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Advances in Microbiology

Volume II

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Dr. M. Poornima
Aquatic Animal Health and Environment Division, ICAR-Central Institute of Brackishwater aquaculture, Chennai, Tamil Nadu

Dr. Sunita Ramlu Mukkawar
Department of Microbiology, B. Raghunath ACS College, Parbhani, M. S.

Dr. Kavita Chahal
Department of Botany, Government College Bichhua, Chhindwara, M. P.

Dr. Sulochana Ramsing Rathod
Department of Botany, Government College of Arts and Science, Aurangabad, M. S.

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PREFACE

We are delighted to publish our book entitled ***"Advances in Microbiology Volume II"****. This book is the compilation of esteemed articles of acknowledged experts in the fields of microbiology and life science providing a sufficient depth of the subject to satisfy the need of a level which will be comprehensive and interesting. It is an assemblage of variety of information about advances and developments in microbiology and life science. With its application oriented and interdisciplinary approach, we hope that the students, teachers, researchers, scientists and policy makers will find this book much more useful.*

The articles in the book have been contributed by eminent scientists, academicians. Our special thanks and appreciation goes to experts and research workers whose contributions have enriched this book. We thank our publisher Bhumi Publishing, India for compilation of such nice data in the form of this book.

Finally, we will always remain a debtor to all our well-wishers for their blessings, without which this book would not have come into existence.

- Editors

Advances in Microbiology Volume II

CONTENTS

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INFORMATICS IN CLINICAL MICROBIOLOGY

Anubhuti Jha
P.G. Department of Biotechnology and Microbiology,
St. Thomas College Bhilai, Durg, Chhattisgarh
Corresponding author E-mail: anubutijha21@gmail.com

Abstract:

From defining the causative agent in a patient's infection to assisting in the detection of worldwide disease outbreaks, the clinical microbiology laboratory has a wide range of tasks. All of these procedures are getting increasingly intertwined with informatics. The use of informatics technologies effectively can improve the accuracy, timeliness, and completeness of microbiology testing while reducing laboratory burden, resulting in improved laboratory efficiency and lower costs. With the advent of comprehensive laboratory automation, complicated instrument interfaces, electronic health records, clinical decision support systems, and the practical use of microbial genome sequencing, informatics is poised to become increasingly relevant in clinical microbiology. From defining the causative agent in a patient's infection to assisting in the detection of worldwide disease outbreaks, the clinical microbiology laboratory has a wide range of tasks. All of these procedures are getting increasingly intertwined with informatics. The use of informatics technologies effectively can improve the accuracy, timeliness, and completeness of microbiology testing while reducing laboratory burden, resulting in improved laboratory efficiency and lower costs. With the advent of comprehensive laboratory automation, complicated instrument interfaces, electronic health records, clinical decision support systems, and the practical use of microbial genome sequencing, informatics is poised to become increasingly relevant in clinical microbiology.

Introduction:

Since the introduction of genomes, transcriptomics, and proteomics, microbiology has expanded in relevance tremendously, allowing researchers to better understand microbial biogeochemical processes and their interactions with macroorganisms in both health and disease. Microorganisms, their activity, and metabolites have profound influence on the functioning of humans and the entire biological world, as they are the biosphere's life-support system. Microbiology, the study of interactions between micro- and macro-organisms in health and illness, is an area of the biological sciences that has exploded in popularity since the introduction

of genomes, transcriptomics, and proteomics. Applied microbiology is the branch of biology concerned with the use of microorganisms in specific endeavours such as crop improvement, food and supplement production, fermentation-based chemical and biomaterial production, natural resource recovery and energy production, waste treatment and bioremediation of polluted sites, drug development, vaccine development, diagnostic tools, and biosensor systems, and the production of drugs, vaccines, diagnostic tools, and biosensor systems [1].

The clinical microbiology laboratory's tasks include everything from determining the cause of a patient's infection to assisting in the detection of worldwide disease epidemics. All of these procedures are getting increasingly intertwined with informatics. The use of informatics technologies effectively can improve the accuracy, timeliness, and completeness of microbiology testing while reducing laboratory burden, resulting in improved laboratory efficiency and lower costs. With the advent of comprehensive laboratory automation, complicated instrument interfaces, electronic health records, clinical decision support systems, and the practical use of microbial genome sequencing, informatics is poised to become increasingly relevant in clinical microbiology [2].

The microbiology laboratory information system, decision support tools, expert systems, instrument interfaces, total laboratory automation, telemicrobiology, automated image analysis, nucleic acid sequence databases, electronic reporting of infectious agents to public health agencies, and disease outbreak surveillance are all covered in this review. Clinical microbiology informatics technologies have become vital in modern clinical and laboratory practise due to their scope and usability. In the future, continued technological advancements and the development of these informatics tools will improve patient and public health care even more [3].

The responsibilities of the local clinical microbiology laboratory range from characterising the causative agent of a patient's infection to assisting in the detection of worldwide disease epidemics. These procedures are becoming more complicated. Every laboratory is required to maintain a high level of quality while increasing efficiency. Clinical microbiology laboratories are being asked to conduct more work, identify more germs, report complicated and changing drug-related information, automate operations, integrate traditional lab data with molecular results, and assist in public health reporting and epidemic monitoring [4].

The use of information (e.g., data, knowledge, and results) and information tools (e.g., software, databases, and rules) in the "science and service dealing with detection, identification, and antimicrobial susceptibility testing" of clinically relevant microbes and the communication of these results to clinicians" is referred to as "clinical microbiology informatics" (8). A more

practical definition includes the use of information technology (IT) to improve workflow, efficiency, dependability, and, ultimately, patient care in clinical microbiology. It's crucial to note that informatics isn't only about technology; it also encompasses the people who use, build, and manage information systems, as well as the workflow processes that are influenced by it[5]. The various informatics components that are specifically important to the clinical microbiology laboratory are discussed in this review. The microbiology laboratory information system (LIS), decision support tools, expert systems, instrument interfaces with the LIS, total laboratory automation, remote and automated image analysis, nucleic acid sequence databases, infectious agent reporting to public health agencies, and outbreak surveillance are all covered in detail. This review does not go over all of the systems, algorithms, and published papers in detail. Instead, the focus of this essay is on the numerous links that informatics and clinical microbiology have, as well as the potential benefits that might be gained by increasing the use of informatics tools in clinical microbiology [6].

Some of these informatics solutions have only been implemented in a few hospitals or laboratories, whereas other informatics components are more likely to be familiar to many microbiologists since they are ingrained in clinical microbiology practise. It's critical to keep looking for innovative informatics technologies that have the potential to improve clinical microbiology care efficiency, accuracy, cost, and quality.

Expert systems:

Artificial intelligence takes the form of an expert system. It is divided into three sections: a knowledge base (known facts), an inference engine (rules), and a user interface. An expert system is a piece of software that combines a database of data with a set of rules to assist in reaching a decision regarding a given input. A human may theoretically reach the same conclusion. The advantage of adopting an expert system is that it "remembers" all of the rules involved in the decision-making process, allowing it to produce the same objective output for a given input quickly and consistently. Microbiologists have traditionally utilised simple principles to link an organism's identified phenotype to a therapeutically relevant discovery. The discovery of cefoxitin resistance in Staphylococcus aureus, for example, is used to infer the organism's resistance to penicillins and cephalosporins. In some cases, such as detecting and separating extended-spectrum-beta-lactamase (ESBL)-producing, AmpC-hyperproducing, and wild-type Enterobacteriaceae bacteria, finding resistance mechanisms can be more difficult. A simple flow chart or table can soon expand into a complex algorithm due to the variety of microorganism

morphologies and in vitro test findings, as well as the possibility of numerous resistance mechanisms [7].

Expert systems are most widely used in automated antimicrobial susceptibility testing in clinical microbiology laboratories (AST). These expert systems can notify the user of an unusual AST pattern for an identified organism, change the AST interpretation of one antimicrobial based on the interpretation of test results for a second antimicrobial, suppress the report for an antimicrobial's AST if necessary, add a footnote to an interpretation, and allow laboratories to customise the expert system's rules. Winstanley and Courvalin published a comprehensive evaluation of clinical microbiology expert systems lately [8].

In practise, MIC values are entered into an expert system by a user (or instrument). Using the inference engine, the software compares the input to the knowledge database, and the system then outputs the appropriate susceptible, intermediate, or resistant (SIR) interpretation. By comparing a clinical isolate's MIC values to a curated database of isolates with their MIC values and mechanism(s) of resistance defined, expert systems can infer the mechanism of antimicrobial resistance. The expert system not only includes antimicrobial breakpoints in its database, but it also has criteria that check to see if particular medication results should be labelled as resistant even though the organism tests as sensitive [9].

Expert systems can also be used to send fresh or relevant information to the appropriate employees. When extra work-up on an isolate is required, an expert system can warn a laboratory scientist on the bench. A system can also notify a laboratory scientist if the laboratory has recently worked up a comparable isolate, indicating that the current isolate may not require a repeat AST. When a patient needs to be placed on contact precautions, an expert system can send an email notification to hospital staff, or it can email the microbiological director when the health department needs to be notified [10].

Patient demographics, specimen source, and antibiotic resistance can all influence how results are reported. The rules can be embedded into the system to selectively distribute results, rather than relying on humans to research these criteria, recall the associated rules, and accurately implement the rules. It's no surprise that the microbiology lab is using expert systems (also known as knowledge-based systems) to try to organise algorithms into more useable and useful solutions [11].

Expert systems can be used to compile and report on a variety of metrics. For example, an organism's MIC trends can be viewed, infection rates in a certain hospital unit can be determined, and blood culture contamination rates connected with a specific clinical location or phlebotomist of interest can be investigated [12].

To streamline the flow of microbiology data and transform those data into enhanced patient care, there is untapped potential for full integration of the microbiology laboratory's expert systems with clinician alarms and advanced clinical decision support tools. Antimicrobial resistance and therapy-related problems can be reduced by reducing poor or unneeded antimicrobial therapy. Expert systems could also be integrated into total laboratory automation systems, with expert systems being able to "read" plate cultures and report autoverified results in some cases [13].

According to Williams and Trotman in the 1960s, the clinical microbiology laboratory faced "an ever-increasing load of work not matched by an increase in the number of workers available to execute it," which still appears to be true today. For the past half-century, the laboratory has looked to automation to help it overcome this problem. Williams and Trotman envisioned a more fully automated laboratory in which specimen inoculation and transfer to the incubator could be automated, and when the bacteriologist has chosen the colony of interest and selected its identification programme, it should be possible to transfer it to various identification media or set up cultures for antibiotic sensitivity determination mechanically without undue difficulty.

Many clinical laboratories have adopted automated devices as workhorses, although automation has usually been limited to discrete segments of the analysis pipeline, such as detecting the presence of growth in blood cultures and determining the identity and susceptibility of isolated organisms. Williams and Trotman's ideal of more full laboratory automation has only recently begun to come true. This method is known as "complete laboratory automation" (TLA)[14].

Outbreak detection:

Infectious illness epidemics must be detected quickly on a global, regional, and local level. State public health organisations, the CDC, and the World Health Organization are primarily responsible for detecting widespread epidemics. In academia and public health, there is still a lot of interest in detecting, tracking, and modelling epidemics. Rapid detection is critical because reducing the time it takes to notice an outbreak can dramatically reduce its negative impact.

Antimicrobial-resistant organisms' introduction or rising prevalence is a worry for both local and worldwide clinical microbiology. There is software that can connect a local laboratory to other regional and worldwide laboratories for bidirectional antimicrobial susceptibility data

transmission. WHONET and The Surveillance Network are two of these programmes that have been in operation since the 1990s (TSN). Both WHONET and TSN were formerly used to evaluate antibiotic susceptibility data from many laboratories in order to establish patterns of microbial susceptibility, and both networks are still in use to disseminate information. The TSN, however, is no longer supported and is no longer available as of 2014 [15].

Conclusion:

An ever-increasing amount of data must be generated, analysed, and interpreted in the clinical microbiology laboratory. It is critical to incorporate the usage of informatics technologies to improve the quality of laboratory workflow and processes in order to evaluate and disseminate data effectively and efficiently. Microbiologists can use informatics tools to keep track of specimen work-ups in the lab, automate their workloads, identify clinically relevant microorganism characteristics, remotely share digital images for teleconsultation, quickly distribute accurate and appropriate results, perform more thorough and rapid disease surveillance, and (most importantly) provide better health care to patients and the general public. In order to continue to assist the laboratory in producing, interpreting, and communicating the most beneficial information, informatics tools must be developed and implemented. In order to develop and deploy these informatics tools to their full potential, guidance is required, particularly in the fields of telemicrobiology and microbial MGS and WGS. Informatics tools, when utilised correctly, can enable the clinical microbiology laboratory do more with less while enhancing patient and public health care quality.

Although municipal laboratories are not normally held directly responsible for finding regional and global epidemics, they are critical in supplying information to public health organisations so that outbreaks can be identified. The local laboratory's participation in the identification of worldwide epidemics is usually limited to reporting the finding of notifiable infectious agents to the public health agency in charge of the laboratory's territory. However, the detection of some local outbreaks has worldwide public health implications, and every global outbreak detection starts locally. One strategy to help regional public health organisations identify regional epidemics more quickly is to provide clinical and microbiological information from the local laboratory as soon as possible. The use of electronic reporting by local laboratories can improve the speed with which regional outbreaks are identified.

Local clinical microbiology laboratories play an important role in the detection of disease epidemics in their communities. Active surveillance by infection preventionists in collaboration with the clinical microbiology laboratory, as well as "virtual surveillance," in which

mathematical algorithms are used to identify possible outbreaks independently of hypothesis-based or query-based investigations, is the best way to identify local outbreaks. Community-acquired diseases (e.g., yearly seasonal influenza epidemics) or hospital-acquired infections could be the source of these local outbreaks (e.g., nosocomial infections). The data of clinical microbiology laboratories has been effectively mined for anomalies that could indicate a community outbreak using software. Prospective organism surveillance, laboratory surveillance (including virtual surveillance), and syndromic surveillance are three methods for detecting outbreaks. The most quick method of detection is prospective surveillance utilising environmental biosensors.

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MEDICINAL USES OF *CATHARANTHUS ROSEUS*

Neelanka De[1], Sukanya Bhowmik[2] and Biswajit Saha*[3]

[1]Department of Microbiology, T. H. K. Jain College, Kolkata

[2]Department of Microbiology, Asutosh College, Kolkata

[3]Department of Microbiology, Bijoy Krishna Girls' College, Howrah

*Corresponding author E-mail: biswajit.saha1402@gmail.com

Abstract:

Herbal therapy is a holistic therapy, having no adverse effect than the other synthetic drugs. This can be a reason that scientists are more focusing on the discovery of medicinal advantages of many plant parts. Medicinal plant has a great importance and their usage is increasing day by day. *Catharanthus roseus* is one of the commonly known medicinal herbs, which have a great importance in cancer treatment. This plant extract contains many alkaloids, which have a great importance in medical science. Plant parts including leaf, root, shoot and stem contains more than 200 alkaloids. Some important alkaloids include Vinblastine, Vincristine, Rubacine etc. These alkaloids have several pharmacological activities like anti-oxidant, anti-microbial, anti-diabetic, wound healing property, anti-ulcer, hypotensive, antidiarrheal, hypolipidemic and memory enhancement property also. Root extract can also be used as an anti-arrhythmic agent. In this review more information of about this plant, its traditional uses, about its alkaloids and their pharmacological activities is discussed.

Keywords: Synthetic drug, *Catharanthus roseus*, Alkaloid, Anti-ulcer.

Introduction:

The use of medicinal plants is as old as mankind itself. In ancient times to get cured from the disease, people used to look for drugs in the nature. In traditional medicine, as well as ethnomedicine, medicinal plant has a long history of usage. The knowledge of medicinal plant transferred from one generation to another, and is increasing day by day [1]. Preparation of drug (herbal medicine) can be done in several ways. Powdered form of drug is thought to be simplest method to take up those drugs [1, 2].

Various plant parts are used for medicinal purposes, like- leaf (alone or mixture of leaves with twigs or stems or in some cases buds, for example- Maidenhair tree, wood (Sandal wood), flower (Chamomile flower), bark (Oak or Willow bark), roots and seeds, tubers (African potato,

Autumn crocus), rhizome (Ginger), bulb (Onion, Garlic) and in some cases the whole plant. Sometimes, the secondary products are also used for medicinal purpose, like- resins, latex or gums [3].

The effectivity of medicinal plants are due to presence of various biologically active substances, which includes-

Alkaloids- molecules bearing nitrogen, can be used in treating cancer (eg. *Catharanthus roseus*).

Flavonoids- consist of anti-inflammatory and anti-viral properties (example- lemon, buckwheat).

Phenols- consist of anti-inflammatory, anti-viral and antiseptic properties (example- mint family members).

Bitters- can improve digestive system and appetite (example- wormwood).

Various other important biologically active substances are also present like saponins, volatile oil, vitamins and minerals [4].

Out of many therapeutically important herbs, *Catharanthus roseus is* one of the very famous herbs in cancer treatment. It is very commonly seen in Indian gardens as an ornamental plant. It can grow in coastal areas, sandy soils as well as in bushland. *C. roseus* has many beneficial pharmacological properties like anti-oxidant, anti-diabetic, anti-ulcer, anti-microbial, anti- diarrhoeal, healing wound, hypotensive, memory boosting and hypolipidemic activity [1].

C. roseus is known by different names in different languages, for example- in English it is known as ‘periwinkle’, in Hindi it is called ‘sadabahar’, in Sanskrit it is ‘nityakalyani’ and in Bengali it is called ‘noyontara’ [5].

Background:

Classification:

Kingdom – Plantae; Division –Magnoliophyta; Class - Magnoliopsida (dicotyledons)

Order – Gentianaes; Family – Apocynaceae; Genus – *Catharanthus* Species – *roseus* [5]

Morphology:

C. roseus is an evergreen sub-herb plant (around 1m in height) [5]. The flowers can be of various colours – ranging from white, to pale pink to pinkish red, with reddish (or dark coloured) centre [6]. They are actinomorphic in symmetry and consist of 5 petals. The leaves are ovalis in shape, shiny in texture and pinnately compound inflorescence. The leaf abaxial surface is generally seen of dark green in colour with light green leaf stalk. A short petiole is present (1-1.8 cm). Its fruit consists of two follicles (2-4 cm in length, 3 mm in width) [6].

