* Roxb. from juvenile explants. *Indian Journal of Biotechnology*. **7(2):** 246-249

No part of the manuscript should be in Italic except for Latin names of organisms (genus and species), special terms, local names, local terms and names of books and journals in reference section. If abbreviations are absolutely necessary, they should be placed in parentheses after the words/ phrases where they appear first in the text.

Only metric units (SI Units) should be used. Other units may be given in parentheses only if absolutely necessary.

Abbreviations of units are same for the singular and plural. Greek letters and unusual symbols should be identified separately in the margin. Distinction should be made between confusing letters like letter O and zero (0) and between K and *kappa* (k).

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Papers should be submitted to the Dean, Faculty of Science, Ranchi University, Ranchi-834001, Jharkhand. India.

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