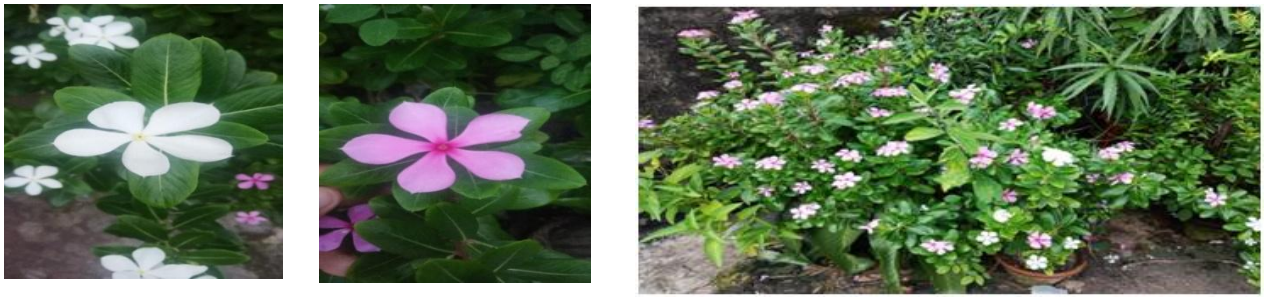


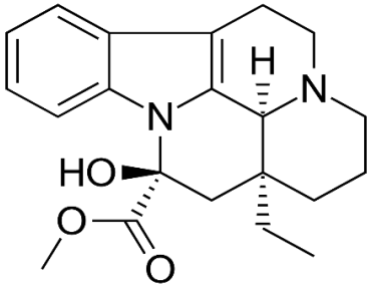
Figure 1: Different flowers of *Catharanthus roseus*

Table 1: Traditional uses

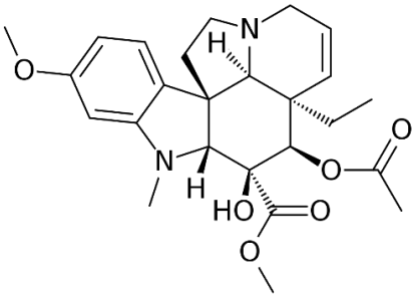
Hot water extract of	Used in treatment of	Country
Whole plant	• Anti-galactagogue • Stomachproblem	France, South Vietnam [7, 8] Mexico [9]
Dried whole plant	• Diabetes • Cancer • Leishmaniasis • Liverdisease	Brazil, England [10, 11] India, Peru [8], [12] Peru [8] Taiwan [13, 14]
Dried leaves	• Menorrhagia • Hypertension • Diabetes • Cancer • Hodgkin's disease	Australia, South Africa [15] Cook-Island [16] Australia, Jamaica, Europe, Kenya, Malaysia [15, 17, 18] Cook-Island [16] India [8]
Leaves	• Ease primaryuterine contractions	Dominica [19]
Root	• Menorrhagia • Hypotensive • Abortion	India [8] Mozambique [20] Philippines [13]
Ovule	• Diabetes	Pakistan [21]
Dried root	• Venerealdisease	Venda [22]
Aerial parts	• Menstrualregulator	China, North Vietnam [7, 8]

Chemical composition:

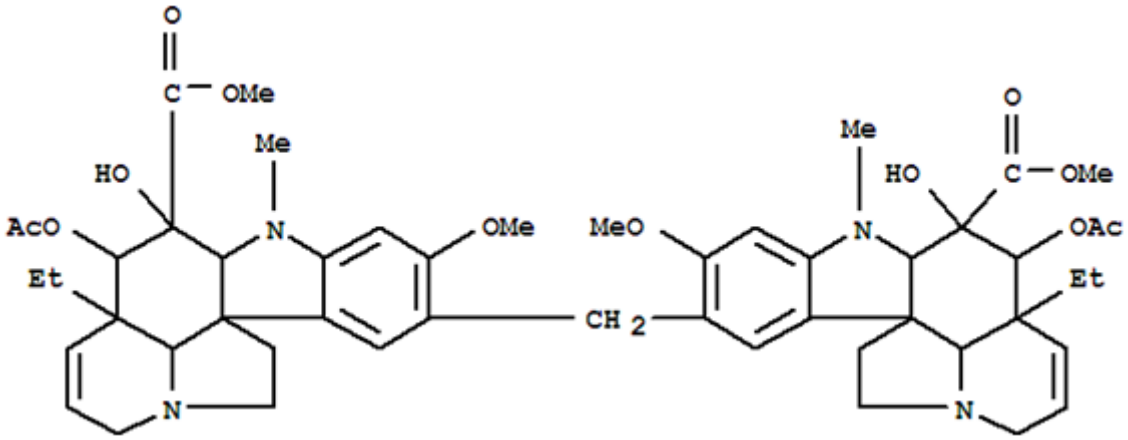
Catharanthus roseus consists of several chemical components which plays vital role in medicinal purposes, like



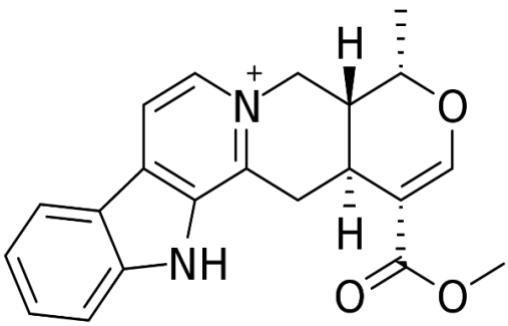
Vincamine ($C_{21}H_{26}N_2O_3$) plays role in treating cerebral disorders



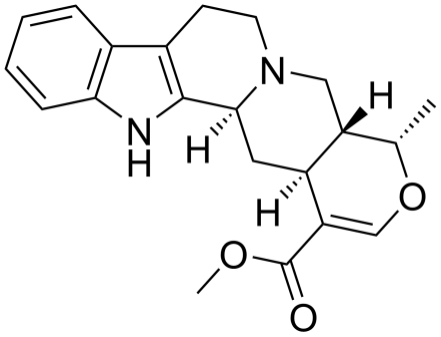
Vindoline($C_{25}H_{32}N_2O_6$) plays role as anti-ulcerative component



Vindolicine ($C_{51}H_{64}N_4O_{12}$)- plays role in diabetes management



Serpentine($C_{21}H_{22}N_2O_3$) plays role intreatinganxiety component [23]



Ajmalicine($C_{21}H_{24}N_2O_3$) plays role as anti-stress

Several other components are Catharanthamine [25], cholesterol [26], choline [27], rosamine [28], epi-vindolinine [29], zeatin [30], virosine [31], stigmasterol [26], mitraphylline [32] and many more.

Flowers and leaves of *Catharanthus roseus* consists of 13 vital elements (macro and micro nutrients) Na, Ca, Mg, K, Zn, Cr, Cu, Cd, Fe, Al, Ni, Pb and Mn. It is experimentally found that leaves contain low amount of Zn and K, whereas flowers contain high amount of Zn and K [24].

Beneficial properties:

Anti-cancer activity

Catharanthus roseus is known as "an anticancerous drug yielding plant". This plant produces some anticancer alkaloids, such as Vinblastine and Vincristine, from stem and leaf. These alkaloids prevent the growth of some human tumors (by altering microtubular dynamic, which ultimately leads to apoptosis) [33]. For treatment of neoplasmas, Vinblastine is used. This drug is particularly recommended for Hodgkins disease (A type of lymphoma, which is a blood cancer that starts in the lymphatic system). It is also used for lymphosarcoma (a malignant tumor of lymphatic tissue), choriocarcinoma (a fast-growing cancer founds in woman's uterus), neuroblastoma (a disease in which malignant cells form in neuroblasts, in the adrenal glands, neck, chest, or spinal cord), carcinoma of breast and lungs and lymphocytic leukemia.

Vincristine, an oxidised from of Vinblastine, which can arrest mitosis in metaphase. It is found to be very useful, in the treatment of leucoblastic leukemia in adults and children. It is seemed that Vincristine is also used for Hodgkins disease also, and used to treat leukemia in children. Anticancer activity of these methanolic crude extracts was depends upon their percentages. Different percentage represents a significant anti-cancer activity. Commercially Vinblastine is found as Velban and Vincristine is sold as Oncovin [1, 34].

Anti-oxidant activity

The extract of *Catharanthus roseus* leaves is considered as one of the effective components which work as anti-oxidant also. The extract consists of various biologically active substances. The leaf extract is fractioned with various solvents, like- ethanol, acetone, butanol or water [35]. The anti-oxidant property of this extract is measured via several assaying methods. These methods include radical scavenging assays- ABTS and DPPH radicals, redox assay -FRAP and CUPRAC, to calculate anti-oxidant capacity- TAC, metal chelating assay- Trolox and EDTA [36]. During analysis of the extract of *C. roseus*, it seemed to be a good source for natural anti-oxidants [35].

Anti-diabetic activity

Diabetes mellitus is one of the disorders of Endocrine system. In this disease our body isn't able to take up glucose into its cells and use it for energy. It leads to increase in sugar concentration in the bloodstream. Pancreas secretes Insulin which allows the glucose to enter into our body cells, so that energy can be produced. In a diseased person Pancreas doesn't secrete enough insulin. Till date there is no completely effective treatment for its healing. Though now a days, artificial insulin and oral hypoglycaemia agents are available, but these treatments show some side effects. Scientists were looking for herbal medicines or plant products having minimal side effects [34, 37].

It is found that the ethanolic crude extract of the leaves and flowers of *Catharanthus roseus* can decrease the sugar level in bloodstream. The hypoglycemic effect occurs due to the glucose utilization in the liver. It increases the glucose utilisation. In diabetic rats, it was shown that the aqueous extract can decrease blood glucose level about 20%. The alkaloids containing hypoglycemic activity have been studied pharmacologically. Drugs are prepared from it for its hypoglycemic activity and are marketed as Vinculin [1, 38].

Anti-microbial activity

Microbes are becoming resistant to anti-microbial agents, due to their excessive use. Microbes are adapted themselves in such a way, so that they can withstand those adverse condition of antimicrobial agents. So, researchers are looking for new microbial agents [39].

It is found that ethanolic extract from different parts of *Catharanthus roseus* such as leaves, stem, roots, and flowers show antimicrobial activity. Leaf extract shows significantly higher effectiveness than others [40]. It is showed that Yohimbine, from *C. roseus* has an antiviral activity, and used against Herpes simplex virus (type I). Yohimbine contains an antifungal activity against *Candida albicans*, an opportunistic pathogen. [34, 41]

Another indole alkaloid, Catharoseumine is found and isolated from the whole plant body of *Catharanthus roseus*. This alkaloid possesses a peroxy bridge. It is found that Catharoseumine is a potential inhibitor against protozoan parasite Falcipain- 2. Vincristine and Vinblastine showed an antiparasitic effect. *Trypanosomacruzi*is a human parasite, causes Trypanosomiasis in human. These drugs affect this parasite by restricting its mitosis or by affecting in its cell shape [34]. Leaf extract of this plant is examined against many microbes, such as *Pseudomonas aeruginosa* NCIM2036, *Salmonella typhimuruim* NCIM2501, *Staphylococus aureus* NCIM5021. It was observed that the extract can be used to treat many of the illness, as it acts like a prophylactic agent to many diseases and bearing anti-microbial activities to many microbes [1].

Memory booster

Catharanthus roseus is known to boost memory as it increases the glucose and oxygen uptake ability of brain, resulting in increased function [42]. This can be used for Alzheimer's patients [1]. The chemical component responsible for this property is Vincamine and Vinpocetine (chemically modified version of Vincamine). The mode of action is still not clear but is thought to be responsible for increasing cerebral blood flow, increasing enzymatic activity of catecholamines and adenosine inhibition [42]. It is also known to potentially inhibit acetylcholinesterase [43]. It is taken orally in many areas [42].

Hypolipidemic activity

Various studies of *Catharanthus roseus* (Linn.) G. Donn showed that it can reduce the serum lipid profile: total cholesterol, LDL (low-density lipoprotein) cholesterol, VLDL (very low- density lipoprotein) cholesterol and triglycerides [44, 45]. The experimental animals used in these are rats, guinea pigs. It is taken in fresh juice (of leaves) form [45].

Anti-ulcer activity

Leaves of *Catharanthus roseus*, produces some alkaloids such as Vincamine and Vindoline, which show anti-ulcer property. It is found that chloroform fraction of *C. roseus* and its active constituentsvincamine and vindoline impart gastroprotective effects. [46]

Anti-diarrheal activity

The ethanolic extract of *Catharanthus roseus* from leaves is seen to be effective on induced diarrhea (by castor oil) in Wistar rats experimentally. The experiment showed that the effectivity of this extract depends on the uptake amount/ dose. The extract contains alkaloids, flavonoids, tannins, saponins and triterpenes [47].

Healing wound

The extract of *Catharanthus roseus* was applied experimentally on rats. This experiment showed that the ethanolic extract can contact the wound increasingly along with increase in hydroxyproline content and tensile strength. Due to the presence of anti-microbial effect in the extract of *C. roseus*, the healing process was even more elevated [48].

Hypotensive activity

The hypotensive effect of *Catharanthus roseus*was experimented on AIHR rats, where AIHR stands for adrenaline induced hypertensive rats. After administering the rat for one week with the biologically active components which are responsible for the hypotensive activity, which was acquired from the extract of the leaves of *C. roseus* , several significant changes were observed in cardiovascular parameters such as level of glucose in blood, triglyceride in serum, cholesterol in serum and weight of heart [49].

Conclusion:

Although for a long time *Catharanthus roseus is* grown as an ornamental plant, it was considered as a medicinal herb from ancient times. With modern advances in science, several beneficial properties of *Catharanthus roseus is* being displayed. Due to its huge amount of benefits, its now a topic of research in various laboratories. Several research papers already proved that it plays a vital role as anti-oxidant, anti-cancer, anti-diabetic because it consists of many biologically active substances. Since it is a natural source, its use will not lead to any carcinogenic or ill-effect (which can appear when people intake chemical compounds. So, *Catharanthus roseus*with more research and proves may lead to a new and safe- alternative for various human diseases.

Future prospects:

1. We can include more use of *Catharanthus roseus*extracts as an alternative to chemically producedmedicines.
2. *C. roseus* can be inserted in daily food habits, for a maintained and healthy lifestyle.

People should be made aware about the beneficial role of *C. roseus*, so that more people can employ it in their dailylife.

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GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM FRUIT PEEL EXTRACTS- A BRIEF REVIEW

Aleena Elsa Mani

Post graduate and Research Department of Zoology,

CMS College, Kottayam, Kerala

Corresponding author E-mail: alina7m@gmail.com

Introduction:

Nanoparticles are molecules in the range of 1- 100 nm. They can be synthesized by physical, chemical and biological methods using two approaches: Top-Down and Bottom-Up. In the former approach, a bulky material is broken down into smaller fine particles by reducing the size with the help of various physical methods such as grinding, milling, sputtering, laser or thermal ablation and so on. On the other hand the latter approach deals with self-aggregation of atoms to new nano-sized molecules by chemical and biological procedures such as chemical reduction, electrochemical methods etc.

The 'green synthesis', a technique under the bottom up approach which involves the production of nanoparticles from living systems like bacteria, virus, fungi, algae, plants and plant extracts. This is an eco-friendly approach to synthesize metal nanoparticles. The root, leaves, flower, fruit, seed and fruit peels can be used to synthesize metal nanoparticles. Usually Ag, Cu, Au, Zn, Fe nanoparticles etc. are synthesized.

Limitations of physico- chemical synthesis of metal nanoparticles:

- Bioaccumulation of toxicants
- Expansive analysis requirements
- Instability in hostile environment
- Laborious task
- Expensive
- Not environment friendly

Because of these limitations of physico- chemical methods, the green synthetic approach is gaining wide popularity. The items which are considered as wastes became the raw materials to synthesize metal nanoparticles having wide scale applications in environmental remediation, medical field, and agriculture and waste water treatments. The large-scale synthesis of metal nanoparticles can then be feasible in economic and ecological means.

Soto *et al*., in 2019 [1] presented a study using fruit peel waste for the green synthesis of silver nanoparticles with antimicrobial activity against foodborne pathogens. From lyophilized extract of grape and orange wastes, the silver nanoparticles were synthesized, monitored by UV-Vis spectroscopy followed by characterization using TEM, Diffuse Reflectance Infrared Spectroscopy (DRIFT) and X- Ray Diffraction (XRD) the agar diffusion method and optical density (OD) method were used to determine the anti-microbial activity.

Kahrilas *et al*., in 2014 [2] had done a work on Microwave assisted green synthesis of silver nanoparticles using orange peel extract. The experimental study was focused on the synthesis of AgNPs from citrus fruit peels including orange, lemon, lime, tangelo and grapefruit. However they found successful in synthesizing it from orange peel extract and determined it using UV-Vis spectroscopy, FTIR, powder XRD and TEM. The analysis of all citrus peel extracts by gas chromatography- mass spectroscopy (GC- MS) indicated that putative compounds responsible for successful AgNPs synthesized with orange extracts were aldehydes.

Ahmad *et al*., in 2012 [3] presented a research work on the biosynthesis of silver and gold nanoparticles using Pomegranate peel extract. The characterization of synthesized AuNP and AgNP were determined using UV-Vis spectroscopy, TEM, Selected Area Electron Diffraction (SAED) and XRD.

Ndikau *et al.,* in 2017 [4] presented a work where AgNPs were synthesized using *Citrullus lanatus* fruit rind extract and characterized by UV-Vis and Cyclic Voltammetry (CV) and TEM.

Ibrahim *et al*., in 2015 [5] reported the biosynthesis of AgNPs using banana peel extract and characterized using UV-Vis spectroscopy, XRD, SEM, FE SEM, TEM and FTIR. The synthesized AgNPs showed effective antibacterial activity against the bacterial and fungal pathogens of specific strains.

Nahar *et al.,* in 2021 [6] presented a research work on AgNP synthesis from *Citrus sinensis* peel extract and characterized using UV-Vis, TEM, SEM and Dynamic Light Scattering techniques. The peel extract was subjected to qualitative analysis to determine the presence of primary and secondary metabolites responsible for AgNP synthesis. The efficiency of synthesized AgNPs as antibacterial compound was determined using *Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli.*

Silver Nanoparticles were prepared through a biological procedure where the essential oils of orange peel were used as a capper and reducing agent. Their characterization was carried out using XRD, FTIR and FE SEM, Energy dispersive XRD(EDS), TEM, UV- Vis and Thermo

gravimetric Analysis and the biosynthesized nanoparticles were used as nanocatalysts for A3 coupling (amines, aldehyde and alkynes) [7, 8].

Much of the research works were focused on the synthesis of silver nanoparticles from Citrus plants. Yet another work where AgNPs synthesized from *Citrus medica* peels gained wide popularity. The synthesized ones were subjected to characterization by XRD, SEM, EDAX and HR- TEM. Also the synthesized nanoparticles were tested for their antioxidant activity rather than the usual antibacterial activity [9].

A single step green synthesis of AgNPs from *Citrus sinensis* peels were done in 2018. But, the study dealt with the hot water extract of sweet orange peels to synthesize AgNPs. Their characterization using EDX, XRD, FTIR, DLS, FE-SEM and HR-TEM were carried out [10].

The synthesis of silver nanoparticles from pomegranate peels and it's biomedical activities were evaluated in 2019.The synthesized AgNPs inhibit 77% of DPPH free radical while the peel extract itself inhibit 100% DPPH free radical. Peel extract cause 70% cell death in treated breast tumor cell lines, BT-20 and MCF- 7, while synthesized AgNPs led to more than 81% and 89% ±cell death in BT-20 and MCF -7 respectively [11].

The Ethiopian cactus pear fruit peels were used as a phytoreducing agent to fabricate silver nanoparticles and their characterization was done by UV- Vis spectroscopy, TEM, EDX, and XRD analysis. The synthesized AgNPs were effective against *E.coli* > *Salmonella* spp.> Bacillus spp.> *Pseudomonas* spp.> *Staphylococcus* spp. They also demonstrated effective anti-cancerous activity against human cervix epithelioid carcinoma cell lines. They have marked applications in medical field [12].

Dragon fruit peel extract was used for the synthesis of silver nanoparticles followed by the characterization using FTIR, UV- Vis spectroscopy, XRD, EDX, TEM, and Zeta potential. FTIR also revealed the possible organic compound in dragon fruit peel responsible for the reduction and stabilization of nanoparticles. AgNPs thus synthesized showed effective antibacterial activity against representative pathogenic bacteria [13].

The silver nanoparticles produced from the fruit peel aqueous extract of *Solanum melongena L*. showed strong antibacterial property against *P. fluoroscens* and *B. amyloliquefaciens*. AuNPs also was synthesized using the same peel under microwave irradiation condition [14].

Opuntia ficus indica fruit peel was used to synthesize AgNPs and characterized by SEM, UV- Vis spectroscopy, EDX, AAS, and DLS. The activity of AgNPs was evaluated in water from the effluent of waste water treatment plants before and after chlorination and inhibition of some specific strains of bacteria [15].

Wound healing potential of green synthesized AgNPs were reported from *Lansium domesticum* fruit peel extract [16]. *Carica papaya* peel extract was used to synthesize AgNPs and was found to be an antibacterial agent against human pathogens [17]. Kinnow mandarian peel was used to synthesize AgNPs and characterized. The shampoo formulations with AgNPs molecules showed enhanced antimicrobial activity as compared to shampoo formulation without any Ag nanoparticles [18].

The extracts of lemon peel, green orange peel and orange peel were used to synthesize AgNPs and the synthesized ones were subjected to characterization by UV- Vis spectroscopy, TEM, and FTIR. The synthesized AgNPs showed antibacterial activity against *E. coli*, and *S. aureus* [19].

In a study silver nanoparticles were synthesized using multiple fruit peel waste (pomegranate, orange, banana and apple (POBA)). The synthesized AgNPs were analyzed by various physicochemical techniques such as UV- Visible spectroscopy, x-ray diffraction (XRD), Fourier transform infrared (FT-IR) spectroscopy and transmission electron microscope (TEM). The AgNPs were checked with human pathogens (*Salmonella*, *E.coli* and *Pseudomonas*), plant pathogen (*Fusarium*) and marine pathogen (*Aeromonas hydrophila*) and also studied the scavenging effect and anticancer properties against MCF-7 cell lines and hence demonstrated the way for its wide biomedical applications [20].

Silver nanoparticles (Ag NP´s) were synthesized using mango peel extract. Prepared Ag NPs were characterized by UV-VIS spectroscopy, dynamic light scattering (DLS) and scanning electron microscopy - energy dispersive spectroscopy (SEM-EDS) [21].

Larvicidal and anti- helminthic properties of silver nanoparticles have been demonstrated against *Culex quinquefasciatus* and *Eudrilus eugeniae*. The insecticidal effect was observed in silver nanoparticles synthesized from POBA fruit peel extracts and were characterized through UV Visible spectroscopy, x-ray diffraction (XRD) and transmission electron microscopy (TEM) [22].

AgNPs were synthesized from *Annona squamosa* peel extract and characterized using UV- Vis, FTIR, XRD, AND TEM techniques. The FTIR Analysis demonstrated that phenolic compounds and protein compounds of Annona is responsible for AgNP synthesis and stabilization. The synthesized AgNPs showed high antioxidant activity that too comparable to standard antioxidants like Ascorbate. They have also showed the anti- diabetic property by inhibiting α- amylase in a dose dependent manner [23].

AgNPs were synthesized from *Ananas comosus* fruit peels and characterized by FTIR, UV- Vis, XRD, FESEM, and TEM. The synthesized AgNPs showed promising inhibitory effect on Gram negative and Gram positive pathogenic microorganisms and the cytotoxic and growth inhibitory effects on different cancer cell lines were examined via the MTT assay [24].

A green approach, including using phytochemicals in pomelo peel extract (PPE) and direct sunlight, was used to synthesize silver nanoparticles (AgNPs). Characterization techniques, including X-ray diffraction, transmission electron microscopy, and scanning electron microscopy, confirmed the formation of AgNPs with sizes of 20–30 nm. The AgNPs synthesized in PPE showed antibacterial activities comparable to those of $AgNO_3$ at the same silver concentration against four pathogenic bacterial strains. The obtained PPE containing AgNPs, pectin, and other phytochemicals can be utilized further to produce antibacterial and antioxidant films in food packaging and medical applications [25]. Silver nanoparticles were synthesized effectively from *Beta vulgaris* peel extract. The transmission electron microscope, SAED and X-ray diffraction studies infer that the nanoparticles formed were spherical/quasi-spherical in shape, which primarily exhibited a face centered cubic crystal (FCC) structure. Amine groups were considered responsible for the reduction of Silver ions [26].

Silver chloride nanoparticles were synthesized using the aqueous extract of outer peel of peach fruit (*Prunus persica* L.) and evaluated its antibacterial activity, synergistic antibacterial and anticandidal potential against five foodborne pathogenic bacteria and five pathogenic *Candida* species respectively along with its antioxidant potential. The synthesized silver chloride nanoparticles (PE-AgClNPs) were visually confirmed with surface plasmon resonance peak at 440nm upon UV- Vis spectroscopy analysis [27].

Conclusion:

This brief review tries to give a glimpse on the green synthesis of Silver nanoparticles from the so called fruit wastes, the fruit peel extracts. This is an economical and time saving method of synthesis of metal nanoparticles. The research is still going on searching for the unexplored methods and sources to synthesize metal nanoparticles in an environment friendly manner and at ease.

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A REVIEW ON MICROBIAL INOCULANTS USED AS BIOFERTILIZERS

Nafeesa Begum and Shaila M

Department of Botany,

Sahyadri Science College, Kuvempu University, Shivamogga-577203

Correponding author E-mail: shailamresearchscholar95@gmail.com,

nafeesabegum30@gmail.com

Abstract

Current soil management strategies are mainly dependent on inorganic chemical-based fertilizers, which caused a serious threat to human health and environment. The exploitation of beneficial microbes as a biofertilizer has become paramount importance in agriculture sector for their potential role in food safety and sustainable crop production. The eco-friendly approaches inspire a wide range of application of plant growth promoting rhizobacteria (PGPRs), endo- and ectomycorrhizal fungi, cyanobacteria and many other useful microscopic organisms led to improved nutrient uptake, plant growth and plant tolerance to abiotic and biotic stress. The present review highlighted microbial biofertilizers are mediated and thereby crop improvement.

Keywords: Biofertilizers, Microorgsnisms, Plant growth, Sustainable

Introduction:

Microbial inoculants or biofertilizers are preparation containing viable algae, fungi, and bacteria alone or consortium together to support the plant growth and increase crop yield. Biofertilizers contain beneficial microbes that improve soil chemical and biological characteristics by fixing nitrogen, cellulolytic activity, or phosphate [1]. When they are applied to seed, plant surfaces, root, or soil, they inhabit the rhizosphere, and through their biological activity, they enhance nutrient bioavailability, promote plant's growth, and increase the soil microflora. Thereby, they are preparations that readily improve the fertility of the soil. Rhizobium has symbiotic associations with legume roots, such as rhizobacteria, that reside on the surface of the root or in the soil of the rhizosphere. Broad-spectrum biofertilizers include Blue-Green Algae (BGA), Rhizobium, and Azolla are crop-specific bio inoculants, such as Azospirillum, Azotobacter, phosphorus solubilizing bacteria (PSB), vesicular-arbuscular mycorrhiza (VAM), and Anabaena, as nitrogen-fixing cyanobacteria. These bacteria are known as biofertilizers and plant growth-promoting rhizobacteria (PGPR). Competition mechanisms and antagonism activity are carried out by the enzymatic activity of PGPR for crop production, such

as the inhibition of phytohormones and phytoparasites; it also helps plants in withstanding stress by heavy metal contaminations and pollutants.

Organic farming is increasing the production of pollutant-free crops. It involves the use of biofertilizers and biopesticides which increases the nutrient quality of the crop and controls any kind of pest and pathogen. Biofertilizers are microorganisms that add to the nutrient quality of the soil. Bacteria, fungi, and algae are some of the beneficial microorganisms that help in improving the fertility of the soil. The rhizosphere, which is the narrow zone of soil surrounding plant roots, can comprise up to 1011 microbial cells per gram of root and above 30,000 prokaryotic species that in general, improve plant productivity [2]. The collective genome of rhizosphere microbial community enveloping plant roots is larger compared to that of plants and is referred as microbiome [3], whose interactions determine crop health in natural agroecosystem by providing numerous services to crop plants viz., organic matter decomposition, nutrient acquisition, water absorption, nutrient recycling, weed control and bio-control [4]. The metagenomic study provides the individual the core rhizosphere and endophytic microbiomes activity in Arabidopsis thaliana using 454 sequencing (Roche) of 16S rRNA gene amplicons [5]. It has been proposed that exploiting tailor-made core microbiome transfer therapy in agriculture can be a potential approach in managing plant diseases for different crops [6]. Rhizosphere microbial communities an alternative for chemical fertilizers has become a subject of great interest in sustainable agriculture and bio-safety programme

Biofertilizers are classified as:

A **biofertilizer** is a substance which contains living microorganisms which, when applied to seed, plant surfaces, or soil, colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant. Bio-fertilizers add nutrients through the natural processes of nitrogen fixation, solubilizing phosphorus, and stimulating plant growth through the synthesis of growth-promoting substances. The microorganisms in bio-fertilizers restore the soil's natural nutrient cycle and build soil organic matter.

- Free-living nitrogen-fixing bacteria like Azotobacter, and Rhodospirillum.
- Free-living nitrogen-fixing Cyanobacteria like Anabaena, and Nostoc.
- Loose association of nitrogen-fixing bacteria like Azospirillum.
- Symbiotic nitrogen-fixing bacteria like Rhizobium, and Frankia

Inoculants are products that have in their composition living microorganisms capable of benefiting the development of different plant species. The most antique microorganisms used as

inoculants are the "rhizobia", diazotrophic bacteria able to colonize the rhizosphere and establish nodules in the roots of their host plants, composed by several species of the Fabaceae family [7]. The symbiosis legumes-rhizobia leads to the process of biological nitrogen fixation (BNF), which very often can fully supply the plant´s demands on N. Moreover, other diazotrophic bacteria, such as *Azospirillum*, establish less straight relationships with the host plant, but are also able to supply, at least partially, the plant's demands on N. Both *Azospirillum* and rhizobia, among other diazotrophic and non-diazotrophic bacteria are named as plant-growth-promoting bacteria (PGPB) or plant-growth-promoting rhizobacteria (PGPR), as they may benefit the plants by a variety of single or combined processes, including the production of phytohormones, siderophores, phosphate solubilization, induction of plant intrinsic systemic resistance to abiotic and biotic stresses, among other microorganisms have also been increasingly used in agriculture for biological control of pests and diseases.

Microbes are used as Biofertilizers:

The following microorganisms are used as bio fertilizers

- **Rhizobium:** They form root nodules in leguminous plants and fix the atmospheric nitrogen into an organic form. Rhizobium also has no negative effect on soil quality and improves the quality, nutrient content, and growth of the plant.
- **Azotobacter:** These are free-living nitrogen fixers found in all types of upland crops. These not only fix nitrogen but also provide certain antibiotics and growth substances to the plant.
- **Azospirillum:** Unlike Azotobacter, these can be used in wetland areas. They are found inside the roots of the plant (non-free-living) where they fix the atmospheric nitrogen.
- **Blue-green algae:** These are free-living nitrogen-fixing Cyanobacteria that are present only in wet and marshy lands. However, they do not survive in acidic soil.
- ***Azolla:*** *Azolla* is a free-floating water fern that floats in water and fixes atmospheric nitrogen in association with nitrogen fixing blue green alga *Anabaena azollae*. *Azolla* fronds consist of sporophyte with a floating rhizome and small overlapping bi-lobed leaves and roots. *Azolla* is used as biofertilizer for wetland rice and it is known to contribute 40-60 kg N/ha per rice crop.
- **Mycorrhiza:** It is a symbiotic association between the fungi and the roots of a plant. The mycorrhizal fungus plays an important role in binding the soil together and improves the activity of the microbes. The fungi draw water and nutrients from the soil thereby increasing the plant productivity. It also helps the plant to survive under various environmental stresses.

- **AM fungi:** An arbuscular mycorrhiza (AM Fungi) is a type of mycorrhiza in which the fungus penetrates the cortical cells of the roots of a vascular plant. Transfer of nutrients mainly phosphorus and also zinc and sulphur from the soil to the cells of the root cortex is mediated by intracellular obligate fungal endosymbionts of the genera *Glomus, Gigaspora, Acaulospora, Sclerocysts* and *Endogone* which possess vesicles for storage of nutrients and arbuscles for funneling these nutrients into the root system.
- **Phosphate solubilizing microorganisms (PSM):** Several soil bacteria and fungi, notably species of *Pseudomonas, Bacillus, Penicillium, Aspergillus etc*. secrete organic acids and lower the pH in their vicinity to bring about dissolution of bound phosphates in soil. Increased yields of wheat and potato were demonstrated due to inoculation of peat based cultures of *Bacillus polymyxa* and *Pseudomonas striata*. Currently, phosphate solubilizers are manufactured by agricultural universities and some private enterprises and sold to farmers through governmental agencies.
- **Silicate solubilizing bacteria (SSB)**: Microorganisms are capable of degrading silicates and aluminum silicates. During the metabolism of microbes several organic acids are produced and these have a dual role in silicate weathering.
- **Plant Growth Promoting Rhizobacteria (PGPR)**: The group of bacteria that colonize roots or rhizosphere soil and beneficial to crops are referred to as plant growth promoting rhizobacteria (PGPR).

Figure 1: represents the different types of Biofertilizers

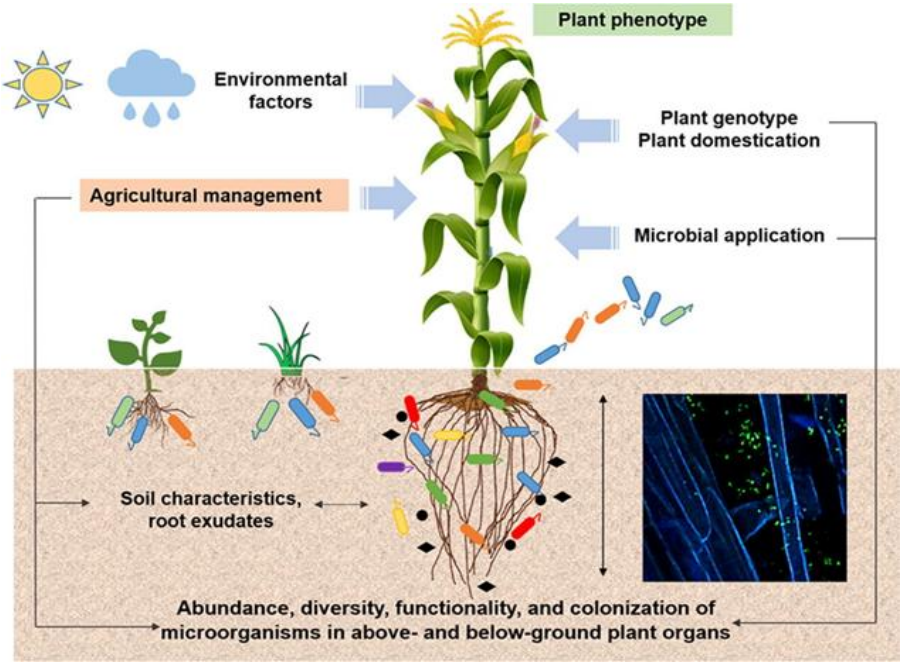

Figure 2: Shows the Interactions of Microorganisms with Plant

Microorganisms displaying plant growth-promoting characteristics are recruited by the plant to inhabit the rhizosphere. The microbial composition of the rhizosphere is optimized through the production and exudation of specific compounds, such as pyrone and sesquiterpenes, that prevent the growth of specific microbes [8]. However, root exudates represent about 20% of the carbon fixed by the plant through photosynthesis and represent an important source of carbon for the microbial community [9], especially in soils with different compositions of organic matter [10]. Bacteria that live in the rhizosphere, generally referred to as rhizobacteria, develop specific communication pathways with the plant and may influence plant physiology [11], including the type and amount of root exudates produced by the plant. Examples of the outputs of these interactions are biotic and abiotic plant stress tolerance induced by microorganisms.

Production of Biofertilizers:

Formulation is a crucial step in the production of a biofertilizer since it has to maintain the viability of the microorganism used while maintaining its activity at low levels. The formulation process involves preparation of inoculum, inclusion of additives, and selection of the best carrier, sterilization of carrier material, scaling up, good quality control measures, and adequate packaging with the best delivery method. Formulation of microbial biofertilizer is regarded as a mixture that comprises one or more viable (active) strains of microorganisms aimed at improving plant metabolic activities at the site of application and is regarded as an

alternative approach to chemical fertilizers. Formulation aims to provide long shelf life to microorganisms. The carrier material serves as a support for the proliferation of microorganisms and ensures that the microorganism establishes itself with the plant. Additives protect the formulation from any unfavorable environmental conditions and improve the properties of the formulation. Scaling up provides optimum growth conditions for the proliferation of the microbe used in the formulation process.

Currently, inoculation of crops with mycorrhizal fungi as biofertilizers is becoming more common because of the reduction in the population of indigenous mycorrhiza fungi in the soil through the application of chemical fertilizer. However, in selecting appropriate mycorrhizal fungi, it is essential to select high-quality mycorrhiza fungi, which will be able to colonize plant roots, act in the presence of bacteria, and have a long shelf life in the field and greenhouse. The easiest way of propagating arbuscular mycorrhizal fungi is through the propagation of a viable spore using sterile soil and a suitable host plant. This requires the cultivation of inoculated host plants in sterile soil and arbuscular mycorrhizal fungi spores being allowed to develop and propagate within the host plant. This method of producing inoculum is referred to as soil-based inoculum, which is the most common method used in the multiplication of arbuscular mycorrhiza spore.

The successful use of arbuscular mycorrhizal fungi depends on the strain utilized, the host plant, and the substrate used for propagation. More importantly, host plants that are commonly used in the propagation of arbuscular mycorrhizal fungi are sorghum and maize because of their high infectivity by mycorrhizal fungi. Roots and soil containing mycorrhizal fungi are harvested at the growing cycle, dried, and used as an inoculum.

Recently, new technology has been introduced in the formulation of biofertilizer, which involves the amendment of plant growth-promoting microbes with nanoparticles. This technique involves the use of nanoparticles made from organic or inorganic material with at least 100 nm in size. In agriculture, this technique is referred to as the agro–nanotechnology approach. Plant growth-promoting microbes are integrated into the nanostructure to enhance yield performance in plants. The formulation of nano-biofertilizer has efficiently enhanced agricultural productivity by increasing high retention in soil moisture content and increasing essential nutrient due to the direct and indirect effects of nanomaterial coating on plant growth-promoting microorganisms and its application has been reported to increase yield performance in cereal and leguminous plants by stimulating the germination potency in plants.

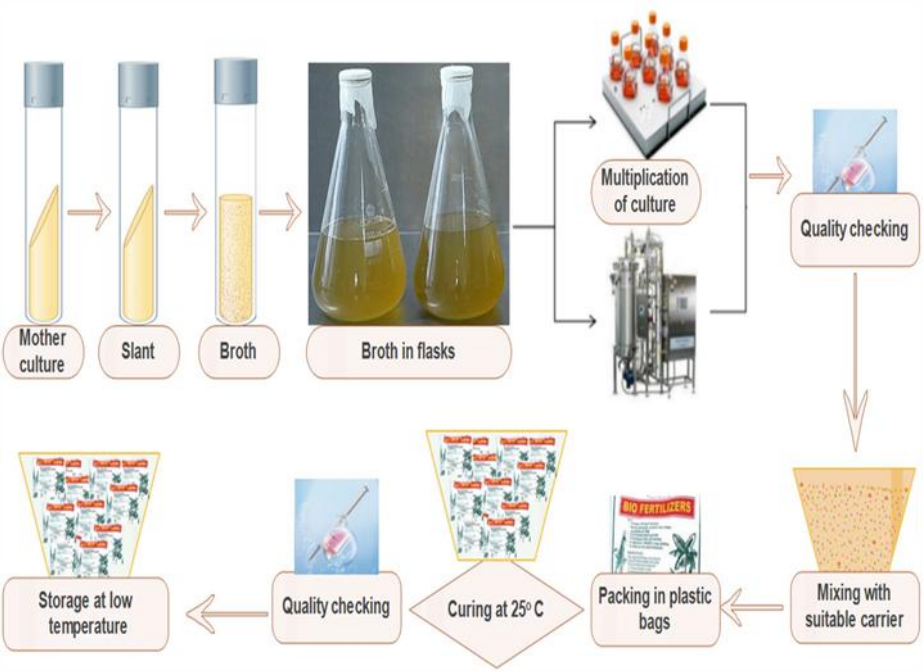

Figure 3: Production of Biofertilizers

Functions of Plant Microbiota as Biofertilizers:

The individuals from plant microbiome include advantageous, neutral, or pathogenic microorganisms. The following are the significant functions which are performed by plant microbiota as biofertilizers:

(a) Plant growth-promoting bacteria secrete auxin, gibberellins, and cytokinin affecting growth of plants through endogenous modulation of hormone levels, thereby advancing plant development by either immediate or aberrant mechanisms.

(b) Nitrogen fixation, phosphate solubilisation, indole acetic acid, growth and stress tolerance, and mechanisms involved in improved uptake of nutrients are some important plant growth promotion properties that have been found in soybean roots and wheat harboring *Pantoea* spp., *Paraburkholderia* spp., and *Pseudomonas* spp. [12].

(c) Arthrobacter spp. and Bacillus spp. are some of the plant growth-promoting bacteria that secrete an enzyme, 1-aminocyclopropane-1-carboxylate deaminase, which enhances growth of plant by reducing ethylene level, i.e., the main stress hormone in the plant [13].

(d) Some microorganisms can cause infection manifestations through the secretion of phytohormones, phytotoxic mixes, and proteins. Erwinia amylovora is a pathogenic bacterium that causes fre blight disease of ornamentals plants and fruit trees. Pseudomonas syringae is a notable plant pathogen having an exceptionally expansive host range including olive, tobacco, green bean, and tomato. *Xylella fastidiosa, Xanthomonas* species, and *Ralstonia solanacearum* are likewise connected with numerous signifcant maladies of banana and potato. The seriousness

of plant infection relies upon the combination of numerous components like pathogen populace size, susceptibility of host, plant microbiota, and favorable environment that by and large decide the result of plant-pathogen interaction [14].

(e) Both subterranean and aboveground plant-related microorganisms have been found to improve resistance of host against pathogen disease either through interaction of commensal pathogen or through plant defence modulation. Production of siderophores, antibiotics, pathogen inhibiting volatile compounds, and lytic enzymes are some of the activities which protect the plant against disease progression and invasion of pathogen. Specifically, genera like *Paraburkholderia, Paenibacillus, Pseudomonas, Bacillus, Enterobacter, Pantoea, Streptomyces,* and *Burkholderia* have been reported for their role in pathogen suppression. The nonstop utilization of agricultural soils can facilitate pathogen pressure and can likewise create malady suppressive soils containing microorganisms interceding suppression of disease [15]. *Firmicutes, Acidobacteria,* and *Actinobacteria* controlled the attack of *Fusarium* wilt at a continental scale. Soil disease suppressiveness of *Paraburkholderia graminis* PHS1 against fungal root pathogen was accounted to synthesis of cysteine desulfurase and dimethyl sulfoxide reductase. *Enterobacter* and *Serratia* were found to be the best possible endospheric community of bacteria for suppression of take-all diseases, i.e., *Gaeumannomyces graminis*.

Conclusion:

Biofertilizers are the perfect alternative to chemical fertilizers. The chemicals not only harm the soil and its productivity but also harm the living organisms consuming the crops grown on that soil. Therefore, the scientists had discovered the use of microorganisms as fertilizers. They have gained recognition over the years and are being implemented on a large scale to increase agricultural productivity without harming human health. Biofertilizers can help solve the problem of feeding an increasing global population at a time when agriculture is facing various environmental stresses. It is important to realise the useful aspects of biofertilizers and implement its application to modern agricultural practices.

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POST-HARVEST FUNGAL DISEASES OF FRUITS AND VEGETABLES IN THE MARKETS OF CHIKKAMAGALURU, KARNATAKA

Annapurneshwari. H

Department of Zoology,

IDSG Government College, Chikkamagaluru -577 102, Karnataka

Corresponding author E-mail: annapurneshwari3@gmail.com

Abstract:

The Survey of post-harvest fungal diseases of some fruits and vegetables in the markets of chikkamagaluru was undertaken to understand the cause for the post-harvest loss of fruits and vegetables. Spoilage occurs at the time of harvesting, handling, transportation, storage, marketing and processing. Fruits and vegetables were collected from different vegetable markets of chikkamagaluru. Previously infected or rotten fruits and vegetables were collected in polythene bags. They were kept in isolated conditions for proper growth of fungal hyphae. The casual organisms infecting the samples were observed directly by preparing lacto phenol cotton blue mounts. The identification is based largely on the morphological characters of spores bearing structure using direct microscopy. In this study 22 fruits and vegetables were studied and 9 fungal pathogens were observed. Among them *Alternaria, Aspergillus, Fusarium, pencillium, Rhizopus, Geotrichum, Sclerotinia, Colletotrichum* and *Trichoderma* were found to be the disease causing organisms. The post-harvest losses can be minimizing by adopting necessary culture techniques such as careful handling, packing and the use of appropriate chemicals at pre and post-harvest stages.

Keywords: Post-harvest, fungal diseases, vegetable markets, spores, rotten fruits.

Introduction:

Fruits and vegetables are commercially and nutritionally important food products. Fruits and vegetables play an important role in human nutrition by contributing the necessary growth factors such as vitamins and essential minerals in human daily diet maintaining a good and normal health. Post-harvest activities include harvesting, handling, storage, processing, packaging, transportation and marketing (1). These post-harvest losses are caused by the disease which occurs on fruits and vegetables (2). Fungi are the most crucial and common pathogens and the mean cause of crop diseases. It Infect a wide range of fruits and vegetables during storage

and transportation (3). The mechanical damages during and after the harvest can result in their spoilage by microorganisms such as fungi (*Alternaria, Aspergillus*, *Botrytis*, *Ceratocystis, Cladosporium, Colletotrichum, Fusarium, Geotrichum, Lasiodiplodia, Monilinia, Mucor, Penicillium, Pestalotiopsis*, *Phoma*, *Phomopsis*, *Phytophthora*, and *Rhizopus*), bacteria (*Erwinia, Pseudomonas, Xanthomonas, Acetobacter,* and *Enterobacter*), and yeast (*Candida* and *Saccharomyces*).

Postharvest losses of fruits and vegetables vary from an estimated 5 percent to more than 20 percent which can be more than 50 percent in the developing countries (4, 5). Although India is the largest producer of fruits in the world, its production per capita is only about 100 g per day. Between 20 and 30% of total fruit production goes to waste owing to spoilage at various steps of the postharvest chain, reducing per capita availability of fruits to around 80 g per day which is almost half the requirement for a balanced diet. The fruit processing sector has grown at a rate of about 20% per annum. India is the second largest producer of vegetables in the world, ranking next to China, and accounts for about 15% of global vegetable production. It is estimated that between 30 and 35% of India's total vegetable production is lost because of poor postharvest practices. Less than 2% of the total vegetable production in the country is commercially processed as compared to 70% in Brazil and 65% in the USA. The objective of the present study was to investigate and document the prevalence offungi responsible for the spoilage of some fruits and vegetables in the vegetable markets of Chikkamagaluru.

Materials and Method:

In the present study different fruits and vegetables were collected from vegetable markets and fruit shops of Chikkmagaluru. Previously infected or rotten fruits and vegetables were collected in polythene bags from the market to analysepost-harvest fungi. They were kept in isolated conditions for proper growth of fungal hyphae about 2-3 days. Vegetables and fruitswere taken to laboratory and the casual organisms infecting the samples were observed directly by preparing lacto-phenol cotton blue mounts. Identification of fungi was also based on the color of mycelia and microscopic examinations of vegetative and reproductive structures. The causal organisms infecting the samples were identified from the hand book of 'Soil fungi" by Nagamani *et al*. (6).

Results and discussion:

In the present study fungal diseases of fruits and vegetables commonly available in chikkamagaluru were studied and 10 fungal pathogens were observed. Among these *Alternaria,*

Aspergillus, Fusarium, Penicillum sp., *Geotrichum, Colletotrichum and Rhizopus* (Table 1) were found to be major disease causing organisms**.**

Table 1: Common postharvest diseases and pathogens of fruits and vegetables

Sr. No.	**Fruits and vegetables**		**Pathogen**	**Diseases**
1	Lemon	*Citrus aurantifolia*	*Geotrichum* *Penicillium digitatum*	White rot or sour rot Brown rot
2	Tomato	*Lycopersicum esculantum*	*Alternaria sp*	Alternaria rot
3.	Ginger	*Zingiber officinale*	*Fusarium sp*	Fusarium rot
4	Apple	*Malus pumila*	*Alternaria alternata*	Alternaria rot
5	Papaya	*Carica papaya*	*Geotrichum* *Colletotrichum papayae*	White rot or sour rot Anthracnose of Papaya
6	Chayote	*Sechium edule*	*Geotrichum sp*	soft rot
7	Lady's finger	*Abelmoschus esculentus*	*Sclerotinia*	Sclerotinia rot or white rot
8	Onion	*Allium cepa*	*Aspergillus niger*	Black mold rot
9	Ground nut	*Arachis hypogaea*	*Sclerotinia*	Sclerotinia rot
10	Beetroot	*Beta vulgaris*	*Fusarium sp*	Fusarium rot
11	Pomegranate	*Punica granatum*	*Aspergillus* *Penicillium*	Black mold rot Blue Green rot
12	Mango	*Mangifera indica*	*Colletotrichum*	Anthracnose
13	Bel fruit	*Aegle marmelos*	*Trichoderma*	Green mold
14	Banana	*Musa paradisiaca*	*Rhizopus*	Rhizopus soft rot
15	Green bean	*Phaseolus vulgaris*	*Colletotrichum*	Anthracnose
16	Sapota	*Manilkara zapota*	*Penicillium*	Blue Green rot
17	Coconut	*Cocos nucifera*	*Rhizopus stolonifera*	Rhizopus soft rot
18	Yellow cucumber	*cucumis sativus*	*Rhizopus sp*	Rhizopus soft rot
20	Brinjal	*Solanum melongena*	*Rhizopus sp*	Rhizopus soft rot
21	Potato	*Solanum tuberosum*	*Fusarium sp* *Geotrichum*	Fusarium dry rot Soft rot
22	Carrot	*Daucus carota*	*Rhyzopus sp*	Rhizopus rot

The findings of this study showed that fungal infection is mainly due to injury during storage and transportation (7). *Rhyzopus* was the most common genera that infected Yellow cucumber, Brinjal, Coconut, Carrot and banana. *Geotrichum* was the second most frequent genus on the tested fruit samples (Lemon, Papaya, Chayote and Potato) (8). Our data showed that could be *Colletotrichum* regarded as the third most frequent genus on the tested fruits (Papaya, Mango, Green bean). *Aspergillus* was isolated from Onion and Pomegranate. *Alternaria* was isolated from tomato and apple (9). *Penicillium* was isolated from Pomegranate and Sapota. *Fusarium* was isolated from Beetroot and potato. *Sclerotinia* was isolated fromLady's finger. Fungal species belonging to *Aspergillus, Trichoderma, Alternaria, and Penicillium* were generally rare on the different kinds of the tested fruits. When food and feed contaminated with fungi, and the toxins they may produce, are ingested by humans or animals, a wide variety of debilitating diseases can occur with some leading to death.

Conclusion:

In the present study 10 fungal genera could be regarded as most common causes of post-harvest deterioration of common fruits and vegetables available in chikkamagaluru market. A wide variety of pathogens cause postharvest disease in fruits and vegetables and can cause serious damage to them. Some of these pathogens contaminate before harvest and then remain silent or dormant until the conditions aremore favourable for disease development afterharvest. Other pathogens contaminate during andafter harvest through surface injuries. The post-harvest losses can be minimizing by adopting necessary culture techniques such as careful handling, packing and theuse of appropriate chemicals at pre and post-harvest stages.

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A CRITICAL REVIEW ON PHYTOCHEMICAL PROPERTIES AND ANTI-MICROBIAL ACTIVITIES OF GENUS *OCIMUM* L.

Mamita Kalita

Department of Botany,

Gauhati University, Guwahati, Assam, India

Corresponding author E-mail: mamita.bot@gmail.com

Abstract:

Ocimum L. being highly medicinal is rich in essential oil. A varied number of essential oil makes it important from a phytochemical point of view. Further, these Phytochemicals are paving the way for anti-microbial activities. These properties are fundamental to healthy society. These are effective, and the microbial growth percentage was reduced when the microbes were treated with Basil oil. The present study has been carried out based on a literature study. The study enumerates the associated microbial studies carried out so far in genus *Ocimum* L. The Basil plants have very much potential in being used as an anti-microbial agent.

Keywords: Phytochemical, Anti-microbial, Basil.

Introduction:

The genus *Ocimum* L. belongs to the tribe *Ocimoideae* of the family *Lamiaceae*. *Ocimum* is derived from the Greek word ‘ozo,' meaning smell. The plants are called Basil or Tulsi is recognized as Queen of herbs, Mother medicine of Nature (Singh *et al.*, 2010). A total of 150 species are allied to *Ocimum* L. (Dzoyem, 2017). The plant holds a special place in Hinduism. It was found to be grown in the courtyard or pots, especially Vrindavan. People were found to wear rosaries of the tulsi stem. In Hinduism, the Tulsi plant symbolizes Goddess Lakshmi's avatar and thus consort of Lord Vishnu. The leaves and inflorescence are offered in Hindu temples, and leaves sink in water, are consumed by worshippers, and are considered holy in nature. Medicinal Plants have long been used by humanity. The plant's parts such as leaves, fruits, seeds, roots, bark, and stem have medicinal properties. The bioactive compound that makes them essential is secondary metabolites. A single secondary metabolite can act as therapeutic, or there might exist a bunch of such metabolites that make the plants highly medicinal in nature. Since the rise of human civilization, medicinal plants have been used. The earliest mention of medicinal plants was found in Rigveda. Medicinal plants have long been part of study and research. They are the

focal area of interest by microbiologists, botanists, Ayurvedic experts, pharmacologists. Such research is vital for understanding the health issues and well-being of the growing human population. The holy Basil called *Ocimum tenuiflorum* (Synonym: *Ocimum sanctum*) is crucial from the medicinal and religious point of view (Gupta *et al.,* 2002). The holy Basil has anti-inflammatory, anti-pyretic, anti-stress, anti-arthritic properties (Singh *et al.,* 1996). *O. tenuiflorum* is among the herbs used for a healthy immune system and to treat gastrointestinal conditions (Anjaaria, 2002). The leaves are primarily used fresh or in dry powdery form. Herbal plants have great potential to use them as medicinal sources and control various pathogens (Doss., 2009). The basil plant is traditionally used to treat cough and cold, stomaches, headaches, inflammation, malaria (Singh *et al.,* 2010).

Phytochemicals as Anti-microbial agents:

Phyto-means plants are biologically active compounds present in plants and aid in anti-microbial activity. The essential oil, mainly the Eugenols, is present in *Ocimum* L. responsible for the antibacterial and antifungal activities of the genus (Pandian *et al.,* 2016). The *Ocimum* L. anti-microbial action constitutes coagulation of cytoplasm, damage to cytoplasmic membrane and membrane proteins, degradation of the cell wall, leakage of cell contents, depletion of proton motive force. The polyphenols in *Ocimum* L. oil are also effective against human cancer cell proliferation and melanoma (Warrier, 1995). The phytochemicals have inhibitory effects in all microorganisms (Cowan., 1999). Benzene, 1, 2-dimethoxy- 4-(1- propenyl) (Methyl-Isoeugenol) has Antifungal activity (Kurita *et al.,* 1981), Antifeedant activity (Katsumi, 1987), Nematicidal activity (Park *et al.,* 2003). *Ocimum* oil and its derivatives show an antiproliferative effect on Gram-positive and negative bacteria, antiprotozoal and *anti-Trichomonas vaginalis* (Bhattacharya *et al.,* 2019). The phytochemical study reveals the presence of alkaloids, terpenoids, steroids, flavonoids, glycosides, tannins, reducing sugar in medicinal plants. The percentage yield (29.08%) of such phytochemicals was higher in *O. tenuiflorum* (Joshi *et al.,* 2011). The main constituent of *O. tenuiflorum* includes Eugenol, carvacrol, β-caryophyllene, rosmarinic acid, oleanolic, and ursolic acid. Besides these, the plant also contains α-camphor, α-humulene, α-pinene, β-pinene, apigenin, 3-carene, carvacrol, caryophyllene, cirsilineol, cirsimaritin, citral, decyladehyde, Eugenol, isothymusin, isothymonin, limatrol, luteolin, methyl chavicol, methyl ester, rosmarinic acid, and terpinene-4-ol (Prakash and Gupta, 2005). β-caryophyllene possesses anti-microbial activity (Alma *et al.,* 2003).

Eugenol was the main constituent of tulsi found in India, Bangladesh, Brazil, Cuba, and Germany. Also, it is a significant component 67% in *Ocimum gratissimum*, responsible for antibacterial activity (Yamani *et al.,* 2016). *Ocimum gratissimum* has phytochemicals such as Eugenol, Geraniol, Thymol (Vieira., 2001). *O. gratissimum* is ample in Polyphenols such as Rosmarinic acid, Salvigenin, Trans-ferulic acid, and the Flavonoids being Apigenin 7-*O*-glucoside, Cirsimaritin, Isothymusin, Isovitexin, Kaempferol 3-*O*-rutinoside, Luteolin 5-*O*-glucoside, Luteolin 7-*O*-glucoside, Quercetin 3-*O*-glucoside, Rutin, Vicenin-2, Vitexin, and Xanthomicrol (Grayer *et al.,* 2000, Costa *et al.,* 2012, Ouyang *et al.,* 2013). Eugenol has Antimycotic activity (Azzouz *et al.,* 1982), Disinfection (Konstantopoulou *et al.,* 1992), Antiviral (Bishop, 1995), Antiparasitic (Pandey *et al.,* 2000), Antioxidant (Ou *et al.,* 2006) Anticancer (Hussain *et al.,* 2011). *Ocimum basilicum* is one of the constituents of proprietary digestive tonic used to treat Giardiasis and hepatic problems (Sodhi, 2003). The enteric pathogens Shiga toxin-producing *E.coli* (STEC) and *Salmonella* were found in fresh herbs of a few *Ocimum basilicum* which can impact human health (Delbeke *et al.,* 2015). The in-vivo studies of *O. tenuiflorum* suggest its effectiveness in decreasing blood pressure. The specific phytochemicals in *O. tenuiflorum* are anthocyanins, Eugenol, ursolic acid, flavonoids such as apigenin, polyphenols, and luteolin, thymol, or sesquiterpene alcohols. The pharmacological activity of *O. tenuiflorum* includes radioprotective, anti-microbial, hepatoprotective, cardioprotective, antifertility, anti-diabetic, analgesic, anti-pyretic, anticoagulant, anticataract, anti-hypertensive (Vijayasree, 2015). The Eugenol present act as Antibacterial, Anticancer, Anti-inflammatory, Antioxidant, Antispasmodic, Antiviral, Insecticide; α – Farnesene as Acaricide, Allergenic, Anaesthetic, Analgesic, Antibacterial, Antiedemic, Anti-inflammatory, Antioxidant, Antitumor, Antiulcer, Antiviral; Benzene, 1, 2- dimethoxy4-(1-propenyl) as Antibacterial, Insect-attractant, Nematicide; Cyclohexane,1,2,4- triethenyl as Antibacterial, Antiedemic, Anti-inflammatory, Antispasmodic (Borah and Biswas., 2018). The crude extract of *Ocimum tenuiflorum* leaves consists of phenolic compounds such as 3,4-dimethoxycinnamic acid, Caffeic acid, Permethrin, and Rosmarinic acid. Some other compounds have also been found being Chrysoeriol (flavon), Isosakuranetin (Flavanone), Kaempferol, Kaempferide (flavonol), Luteolin (flavonoid), Robinetin trimethyl ether (flavonoids), Xanthomicrol (flavonoid). Also, compounds identified from *Ocimum tenuiflorum* extract are Anthocyanidins, Diosmetin, Flavones, Flavone glycoside, Glycosides, Nevadensin, and Peonidin. (Mousavi *et al.,* 2018). The qualitative phytochemical investigations reveal the presence of alkaloids, flavonoids, and tannins, which might be responsible for antibacterial activity. Also, compounds with anti-microbial activity are cirsilineol, circimaritin, isothymusin, apigenin, and rosameric acid, isolated from aqueous extract

of *Ocimum tenuiflorum* (Panchal and Parvez, 2019). The leaf extract of Holy Basil (*O. tenuiflorum*) was effective against the paddy brown spot causal organism *Helminthosporium oryzae* (Sharma, 2021).

Examination of Anti-microbial activity:

The following methods can test the in-vitro anti-microbial activity of essential oil.

Agar diffusion method- This is also known as the Agar contact method. In this method, the anti-microbial agent is transferred through diffusion from chromatogram (PC and TLC) to an agar plate previously inoculated with microorganisms tested. After some diffusion time, the agar plate was incubated, and the chromatogram was removed. The inhibition zones appear where the agar layer contact with anti-microbial compounds.

Direct bioautography- It can be utilized for bacteria or fungi. The simplest form helps detect spore-producing fungi such as *Aspergillus, Cladosporium, Penicillium,* and bacteria such as *E.coli, Bacillus subtilis, Staphylococcus aureus.* In this method, the TLC plate is dipped with microbial suspension. The bioautogram is incubated for 48 hours at 25ºC. Tetrazolium salts help in the visualization of microbial growth.

Immersion bioautography- This method can be utilized for all microorganisms and hybrid the previous two methods. The TLC plate is covered with a molten seeded agar medium. The plates are kept for incubation and then placed under low temperatures. Then the desired microorganisms can be visualized by staining with tetrazolium dye. This method is also known as Immersion autography.

Dilution method- The dilution method can be performed using Agar dilution or broth medium. This method helps determine MIC (Minimum inhibitory concentrations) values. And so, it helps in testing anti-microbial agent concentrations.

Broth dilution method- The broth dilution method can be either micro- or macro dilution. The microdilution is performed in a 96-well microtitration plate. In contrast, the macro dilution can be completed in the minimum volume of 2 ml. Each dilution, whether micro or macro, is inoculated with the microbial agent (inoculum) prepared in the same medium.

ATP bioassay- The quantification of ATP is done in the process called ATP bioluminescence assay since ATP is the chemical energy form stored in all living cells. And so microbes ATP can also be tested using this method. In this method, D-luciferin undergoes conversion into oxyluciferin in the presence of ATP with the help of enzyme luciferase. The light quantity is expressed as RLU (Relative light Unit), converted into RLU/mole of ATP.

Ocimum L. and its Anti-microbial attributes:

Ocimum americanum:

The in-vitro study of *Ocimum americanum* essential oil was effective against oral microbes such as *Streptococcus mutans, Lactobacillus casei*, and *Candida albicans* (Thaweboon and Thaweboon, 2009). The main important oil present in *O. americanum* is Linalool. *Ocimum americanum* essential oil (however not Linalool) inhibited 90% hemolysis in fish erythrocyte caused by *Aeromonas hydrophila* and promoted the fish's survival (Sutili *et al.,* 2016). *Using the agar well diffusion method, Ocimum americanum* exhibits anti-microbial activity against all tested pathogens. In this, the highest inhibition was seen against *Bacillus cereus* (17 mm) and lowest in *Clostridium pentrigens* (07 mm). The ethyl acetate leaf extract of *O. americanum* was more potent than other extracts (Vidhya *et al.,* 2021).

Ocimum basilicum:

Ocimum basilicum shows anti-microbial activity against *Aspergillus flavus*. *O. basilicum* has shown MIC against antibacterial activities of 1024, 4, and 2 μg/mL against *S. aureus.* And the antibacterial activity of *Ocimum basilicum* may be associated with Linalool. The *O. basilicum* oil MIC value was 1024 μg/mL against *P. aeruginosa* isolate, and for Imipenem and Ciprofloxacin, 4 μg/mL, 2 μg/mL, respectively (Silva *et al.,* 2016*). Ocimum basilicum* was tested against *Salmonella enteritidisa* was found to be sensitive to Basil essential oil (Rattanachaikunsopon and Phumkhachorn., 2010). Total 36 chemical compounds were isolated from *Ocimum basilicum*, and Linalool was the principal constituent. These showed antibacterial activity against Gram-positive and Gram-negative bacteria (Abbasy *et al.,* 2015). *O. basilicum* shows tremendous anti-microbial activity against *S. aureus, E. coli, P. vulgaris* (Ilic *et al.,* 2021)

Ocimum gratissimum:

The essential oil of *Ocimum gratissimum* was found to inhibit *Staphylococcus aureus* at the concentration of 0.75 mg/ml. The Minimum Inhibitory concentration was in the range of 3-12 mg/ml for *Shigella flexineri, Salmonella enteritidis, Escherichia coli, Klebsiella sp.,* and more than 24 mg/ml for *Proteus mirabilis* (Nakamura *et al.,* 1999). *Ocimum gratissimum* revealed antibacterial activity against Gram-positive bacteria such as *Bacillus spp., Staphylococcus aureus,* and Gram negative being *Escherichia coli, Klebsiella pneumonia, Proteus mirabilis Pseudomonas aeruginosa, Samonella typhi* bacteria, and a pathogenic fungus *Candida albicans*. The highest antibacterial activity was seen in Gram-positive bacteria *Staphylococcus aureus* and Gram-negative bacteria *Pseudomonas aeruginosae* and *Proteus mirabilis* by *O. gratissimum* (Meru) oil (Matasyoh *et al.,* 2008).

Ocimum tenuiflorum:

O. tenuiflorum enhances antibody response (Dugenci *et al.,* 2003). *O. tenuiflorum* essential oil was more effective against *P. vulgaris, S. aureus, P. aeruginosa,* and *E. coli* (Helen et al, 2011), (Rasavi and Hosseinzadeh, 2019). The *Ocimum tenuiflorum* ethanolic extract showed a zone of inhibition of 6 mm against *Prevotella* and 7.33 mm against *S. mitis.* The extract was also averse for *Candida albicans* (10.67 mm) and *S. mitis* (7.33 mm). However, it failed to show antibacterial activity against *Lactobacillus* (Parida *et al.,* 2018). The zone of inhibition was 22 mm against *S. mutans* for *O. tenuiflorum* extracts (Agarwal *et al.,* 2010). The alcoholic extract of *O. tenuiflorum* was effective against periodontal pathogen such as *Aggregatibacter actinomycetemcomitans* but ineffective against *Porphyromonas gingivalis* and *P. intermedia* (Mallikarjun *et al.,* 2016). *Ocimum tenuiflorum* essential oil has antibacterial activity against Gram-positive (Staphylococcus aureus, *Enterococcus faecalis*, *B. cereus* at the concentrations of 0.5-32 µL/mL and also against Gram-negative bacteria *E. coli*, *Pseudomonas aeruginosa*, *Shigella flexneri* at concentrations of 0.25-4 µL/mL, except *P. aeruginosa* (Saharkhiz *et al.,* 2013). *Ocimum tenuiflorum* showed anti-microbial activity against *Actinobacillus actinomycetemcomitans* (Eswar *et al.,* 2016). *Ocimum tenuiflorum* acts against fungi and bacteria by affecting specific and non-specific immune responses (Santra *et al.,* 2017). The methanol extract of *O. tenuiflorum* successfully inhibited *Bacillus subtilis* with an inhibition zone of 35 mm and 37 mm against *E. coli* (Tyagi *et al.,* 2021).

The seven *Ocimum* species show solid anti-microbial activity against 08 microorganisms tested by Carovic Stanko *et al.,* 2010. These species are *Ocimum americanum, Ocimum basilicum, O. basilicum var difforme, O. basilicum var. purpurascens, O. basilicum cv Genovese, Ocimum campechianum, Ocimum citriodorum, Ocimum kilimandscharicum.* The minimum inhibitory concentrations (MIC) were 4.5% and 2.25% against *Staphylococcus aureus* and *E. coli*, respectively. The oil shows better inhibition against *S. aureus* in Gram-positive and Gram-negative bacteria (Mishra and Mishra, 2011). The essential oil of *O. basilicum, O. gratissimum,* and *O. kilimandscharicum* shows inhibition diameter of 15-24 mm against *Bacillus subtilis, Micrococcus luteus, Pseudomonas aeruginosa, Shigella dysenteriae, Escherichia coli, Vibrio cholera, Shigella flexneri*, whereas *O. americanum* and *O. tenuiflorum* shows substantial inhibition diameter 09-22 mm. The methanol extracts of *O. basilicum, O. gratissimum,* and *O. kilimandscharicum are* effective against *Bacillus subtilis, Escherichia coli, Vibrio cholera* only. And no antibacterial activity was shown by methanol extracts of *O. americanum* and *O. tenuiflorum* (Saha *et al.,* 2013).

Discussion:

The Basil (*Ocimum* L.) genera constitute annual, aromatic, herbaceous, and few suffruticose species. Humans have long used them for healing stomaches, cough-cold, wounds, warts, diarrhea, constipation, sexual problems. The plants have huge potential of being anti-microbial in nature. The anti-microbial properties are due to the phytochemicals present in them. These phytochemicals make the plants highly aromatic and medicinal. The chemical compounds such as Eugenol, Linalool, Camphor, Estragol, 1-8 Cineole are present in these species. The phytochemical and essential oil or Basil oil differs from *Ocimum* L. species. They might be in combination with one another since most Basil plants parts are used for medicinal purposes, as each of them is rich in Basil oil. Flowers, Leaves, Stem can be made into various extracts and avail for studying their anti-microbial features. A good practice of having *Ocimum* L. species around us and using them for different medicinal and culinary preparations can help humans achieve maximum health benefits.

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STUDY ON PANTRY PESTS AND THEIR CONTROL

Aleena Elsa Mani

Post graduate and Research Department of Zoology,

CMS College, Kottayam, Kerala

Corresponding author E-mail: alina7m@gmail.com

Abstract:

This study deals with the prevalence and control of pantry pests. Storing food grains at different conditions and surveying of pest infested samples collected from different localities yielded various pantry pest species. By analyzing these samples, conditions that favour the growth and multiplication of pests and the most prominent pest species were recorded. The life cycle of the most prominent species was studied in detail and an effort to determine an effective natural control measure against the prominent pests was carried out. This project suggests some effective biological control methods which will be applicable in our daily life to get rid of major pantry pests. The pest species observed were *Sitophilus oryzae, Sitophilus granarius, Tribolium castaneum, Callosobruchus chinensis, Acanthoscelides obtectus, Cryptolestes pusillus, Rhyzopertha dominica, Sitotroga cerealella,* and *Trogoderma granarium.* Conditions that determine the growth and multiplication of pantry pests were found to be, oxygen content, water and moisture content, temperature, duration of storage and the physical environment. The most prominent pantry pests obtained from survey were *Tribolium castaneum* and *Sitophilus oryzae*.

Keywords: Stored food grains, pantry pests, biological control, *Sitophilus oryzae, Tribolium castaneum.*

Introduction:

An insect whose population increases to the extent that it starts causing annoyance, inconvenience or injury to man, his animals, plants and material possessions can be called as an insect pest (Srivastava, 1988). The pests that infest stored foods are referred to as pantry pests. The prominent bio-deteriorating agents are rodents, insects and microorganisms. Among these, insects are the major actors. About 500 species of insects have been associated with stored grain products mostly Coleopterans (beetles and weevils), a few Lepidopterans (moths), and Psocopterans. Nearly 100 species of insect pests of stored products cause economic losses (Bhargava *et al.*, 2015).

Pantry pests are of different types which includes, external feeders that feed on germ and endosperm from outside and attack the whole seed and internal feeders which lay egg inside or

on the seeds and spend a part or entire larval and pupal life inside the seed and only emerges out as adults. The insect pest infestation can be a horizontal infestation in which the infestation starts from the mature standing crop and continues during storage through transportation, crop-drying, threshing or any other post-harvest operations by some pests like Angoumois grain moth, lesser grain borer etc.; a cross infestation where the lateral infestation of storage is commenced during the post-harvest operations from other sources like previously stacked and infested crop on threshing, floor previously used and infested transport etc.; a latent infestation where the insect pests infest the grains through the latent or hidden stages from the cracking walls and surface of storage containers and a vertical infestation in which the infestation starting from top may reaches bottom of bulk or vice-versa by a number of coleopteran beetles and weevils.

The present study focusses on the diversity and abundance of pantry pests from collected and conditioned samples, the factors responsible for their multiplication and their control measures.

Methodology:

Pests of stored grains from 35 houses at different locations in Kottayam were collected and the conditions that promote growth of pests of stored grains were analyzed by keeping three sets of clean, dry bottles with different types of grains such as rice, wheat, rice flour, wheat flour, oats, green gram, Bengal gram, ragi powder, chick pea, cashew nuts, almonds, semolina, urad dal and moong dal under different conditions as follows:

- Bottles with grains kept open for three weeks.
- Bottles with grains kept airtight.
- Bottles with grains soaked or immersed in excess water.

Biopesticidal action of certain botanicals was tested against *Sitophilus oryzae* and *Tribolium castaneum.* For this, the two pest species with definite number were kept in separate bottles containing ginger, garlic, neem leaves, ocimum and pepper. Ginger and garlic were slightly crushed, pepper was grinded well, neem leaf and ocimum were made into a paste and applied and then observed for about one week. Observations were recorded after each day's exposure.

Result and Discussion:

Different samples obtained from the 35 localities in and around Kottayam were analyzed to identify the pests which caused damage to the grains. The major insect pest species found responsible for loss in stored grains are shown in **table 1.**

Table 1: Taxonomic position of observed pantry pests

Sr. No.	Scientific name of species	Order	Family
1	*Sitophilus oryzae*	Coleoptera	Curculionidae
2	*Sitophilus granarius*	Coleoptera	Curculionidae
3	*Tribolium castaneum*	Coleoptera	Tenebrionidae
4	*Callosobruchus chinensis*	Coleoptera	Chrysomelidae
5	*Acanthoscelides obtectus*	Coleoptera	Chrysomelidae
6	*Cryptolestes pusillus*	Coleoptera	Laemophloeidae
7	*Rhyzopertha dominica*	Coleoptera	Bostrichidae
8	*Sitotroga cerealella*	Lepidoptera	Gelechiidae
9	*Trogoderma granarium*	Coleoptera	Dermestidae

The prominent pests were determined by calculating the abundance of pantry pests obtained from the conditioned samples. The abundance calculation is done during the time of peak infestation. Thus the most prominent pantry pests occurring in repeated frequency are *Tribolium castaneum* and *Sitophilus oryzae.*

Conditions that favour the growth and multiplication of pantry pests are oxygen, water and moisture content, temperature, duration of storage and physical environment. The experiments on the pesticidal effect of certain botanicals against pantry pests reveal that Pepper powder, Ocimum leaf paste and neem leaf paste are very effective against *Sitophilus oryzae*. Ginger extract and garlic extract are effective against *Sitophilus oryzae* but slow in action. All plant extracts applied here are effective against *Tribolium castaneum*. The alkaloids present in these plant extracts are responsible for the pesticidal action. Pipperin in pepper, azadirachtin in neem, diallyl trisulphide in garlic, zingiberene in ginger, methyl eugeniol in ocimum, are some alkaloids of pesticidal action. Neem leaves contain large number of bitter compounds like Nimbin, Nimbidin, Nimbidiolquercetin, Azadirachtin, Gedunin, Salannin, etc. Active principle in neem is Azadirachtin (Praveen Chandra, 2006). Huang (1995) reported that Ginger causing adult mortality in *Calloso bruchus chinensis* and had a repellent effect on *Tribolium castaneum*.

Pepper powder is found as the most suitable botanical to get rid of the most common pantry pests since its powder form itself has a pesticidal action and it do not cause any distaste to the stored grains. Garlic and ocimum act as toxicants and neem, a grain protectant and pepper acts as a contact insecticide, pepper and neem are found to be highly effective biological control methods (Rajashekhar *et al.*, 2012). Mixing of plant leaves of *Ocimum sanctum*, Eucalyptus and Citrus sp, which contain essential oils with stored paddy at 1% , effectively controlled primary

and secondary insect pests like *Sitophilus oryzae* (Abeysekera *et al.*, 2004). So the observations here are matched with the observation of an early experiment.

Table 2: Distribution of pantry pests under different conditions

Stored items	Species of pantry pests obtained when kept under different conditions		
	Aerated condition (2-3 weeks)	**Non aerated condition (3-4 months)**	**Medium with moisture content (1-2 days)**
Rice grains	*Sitophilus oryzae*	*Sitophilus oryzae*	*Aspergillus*
Wheat	*Sitophilus granarius*	*Sitophilus* species	*Aspergillus*
Oats	*Trogoderma granarium*	*Tribolium* species and *Sitophilus* species	Fungus
Semolina	*Rhyzopertha dominica*	*Tribolium castaneum*	Nil
Rice flour	*Sitophilus oryzae*	*Sitophilus oryzae*	Scavenger pests
Wheat flour	*Tribolium castaneum*	*Sitophilus and Tribolium species*	Fungus
Green gram	*Acanthoscelides obtectus*	*Rhyzopertha dominica*	*Aspergillus flavus*
Bengal gram	*Sitophilus oryzae*	*Callosobruchus chinensis*	*Aspergillus flavus*
Black gram	*Sitophilus oryzae*	*Callosobruchus chinensis*	*Aspergillus flavus*
Cow peas	*Callosobruchus chinensis*	Nil	*Aspergillus flavus*
Ragipowder	*Acanthoscelides obtectus*	Nil	Fungus
Cashew nut	*Sitophilus oryzae*	*Sitophilus oryzae*	Nil
Almonds	*Tribolium species*	Tribolium species	*Aspergillus flavus*

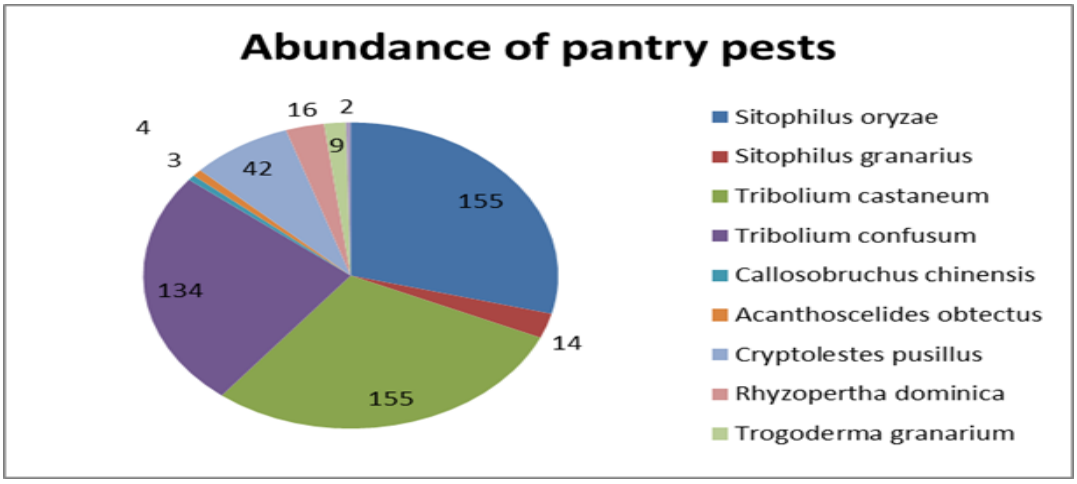

Figure 1: Pie diagram showing the abundance of pantry pests

Table 3: Abundance of pantry pests obtained from the conditioned samples

Name of species	Number obtained	Abundance in %
Sitophilus oryzae	155	29.02
Sitophilus granarius	14	2.62
Tribolium castaneum	155	29.02
Tribolium confusum	134	25.09
Callosobruchus chinensis	3	0.56
Acanthoscelides obtectus	4	0.74
Cryptolestes pusillus	42	7.8
Rhyzopertha dominica	16	2.99
Trogoderma granarium	9	1.68
Sitotroga cerealella	2	0.37
Total	534	

Table 4: Pesticidal effect of certain botanicals on *S. oryzae* and *T. castaneum*

Botanical used	***S. oryzae (Pest mortality)***					***T. castaneum (Pest mortality*)**				
	initial	**1day**	**2days**	**3days**	**>3**	**initial**	**1day**	**2days**	**3days**	**>3**
Pepper	12	-	12	-	-	12	12	-	-	-
garlic	12	-		9	12	12	9	-	12	-
ginger	12	-	9	-	12	12	9	-	12	
Neem	12	12				12	-	-	-	12
ocimum	12	12				12	-	-	-	12

Conclusion:

Most of the pests collected were insects including both external and internal feeders and also primary and secondary pests. Both larvae and adult were found to be active feeders in many cases. Moisture, oxygen, temperature and duration of storage enhance pest growth and multiplication. Since human population is expanding, we should be very careful in storing food grains in order to prevent its wastage. All precautionary steps must be taken before storing grains. The use of airtight containers and fumigation procedures must be done to prevent pest infestation on stored grains. To conclude, storing of food grains requires great attention as it may adversely affect health and economy of people and the nation.

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CLIMATE RESILIENCE FOR ENHANCING PRODUCTIVITY AND PROMOTING AGRICULTURAL SUSTAINABILITY

Saipayan Ghosh[1], Vivek Kumar Patel*[2] and Shubhashi Kumar[2]

[1]Department of Horticulture,

[2]Department of Plant Pathology,

PG College of Agriculture, RPCAU, Pusa

*Corresponding author E-mail: vivek10995@gmail.com

Abstract:

Globally, climate change impacts are manifold, severely affecting the agriculture sector. Agricultural systems are extremely vulnerable to climate change (CC), given their sensitivity to variations in temperature, precipitation and occurrence of natural events and disasters such as droughts and floods. Climate-Resilient Agriculture (CRA) measures at scale to address the impending impact of CC on agriculture. Hence, enabling CRA in the region constitutes one of the four fundamental pillars of Action on Climate Today CRA practices mainly include systematic strategies for management of critical inputs, namely, land and water, cropping systems and livelihood management. CRA is a subset of Climate-Smart Agriculture (CSA) interventions with the specific objective of enhancing the resilience of agricultural systems and the social systems that depend on these. While CSA focuses on both mitigation and adaptation measures, CRA is concerned only with adaptation and resilience. The approach goes beyond on-farm activities and considers off-farm options such as livelihood diversification, when viable in the context of enhancing resilience.

Keywords: Agriculture, climate change, resilience, sustainability

Introduction:

Agriculture and food production are highly vulnerable to climate change. Extreme weather events such as droughts, heat waves and flooding have far-reaching implications for food security and poverty reduction, especially in rural communities with high populations of small-scale producers who are highly dependent on rain-fed agriculture for their livelihoods and food. Climate change is expected to reduce yields of staple crops by up to 30% due to lower productivity and crop failure (Jain *et al.*, 2015). To cope with climate change, farmers need to modify production and farm management practices, such as adjusting planting time,

supplementing irrigation (when possible), intercropping, adopting conservation agriculture, accessing short- and long-term crop and seed storage infrastructure, and changing crops or planting more climate-resilient crop varieties. Indian agriculture, almost 60% of its net cultivated area as rainfed, is exposed to stresses arising from climatic variability and climate change. India has the unenviable problem of ensuring food security for the projected most populous country in 2050 with one of the largest malnourished populations.

a. Definition

Climate resilience can be generally defined as the adaptive capacity for a socio-ecological system to: (1) absorb stresses and maintain function in the face of external stresses imposed upon it by climate change and (2) adapt, reorganize, and evolve into more desirable configurations that improve the sustainability of the system, leaving it better prepared for future climate change impacts (Folke *et al.,* 2006 and Nelson *et al.,* 2007). The three basic capacities that are understood (Sotelo Reyes *et al.,* 2017). Under the common definition are absorptive, adaptive, and transformative, each of which contribute different factors to the efforts of resilience work.

b. Comparison between climate resilience and climate adaptation

Climate change adaptation includes planned or autonomous actions that seek to lower the risks posed by climatic changes, either by reducing exposure and sensitivity to climate hazards or by reducing vulnerabilities and enhancing capacities to respond to them. Adaptation also includes exploiting any beneficial opportunities presented by changing climates. On the other hand, Climate resiliency is the capacity for a socio-ecological system to absorb stresses and maintain function in the face of external stresses imposed on it by climate change, and adapt, reorganize and evolve into more desirable configurations that improve the sustainability of the system, leaving it better prepared for future climate change impacts.

Climate-resilient crops are crops and crop varieties that have enhanced tolerance to biotic and abiotic stresses. They are intended to maintain or increase crop yields under stress conditions such as drought, flooding (submergence), heat, chilling, freezing and salinity, and thereby provide a means of adapting to diminishing crop yields in the face of droughts, higher and lower than seasonal temperatures, and other climatic conditions (Dhankher *et al.,* 2018 and Saab *et al.,* 2016).

Climate-smart agriculture is an approach or set of practices aimed at increasing agricultural productivity and incomes sustainably, while building resilience and adapting to climate change conditions and reducing and/or removing greenhouse gas emissions where possible (Lipper *et al.,* 2014).

1. Elements of Resilience

Climate change resilience requires the following elements:

a. **Flexibility** at an individual, organizational, and systemic level, with each level able to respond and contribute to each situation, and to respond to shifting and unpredictable circumstances.

b. **A multi-faceted skill set**, including abilities that enable thorough preparation, such as comprehensiveness and detail-orientation; survival, such as quick decision-making and resourcefulness; or rapid recovery, such as innovation and diligence.

c. **Redundancy of processes**, capacities, and response pathways within an institution, community, or system, to allow for partial failure within a system or institution without complete collapse.

d. **Collaborative multi-sector approaches** to planning, execution, and recovery, since no one sector has a monopoly on a particular impact and thus understanding the overlaps and gaps between sectors is critical.

e. **Planning and foresight** to prepare for identified impacts and risks. While it is impossible to plan for every possible set of impacts, and in many cases the cumulative effect of impacts is unknown, the process of planning brings learning, builds skills, and helps to create resilience.

f. **Diversity and decentralization** of planning, response, and recovery activities. A diversity of options has greater potential to match the particular scenario of impacts that occurs, while decentralization allows for parts of the system to continue operations even if other parts of the system are down.

g. **Plans for failure** so that break-downs happen gracefully, not catastrophically—for example, when flood gates break, they do so in a way that channels floodwaters to uninhabited floods zones, perhaps damaging property, but protecting human lives. Accepting that the unpredictability and uncertainty of climate risks and responses will ultimately lead to failure of some element of the system allows for failure-planning. In some cases returning to a pre-existing state will not be possible or will not be appropriate. Incremental failures and planning for failures will allow for real-time response and revision and will limit social, environmental, and economic costs. Total system failure limits response options and results in greater suffering.

2. Influence of agriculture on climate change

Through agricultural activities (e.g., land clearing, cultivation of annual crops, irrigation, grazing of domesticated animals), humans are extensively altering the local, national and global land-cover characteristics, including physiological and physical characteristics. It is generally accepted that the expansion of agriculture into natural ecosystems has had a significant climate

impact. Lobell *et al*., 2006) used the National Center for Atmospheric Research (NCAR) general circulation model to demonstrate that a reduction in tillage can have a significant cooling effect by increasing the albedo. The NCAR model predicted that increases in soil albedo by reduced tillage have a potential global cooling effect of 0.2°C. This value is comparable to the biogeochemical cooling from the expected global soil carbon sequestration potential. Boucher *et al.* (2004) examined the human influence of irrigation on atmospheric water vapor and climate. They estimated a global mean radiative forcing in the range of 0.03 to 0.1 Wm^{-2} due to the increase in water vapor in the atmosphere, but a cooling of up to 0.8°C over irrigated areas. Conversion of land from summer fallow to crops decreases air temperature and increases the water content of the air, potentially resulting in greater precipitation.

3. Influence of climate change on agriculture

Sea level rises in the Indian Ocean pose a threat to coastal communities, especially when linked to cyclones (IPCC, 2014b). Low-lying islands in the Sundarbans have already been submerged, with thousands of people displaced (Aalst *et al.*, 2003). Sea level rises can also increase salinity in water and land in coastal areas, severely disrupting agricultural production.

Many parts of South Asia are likely to experience a decline in crop productivity unless there is a shift to different crop varieties and management practices (Ban *et al.*, 2008). Climate change may trigger pests and diseases, which have the potential to severely limit production (Chakraborty and Newton, 2011). Crop production is likely to shift northwards, with cooler regions likely to benefit from warmer temperatures and an increase in their arable area (Altdorff *et al.*, 2018). Rice is vulnerable to heat stress in parts of South Asia but warming may boost wheat production in upland Pakistan.

Precipitation anomalies have detrimental effects on agriculture, mainly in developing nations. Apart from affecting crop yields, it significantly influences cropland areas. There is evidence suggesting that the approximately 9% rate of cropland expansion in the developing world over the last two decades is due to dry anomalies as farmers expand the area to compensate for yield losses (Hijioka *et al.*, 2014). Global warming will pose a severe threat to the world's food security, but if it is limited to 1.5 ◦C, the 76% developing countries' vulnerability will be reduced compared with the same regions at 2 ◦C (Pushpanjali *et al*., 2021). Ensuring food for the world's population in the face of climate change is not an easy task, owing to its huge impact on agriculture production (Sutton *et al.*, 2013). There must be an annual increase in the world's agricultural production by 60% from 2005/2007 to 2050, comprising a rise of 77% in developing and 24% in developed countries, to fulfill the food and nutritional

requirements of the population by 2050. Climate change is known to have an adverse effect on agricultural production, and is projected to reduce the global cereal production of maize and wheat by 3.8% and 5.5%, respectively (Komarek *et al.*, 2020). Because of climatic factors, plants have to face several abiotic stresses such as salinity, drought, heat stress, cold stress, etc.(Rama Rao *et al*., 2018). Shortage of water availability, soil fertility loss, and pest infestations in crops are the significant undesirable impacts of climate change (Kurup *et al.*, 2021).

4. Necessity to adapt to climatic vulnerability

Planned adaptation is essential to increase the resilience of agricultural production to climate change. Several improved agricultural practices evolved over time for diverse agro-ecological regions in India have potential to enhance climate change adaptation, if deployed prudently. Management practices that increase agricultural production under adverse climatic conditions also tend to support climate change adaptation because they increase resilience and reduce yield variability under variable climate and extreme events. Some practices that help adapt to climate change in Indian agriculture are soil organic carbon build up, *in-situ* moisture conservation, residue incorporation instead of burning, water harvesting and recycling for supplemental irrigation, growing drought and flood tolerant varieties, water saving technologies, location specific agronomic and nutrient management, improved livestock feed and feeding methods. Institutional interventions promote collective action and build resilience among communities. Capacity building by extensive participatory demonstrations of location specific agricultural practices helps farmers gain access to knowledge and provides confidence to cope with adverse weather conditions.

5. Adaption strategies for development of climate resilience

Climate change adaptation strategies must focus on development of new land use systems, climate resource conserving smart agro-strategies and resource-use efficient and multiple stress tolerant genotypes.

a. Waterlogging tolerance in crops is primarily associated with two major physiological traits (Flowers *et al.,* 2010; Mullan and Bannett-Lennard, 2010) that enable plants to avoid soil hypoxia. These are, to form root cortex aerenchyma to conduct O_2 and ability to form a barrier for radial oxygen loss that decreases its leakage from root inducing more internal diffusion to the tip.
b. Physiological basis of tolerance to complete submergence in rice involves genetic factors in addition to the Sub1 gene. This suggests the possibility of further improvements in submergence tolerance by incorporating additional traits present in FR13A or other similar land races.

c. Yadav *et al.* (2003) reported dual purpose (fodder and grain) potential of *Horduem vulgare*, under waterlogged saline conditions, which needs morphophysiological characterization for further exploitation in the wake of expected climatic changes.
d. Halophytes are naturally evolved salt-tolerant plants. These have inherent ability to complete their life cycle in salt-rich environment where almost 99% of salt-sensitive species die because of NaCl toxicity. These could be potential new crops (Dagar, 2003; Jardat, 2003), particularly for coastal areas where if necessary sea water may be used for irrigation.
e. Though the salinity and waterlogging stresses can be as hostile for the woody tree species, these are known to tolerate these stresses better than the annual crop species. Therefore, the existing information is collated on site-specific agroforestry systems and appropriate afforestation technologies for saline and waterlogged environments of the varied agroclimatic situations.

Seed and adoption of climate-resilient crops. Seventy-three papers mentioned the topic of seed. Social networks such as farmers' organizations or co-operatives, as well as access to information, were also reported as facilitators of adoption. These themes refer to different social groups and ways in which farmers can exchange seed or get information about seed.

Social differences and adoption of climate-resilient crops. About 53% of studies reported that social differences (such as sex, education and age of household head) influence adoption of varieties or crops as mitigation strategies against the effects of climate change. Of the studies that identified social differences as influencing adoption of climate-resilient crops and crop varieties, education (22%), sex (28%), age (24%) and family size (14%) emerged as the most important factors. The studies largely concur that socio-economic status of farmers plays a large part in their adoption of climate-resilient technologies.

6. Technological approaches towards climate resilient agriculture

a. Building resilience in soil

The majority of soil-water management techniques are centred on rainfall gathering in its broadest terms. Four major groups can be distinguished: (1) in situ water harvesting systems, (2) ex situ water harvesting systems, (3) evapotranspiration management, and (4) suboptimal irrigation. Individually or in combination, the practises that make up a certain group all serve various purposes.

Table 1: Soil - water management strategies for building resilience in soil (Cornelis *et al.*, 2019)

Soil-water management strategy	Purpose	Management options	Management type
In situ water harvesting systems	Maximize infiltration capacity of the soil	Improve topsoil conditions	Protective surface cover: cover crops, residue, mulches against disruptive action of raindrops – No or reduced soil disturbance by tillage – Conservation agriculture – Soil amendments – Fallowing under cover crops or natural vegetation – Temporary closure of grazing land and subsequent protection
		Improve subsoil conditions	– Deep tillage: subsoiler or paraplough to break-up water restricting layers
	Slow down and/or impede runoff	Increase surface roughness	Surface cover: cover crops, residue, mulches, geotextiles – Conservation agriculture
		Apply physical structures across slope or along contour	Terracing: level terraces, bench terraces, Zingg terraces (conservation bench terrace), fanyajuu (Swahili, uphill ridge), fanyachini (Swahili, downhill ridge), murundum (Portuguese, large earth bank), contour bund, graded channel terrace, orchard terrace, platforms, hillside ditches, … – Broad bed and furrow system – Contour field operations

			– Contour ridges and tied ridges – Impermeable and permeable contour barriers: stone bunds, walls, earth banks, trash lines, live barriers
Ex situ water harvesting systems	Harvest and divert rainwater	Harvest flood water	Floodwater harvesting within stream bed – Floodwater diversion: cascade systems
		Harvest groundwater	– Qanat (Persian, gently sloping underground tunnel with vertical shafts)
Evapotranspiration management	Minimize water losses from evaporation and excessive transpiration	Minimize soil evaporation	– Surface cover: residue, mulches – Conservation agriculture – Dry (early) planting – Seed priming – Fertilizer/manure to speedup shading – Adjust plant density and response farming
		Minimize unproductive plant transpiration	– Weed control – Crop rotations – Conservation agriculture – Water efficient crops

b. Building soil carbon

While there are other ways to trap carbon, such as organic manure addition, green manuring, brown manuring, crop rotation, or intercropping with legumes, biochar has a unique quality that is noted below. Biochar is a fine-grained, carbon-rich, porous byproduct of the thermo-chemical conversion process (pyrolysis) of biomass production at cold temperatures (350–600°C) in an environment with little or no oxygen (Amonette and Joseph, 2009).

Table 2: Approaches towards climate resilient agriculture by building soil carbon

Factor	**Impact**
Fertilizer use efficiency	10 -30 % increase
Soil moisture retention	Up to 18 % increase
Crop productivity	20 % increase
Biological nitrogen fixation	50 – 72 % increase

c. Avoid bare soil

The removal of soil particles from the parent body and their transfer must be prevented since it depletes the regional soil fertility. Land contour cultivation, land configuration, and so on. In grasslands, cover vegetation and avoid overgrazing. Mulching Belts that act as a windbreak and a shelter cropping in strips.

7. Climate resilient agricultural strategies

Climate-resilient farming methods are primarily connected to (a) crop management, (b) water management, (c) land management, and (d) livelihood management from a larger viewpoint.

a. **Crop Management Strategies**

Crop rotation, drought-tolerant seed varieties, short-season crops, shift to new crops, legume intercropping, crop diversification, and shifting planting dates are all common climate-resilient crop management practises used by farmers. It's critical to choose proper crops for rotation since this elevates the nitrogen level in the soil, which boosts phytomass production and improves soil organic matter. (Raphael *et al.*, 2016).Increasing soil organic matter is a long-term strategy for improving crop yield while both improving carbon sequestration and keeping the biogeochemical cycles in check.

Herbicide-tolerant crops and drought-tolerant maize helped Africa attain climate change resilience and mitigation (Neate *et al.*, 2013). According to Birthal *et al.* (2015), despite the fact that approximately one-third of India's rice-growing areas are vulnerable to droughts, crop

improvement technology has enhanced rice production's drought resilience. Given the unforeseeable negative effects of climate change on food and agricultural production, genetic modification of climate-resistant crops is becoming increasingly popular as a strategic plan for adaptation. Despite the many benefits of genetically modified (GM) seeds in agriculture, the usage of GM food crops is controversial (Pray *et al.*, 2011; Saab *et al.*, 2016).

a. Water Management Strategies

To tackle climate change, proper irrigation practises are changing dramatically, including the implementation of deficit irrigation, the simultaneous the use surface and groundwater, desalination and water supply, the growth of water management, and the implementation of watershed management strategies. Therefore, choosing an effective irrigation system is critical for ensuring the long-term viability of restricted water supplies. Micro-irrigation methods have been extensively implemented throughout states as a result of national and state regulations encouraging the use of sprinkler and drip irrigation systems to alleviate water stress caused by climate change (Bahinipati and Viswanathan, 2017, 2019a).

Water conservation and correct application via agricultural lakes and community tanks helped boost cropping intensity and build resilience in rainfed regions, according to a research in Bihar. The most significant option for decreasing the potential impacts of climate change and drought is to revitalise existing watersheds. It also allows the farmer to use a variety of farm-level initiatives to improve their resilience in drought-stricken areas of India (Patnaik *et al.*, 2019).

b. Sustainable Land Management Strategies

Sustainable land management is defined by the United Nations as "the use of land resources, such as soils, water, animals, and plants, for the supply of products to fulfil changing people's needs while ensuring the long-term productive potential of these resources and the maintenance of their environmental functions" (Sanz *et al.*, 2017). Despite its lower output than traditional agriculture, organic farming is largely regarded as a sustainable farming approach (Andersen *et al.*, 2015).

8. Policies and Schemes Promoting Climate Resilient Agriculture in India

The National Innovations on Climate Resilient Agriculture (NICRA) programme was created by the Indian Council of Agricultural Research (ICAR) in 2011 with the objective of boosting the agriculture sector's resilience to climatic variation via strategic research and implementation of technology (Sikka *et al.*, 2018). Oil Health Card Scheme, National Mission for Sustainable Agriculture (NMSA), Pradhan Mantri Krishi Sinchai Yojana (PMKSY),

Paramparagat Krishi Vikas Yojana, National Agriculture Market, Agriculture Contingency Plan, Rainfed Area Development Programme, National Watershed Development Project for Rainfed Area (NWDPRA), and Pradhan Mantri Fasal BimaYojana are just a few of the notable national schemes (PMFBY). Because the farmers usually are resource-poor and ill-equipped to deal with climate challenges, it is critical that basic agriculture infrastructure services are provided to them in order to encourage implementation of Climate Resilient Agriculture techniques.

9. Climate-resilient Agriculture: Current Scenario

Farmers and other stakeholders throughout the world are adopting a variety of climate-resilient practises, technology, and initiatives to mitigate the negative impact of climate change. Conventional and intensive farming approaches are used in such strategies. Conventional farming is labor-intensive and is still performed in China (Altieri and Nicholls, 2017), India, Indonesia, Bangladesh, Vietnam (Viswanathan *et al*., 2012) and many regions of Africa (Abate *et al.,* 2000).Smart farming methods, such as using Microsoft's Cortana Intelligence to determine the best sowing dates for crops, are becoming more popular, particularly in India and Colombia. (López and Corrales, 2017), to identify weed growth, unmanned aerial vehicles (UAVs) or drones are used (Lottes *et al.,* 2017). Robotic milking of cattle is also increasing popularity all over the world (Rose and Chilvers, 2018).

10. Challenges

a. Stress tolerance systems could be beneficial in extreme stress conditions, which are nowadays unusual but are anticipated to become more common as a result of climate change. Stress tolerance systems disrupt cellular activities, potentially resulting in performance consequences. For its deployment, more sophisticated alterations, such as stress-specific expression of genes conferring stress tolerance, may be necessary. (Chen *et al*., 2015). On the other hand, various stress response strategies, such as those based on oxidative stress tolerance and cellular functioning protection, may be ideal for adaptability to diverse stresses and conditions. (Ahmad *et al.,* 2010).

b. CO_2 levels in the atmosphere could reach 600 parts per million by the turn of the era, more than double the pre-industrial level. (Van *et al.,* 2011).More environmental stresses tolerance, reduced stomatal closure and oxidative stress, improved root growth, and higher Water Use Efficiency at the expense of lower inorganic nutrient concentrations are all potential benefits. (Myers *et al.,* 2014).

c. Land degradation and harsh environmental conditions increase the likelihood of plants being subjected to multiple stresses at the same time, such as drought and heat, salinity and nutrient deficiencies, or toxicity in various combinations.

d. When opposed to severe stress situations, selected under optimized condition is much more likely to result in better yields, as crossover interactions for yield performance are less likely to happen (Blum *et al.*, 2005).

Conclusion:

Agricultural sustainability, in an era of climate change, concerns the farmers, communities, policy makers and the researchers alike. Scientifically developed indicators for assessing climate change resilience and sustainable development aid in the development of treatments that are best appropriate for a given agro-ecosystem. Climate Resilience Agriculture (CRA) highlights the emergence of global agricultural systems in the context of climate change. According to the study, rural households around the world use a variety of techniques to deal with the effects of climate change on agricultural and farm livelihoods. It might be claimed that CRA has the potential to become a game changer in global agriculture history by introducing an integrated strategy to ensuring food security using existing and continually developing agricultural methods. Despite the fact that many CRA practises are widely used around the world to build climate change resilience, there are substantial differences in adoption between countries due to the difference in social, economic, political, and institutional systems, as well as agro-ecological and hydrological–environmental factors.

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XENOBOTS: THE NEXT STEP IN BIOLOGICAL – ROBOTICS

Shikha Thakur

Department of Microbiology,

Sai Institute of Paramedical and Allied Sciences,

Dehradun, Uttarakhand, 248 001

Corresponding author E-mail: patho.shikha@gmail.com

Regardless of the modernity or complexity, the concept of robots and robotics is something that scientists and researchers have engaged in for the past several decades. When it comes to the assemblage of robots they are generally assembled or created by humans. For this reason, self-replicating robots, capable of creating more copies of them without human intervention, have been a long-standing goal of scientists. As a solution to this problem, scientists at the University of Vermont, Tufts University, and the Wyss Institute for Biologically Inspired Engineering at Harvard University have discovered an entirely new form of biological reproduction with what they call Xenobots. These Xenobots are made from the embryo cells of the *Xenopus laevis* frog.

The word robot tends to supplicate images of metallic constructs performing slowly and in a cumbersome manner. However, a robot need not be necessarily made out of inorganic components like steel and plastics. A robot by definition is simply a tool or an object that performs a set of specific tasks given to it. The Xenobots are one such example. They are biological robots. The embryonic cells themselves have the property of plasticity, to take various forms depending upon the task required. The cells under normal conditions simply congregate until they eventually reach their natural life span and die out. However, the scientists thought to change this fact in a simple yet effective way.

For this task, a specialized Algorithm was used to create the ' Shape ' required for the cells. An artificial intelligence program working on the Deep Green supercomputer cluster at UVM's Vermont Advanced Computing Core is an evolutionary algorithm that was able to test billions of body shapes in simulation - triangles, squares, pyramids, starfish, to find ones that allowed the cells to be more effective at the motion-based "kinematic" replication was reported in the new research. The Artificial Intelligence finally settled on the shape similar to an Old School Arcade Character, ' Pac-Man ' as the most appropriate shape after several months. The shape of the cells is what induces the ability of self-replication by taking in free single cells around them and converting them into more Xenobots. The shape here, thus acts as the ' Program ' for the Robot, helping it to accomplish its task, that is to replicate Itself.

While the current applications of Xenobots are extremely limited and they're confined to lab conditions, scientists hope that they can help to pave the way for complex cellular robotics that may be able to not only aid in issues like regeneration of severe wounds or damaged tissues and organs, but even help in better organ transplant procedures as the organs can be crafted from the recipient's cells.

Xenotransplantation: A technique of using organs of animals for the human body

Recently in New York, a pig that was genetically modified by biotechnologists of the USA was used by the team of New York University researchers and surgeons for a kidney transplant to a human body which turned out to be successful. This process of animal to human transplant is also known as xenotransplantation. The surgeons had taken the consent of the patient's family who acted as the subject. The team of surgeons implanted the kidney of the pig into the patient's body but there were concerns of immediate rejection of the organ but that did not happen, there were no complications. The surgeons monitored the subject for two days, for the time it worked properly, though the research has yet to be peer-reviewed.

Xenotransplantation was a long-term possessed dream by scientists but the human body immediately rejects the organs that do not resemble its own body. So, care has to be taken while choosing the donor. Most scientists have avoided the rejection by using other primates' organs but it did not yield adequate results so the pigs with organs closely resembling human organs were used recently which had a positive impact resulting in successful transplantation and acceptance by the human body. Though the use of non-human organs is dangerous and can raise safety concerns, as medical science progresses, it can be resolved and may turn helpful in the future as there are thousands of deaths due to the limited number of donors and this can save thousands of lives.

A first nano-sized molecular device is capable of sensing and altering cell bioelectric fields. The researchers at USC Viterbi School of Engineering have created the first nano-sized, molecular device capable of sensing and altering the cell's electric field, paving the way for new possibilities for research. Bioelectricity, the current that flows between our cells, is fundamental to our ability to think and function. In addition, there is a growing body of evidence that is recording and altering the bioelectric fields of cells and tissue, plays a vital role in wound healing, and even potentially fights diseases like cancer and heart disease. Now, for the first time, researchers have created a molecular device that can do both: record and manipulate its surrounding bioelectric field.

The triangle-shaped device is made of two small, connected molecules much smaller than a virus and similar to the diameter of a DNA strand. It's a completely new material for reading

and writing the electric field without damaging nearby cells and tissue. Each of the two molecules, linked by a short chain of carbon atoms, has its separate function: one molecule acts as a sensor or detector that measures the local electric field when triggered by red light; a second molecule, the modifier, generates additional electrons when exposed to blue light. Notably, each function is independently controlled by different wavelengths of light. Though not intended for use in humans, the organic device would sit partially inside and outside the cell's membrane for in vitro experiments.

The reporter molecule can insert into the tissue and it can measure electric fields non-invasively, providing ultra-fast, 3-D, high-resolution imaging of neural networks. This can play a crucial role for other researchers testing the effects of new drugs, or changes in conditions like pressure and oxygen. Unlike many other previous tools, it will do so without damaging healthy cells or tissue or requiring genetic manipulation of the system.

This multi-functional imaging agent is already compatible with existing microscopes, so it will enable a wide range of research — from biology to neuroscience to physiology — to ask new types of questions about biological systems and their response to different stimuli i.e drugs and environmental factors. The new frontiers are endless. In Addition, the modifier molecule by altering the nearby electric field of cells can precisely damage a single point allowing future researchers to determine the cascading effects throughout, say, an entire network of brain cells or heart cells. The next steps for this multi-functional new molecule include testing on neurons and even bacteria. A collaborator has previously demonstrated the ability of microbial communities to transfer electrons between cells and across relatively long distances with huge implications for harvesting biofuels.

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MARINE COLLAGEN: ECCENTRIC BIOAVAILABLE SOURCE OF POTENTIAL MOLECULES

Pramitha, V. S.[1]*, Riyas, A.[1], Chithra, J. S.[2], Sreejith arameswara Panicker[2], Raga, S. S.[2] and Sreeja Devi, P. S.[2]

[1]Department of Aquatic Biology and Fisheries, University of Kerala, Thiruvananthapuram 695 581

[2]Department of Zoology, University of Kerala, Thiruvananthapuram 695 581

*Corresponding author E-mail: pramivs_vzm@yahoo.com

Abstract:

Collagen is a kind of biocompatible protein material, widely used in tissue engineering, drug delivery, cosmetics, food and other fields. Because of its wide scope, low extraction cost and good physical and chemical properties, it has attracted much attention from scientific society. However, the application of collagen derived from terrestrial organisms is limited due to the existence of diseases, religious beliefs and other problems. Therefore, exploring a wider range of sources of collagen has become one of the main topics in this current scenario. Marine-derived collagen (MDC) stands out unique because it comes from a variety of resources and comprises a family of genetically distinct molecules with specific triple-helix configurations. In this scenario, present chapterdiscusses the sources, characteristics and the application of MDC in wide spectrum of fields including therapeutic sector.

Keywords: Collagen, Marine, Protein, Types, Sources, MDC

Introduction

Collagen is the most common structural protein, accounting 30% of body protein in all and existsas 90% of the extracellular protein in the tendon and bone and more than 50% in the skin (Piez, 1985). Collagen is built only from amino acids and it belongs to the fibrous proteins and consists of three polypeptide chains twined around one another. Each chain has an individual twist, which are layered in opposite directions, forming a helix (Piez, 1984) and five of these twisted chains crosslink and form a collagen microfibril. The basic collagen molecule is rod-shaped, with a length and width of about 3000 and 15 A, respectively and an approximate molecular weight of 300 kDa.

Different Types of Collagen and Their Distribution

Most of the scaffolding in mammals is composed of collagen and the collagenous spectrum ranges from the tendons to the cornea, including ligaments, blood vessels and teeth.

Hence, different collagen types are necessary to confer distinct biological features to different tissues (Miller, 1998). Currently, at least 29 types of collagen have been isolated, which vary in the lengths of their helices and the nature and size of their nonhelical portions.

Type-I collagen is predominant in higher-order animals, especially in the skin, tendon, bone andcomprises of three chains, two of which are identical and one of which has with a different amino acid composition (Kucharz, 1992). Rarely, it could present as a trimer built of three α-1(1) chains. Type-Il collagen is essentially uniqueto hyaline cartilage and type III is found in limited quantities (10%) in association with type 1. Thus, type-II collagen can be a minor contaminant of type-1 collagen prepared from skin (Piez, 1985). In addition, blood vessels predominantly contain type III. Type IV is a highly specialized form found only as a loose fibrillar network in the basement membrane. The other interstitial collagen types occur in small quantities and are associated with specific biological structures (Miller, 1984).

Degradation of Collagen

Collagen is particularly resistant to attacks by neutral proteases, probably due to its function as the primary structural protein in the body (Stricklin and Hibbs, 1988). Fibrils, as aggregates of collagen molecules are degraded from the outside, collagenase binds tightly to triple helices near the surface and molecules in the interior become accessible to enzymes in the course of the progressive exterior degradation (Welgus*et al*. 1980). Various types of collagen showed different susceptibilities to collagenolytic degradation and facilitated by enzymes such as gelatinases and nonspecific proteinases, which cleave the primary fragments into small peptides and amino acids (Woolley, 1984).

Functions of Collagen

Collagen fibrils have important structural functions in the mechanical properties of tissues such as tendon, skin and bone. They are also involved in numerous biological functions, from the early stages of development to tissue repair and it exhibited biodegradability, weak antigenicity and superior biocompatibility (Maeda *et al*., 1999).

Sources of Marine Collagen

For industrial purposes, collagen is extracted mainly from the skin and bone of cattle and pigs and it causes high infectious and contagious diseases such as mad cow disease (bovine spongiform encephalopathy, BSE), transmissible spongiform encephalopathy (TSE), and foot and mouth disease (FMD). Hence, limited the use of collagen derived from these sources as there is a possibility of transmission of diseases to humans also (Guille *et al*., 2000). Therefore, studies have focused towards finding alternative novel sources of collagen, including marine organisms and marine byproducts. They have found that the skin, bone, fin and scales of both freshwater and marine fish, squid skin, jellyfish, octopus arms and marine sponge and could be used as alternative sources (Li *et al*., 2004).

Fish, Crustaceans, Molluscs, Sponges

Among collagen alternatives, fish provides the best source of raw material because of its high availability, zero risk of disease transmission, lack of religious barriers and possibility of a higher collagen yield. Byproducts from fish processing and by-catch of unutilized and underutilized fish species are also a promising source of collagen. Processing discards from fisheries, including skin, bones, scales, and fins, account for as much as 85% of the total weight of catch (Shahidi, 1994). Collagen is the main component in the skin (Sikorski and Borderias, 1994), which can be collected separately from other byproducts also.About 75% of the total weight of brown-backed toadfish (*Lagocephalus gloveri*) is discarded in the form of skin, bones, fins, heads, guts, and scales during processing (Shahidi, 1994). Shahidi (1994) discussed the possibility of re-utilizing fishprocessing waste in the production of collagen-like biomaterials, which has the potential to increase the economic value of the fish. It was also noted that the total amino acid content of fish collagen is significantly lower than in other species (Morales *et al*. 2000). As a result, fish collagen has a significantly lower melting point, which could affect performance at body temperature. Another potential drawback noted to the use of fish collagen in pharmaceutical preparations is the occurrence of allergic reactions (Piezand Gross, 1984).

The musculature of several crustaceans has been showed to contain collagenous proteins (Yoshinaka *et al*., 1989). Furthermore, Yoshinaka *et al*. (1990) has reported the purification and characterization of genetically distinct types of prawn-muscle collagens. Sivakumar *et al*. (1997) have also reported the simple purification and physicochemical characterization of a homotrimeric type-V collagen from marine prawn *Penaeus indicus*. Similarly, Sivakumar *et al*. (2000) reported a comparative analysis of abdominal and leg-muscle collagens of the marine crab *Scylla serrata.* Studies on crustacean collagens by Kimura *et al*. (1969) were based on their content and biochemical characteristics. Other collagen sources categorized under crustaceans include the muscle of kuruma prawn (*Penaeus japonicus*) (Mizuta *et al*., 1991) and the skin of diamond back squid (*Thysanoteuthis rhombus*) (Nagai, 2004).

Nagai *et al*. (1999) studied the preparation and characterization of edible jellyfish *Exumbrella* collagen. Jellyfish are often considered as gelatinous animals contain mostly water and a developed collagen-rich mesogloea. However, little information is available on collagen in calcified tissues such as fin, scale and bone in molluscs. On the other hand, their increasingly frequent outbreaks also generate ecological and economic consequences in beach closures. Various jellyfish species (*Aurelia aurita*, *Cotylorhiza tuberculata*, *Pelagia noctiluca* and *Rhizostoma pulmo*) has been analyzed as a source of collagen. Dong *et al*. (2011) has also identified sea cucumber (*Stichopus japonicas*) flesh as potential source.

Sponges contain both fibrillar and nonfibrillar collagens, as is the case of vertebrates. Collagen from sponges is biochemically distinct from vertebrate collagens in that it is highly insoluble and difficult to manipulate. Additionally, sponge collagen is highly glycosylated, most of the lysine residues are found in the consensus sequence for hydroxylation and are subsequently glycosylated (Muyonga *et al*., 2004). Extraction of collagen from the marine sponge *Chondrosia reniformis* Nardo has been also reported. Collagen yields of 30% dry weight collagen to dry weight sponge were obtained using a dilute basic extraction medium. Further, the collagen was showed to have potential uses in cosmetic formulations, causing a slight increase in skin hydration. Tough bundles of collagen, called natural collagen fibers, were isolated from different marine sponges including various *Ircinia* species, *Chondrosia reniformis* and *Suberites domuncula*, which showed great applications in pharmaceutical technology, cosmetics, and nutrition and hada high potency in tissue regeneration, especially after injuries (Pallela *et al*. 2011).

Applications of Marine Collagen:

Cosmaceutical Applications

Marine fish skin, bone, and fin byproducts have been used widely for the extraction of cosmaceutical collagen. Proteins and peptides are an important category of cosmaceutical ingredient and collagen is the major structural protein found in human skin. Its structural and physiological properties have been successfully used as moisturizing, nourishing, wrinkle and anti-aging agents. Cosmaceutical products with collagen can be applied to many skin surfaces, including the face and neck also (Kim and Mendis, 2006). Marine collagen has been used as an oral supplement to support not only the skin but also nail and hair health. It contains the amino acid hydroxyproline, a critical building block for collagen synthesis.

Anti-aging/Antiwrinkle Agents

By the age of 40, the natural synthesis of collagen in humans decreases and this accounts for the emergence of fine lines on the skin, which gradually develop into wrinkles. Collagen supplements could help regain the skin's original glow and to revitalize it, especially collagen obtained from marine sources. Products with marine collagen are helped in treating fine lines and wrinkles and rejuvenating the skin. Marine collagen helps in maintaining skin strength and elasticity by initiating synthesis of new collagen in the underlying layers. As the fiber's density increases in the skin, the emergence of fine lines and wrinkles also decreases. Collagen leads to renewal of the cells and minimizes contraction of the facial muscles, which is one of the main causes of wrinkles. The protective effect of marine-collagen peptides on the skin of aged mice induced by D-galactose and showed that marine-collagen peptides mightplayed a protective role in skin aging by proving the activity of antioxidants.

It is certain that oxidative stress can lead to cell and tissue damage. Bioactive peptides derived from fish skin have antioxidant action in D-galactose-induced Sprague-Dawley rats. Hence, it is reasonable that fish protein may be a valuable source of neuroprotective peptides. Pei *et al*. (2010) investigated the effects of marine-collagen peptide, compounds of low-molecular-weight peptides derived from chum salmon (*Oncorhynchus* sp.) It is also investigated that certain cellular and molecular mechanisms of marine-collagen peptides regulated the plasticity of the brain and its ability to repair itself in response to the effects of aging.

The collagen hydrolysates derived from squid skin greatly inhibited polyphenol oxidase activity *in vitro* and decreased the production of lipofuscin, a brown pigment characteristic of aging in fruit fly (Liu *et al*., 2010). The anti-aging effect of collagen hydrolysates might be related to their antioxidant activities. These suggest that the collagen hydrolysate from squid skin exhibits anti-aging activity in fruit fly and may be used as a neutraceutical ingredient in functional foods, cosmetics, and other for health-care purposes.

To prevent environmental damage to skin

Sun-exposed or photo-aged skin is typically coarse and rough, with deep lines and wrinkles and irregular pigmentation (Jenkins, 2002). In addition, free radical damage causes wrinkles by activating the metalloproteinase that breaks down collagen. Several factors start this cascading process, including exposure to UV radiation in sunlight, smoking, and exposure to air pollution. A number of factors, including adverse environmental conditions, can also cause skin dryness. Therefore, accepted practice is that dry skin can be improved by hydrating the outermost layer with a humectant or occlusive agent, smoothing the rough surface with an emollient, and normalizing the stratum corneum by miniaturization (Swatschek *et al*., 2002). Numerous cosmeceuticals have been tested in the treatment of sensitive skin. Consumer-driven demand has led to the development of products that counteract the signs of aging skin and also decrease erythema, and even out tone and pigmentation. These cosmaceuticals can help protect the skin from photo damage and in some ways repair it, through stimulation of new collagen production. Using them in conjunction with sunscreens may enhance their results, especially when used as an adjunct to rejuvenating procedures.

As Moisturizing Products

Collagen is the main marine-derived protein used in the cosmaceutical industry to increase the moisturizing property of a product and moisturizers are employed to treat dry skin. Lipids work on the principle of occlusion, whereas humectants such as collagen attract water in the stratum corneum. In contrast to low-molecular-weight humectants, such as urea and glycerine, higher-molecular-weight humectants such as collagen are not absorbed by the stratum corneum but rather stay on the skin surface. Moreover, they bind water by hydration and increase

the degree of skin humidity. Swatschek *et al*. (2002) found that collagen possesses moisturizing effects which hydrate the skin by binding towater, and it is commonly used in the formulation of cosmetic products for skin care. Collagen thus represents an excellent film-building polymer in cosmetics. According to the study carried by Garrone *et al*. (1975), marine sponge (*Chondrosia reniformis*) can be used as an alternative collagen source, following physicochemical characterization investigated using noninvasive measurement techniques. Studies on the sponge *Chondrosia reniformis* showed that the hydroxyproline content approximately corresponded to a 40% collagen content. On the other hand, the centrifuged sponge extract was easy to incorporate in any tested formulation. Recently, peptide collagen isolated from fish has shown inhibitory activity and therefore potential for use as anti-inflammatory skin care comaceuticals. Gelatin is a heterogeneous mixture of high molecular weight water soluble proteins and is a partially hydrolyzed form of collagen. Enzymatically hydrolyzed fish skin gelatin has shown increased antioxidant activity (Nimni, 1988).

Biomedical Applications

Collagen-based biomaterials have been widely used in medical applications and the primary reasons for the usefulness of collagens are excellent biocompatibility, weak antigenicity, high biodegradability, and good hemostatic and cell-binding properties compared with synthetic polymers (Lee *et al*., 2001). Collagen can form fibers with extra strength and stability through its self-aggregation and crosslinking (Lee *et al*., 2001). It is commonly used in the medical and pharmaceutical industries as a carrier molecule for drugs (drug-delivery system), proteins, and genes (Lee *et al*., 2001). In particular.microfibrous collagen sheets are used as promising drug carriers for the treatment of cancer. They also find use as surgical sutures (Miller *et al*., 1984) and hemostatic agents, and in tissue-engineering as basic matrices for cell-culture systems and replacement/substitutes for artificial blood vessels and valves.The collagen-chitosan sheet was characterized and showed potential for use in the medical field (Bama *et al*., 2010).

As Drug-delivery System

Collagen's use as a drug-delivery system is very comprehensive and diverse. The attractiveness of collagen as a biomaterial rests largely on the view that it is a natural material of low immunogenicity and is therefore seen by the body as a normal constituent rather than as foreign matter (Peiz, 1984). Collagen can be processed into a number of forms such as sheets, tubes, sponges, powders, fleeces, injectable solutions, and dispersions. All of which have found use in medical practice and it can be extracted into an aqueous solution.It is molded into various forms of delivery system, such as collagen shields in ophthalmology (Kaufman *et al*., 1994), sponges for burns/wounds microspheres, mini-pellets, and tablets for protein delivery (Lucas *et al*., 1989), gel formulations in combination with liposomes for sustained drug delivery controlling materials for transdermal delivery, nanoparticles for gene delivery, and basic

matrices for cell-culture systems. Furthermore, it has been used in films for the delivery of humangrowth hormone, immunostimulants, tetracycline, and growth factors (Minabe *et al*., 1989) and as aqueous injection for local cancer treatment (Davidson *et al*., 1995).

Medical devices based on marine-derived collagen shields have numerous applications in ophthalmology as grafts for corneal replacement, suture materials, bandage lenses, punctual plugs and viscous solutions for use as vitreous replacements or protectants during surgery (Devore, 1995). One of the most widely studied drug-carrier applications of marine collagen is as inserts and shields for intraocular drug delivery to the corneal surface or the cornea itself.

Bioengineering

Due to its excellent biocompatibility and safety, the use of marine-derived collagen in biomedical applications has been rapidly growing and has expanded to bioengineering areas, including polymer scaffolds (Angele *et al*., 2004). Polymer scaffolds are central to tissue-engineering technology because they direct a variety of cellular processes based on their structural and biochemical properties (Chen *et al*., 2002). The materials used for scaffold fabrication not only determine such physical properties as biocompatibility, biodegradability, and mechanical stability but also provide the appropriate signals for directing the cellular processes that lead to tissue formation (Yang *et al*., 2001). For this, collagen has become one of the most favored materials in artificial extracellular-matrix tissue-engineering applications.

Marine-collagen skin substitutes are currently optimized for clinical applications. Collagen-populated hydrated gels can be used as a therapeutic option for the treatment of burn patients or chronic wounds (Auger *et al*., 1998). These skin substitutes are produced by culturing keratinocytes on a matured dermal equivalent composed of fibroblasts included in a collagen gel. Jellyfish collagen was found to have an amino acid composition typical of collagen from other sources, with glycine the most abundant amino acid. Song *et al*. (2006) revealed that jellyfish collagen did not induce a significant cytotoxic effect and had higher cell viability than other biomaterials, including bovine collagen. Jellyfish-collagen scaffolds also had a highly porous and interconnected pore structure, which is useful for high-density cell seeding, an efficient nutrient, and an oxygen supply to cells cultured in the three-dimensional matrices. The findings of *in vivo* studies indicate that jellyfish-collagen scaffolds have potential as safe collagen materials for tissue-engineering applications. Pati *et al*. (2010) isolated collagen from the scales of rohu (*Labeo rohita*) and catla (*Catla catla*) and evaluated it using thermogravimetric analysis. Most promising feature was is its close denaturation temperature to that of mammalian collagen, which significantly boosts its applicability. This isolated collagen might find application in the biomedical and pharmaceutical fields for the construction of tissue-engineering scaffolds, wound-dressing systems and drug-delivery devices.

Bone substitutes are one of the more common bioengineering uses of marine-derived collagen. Bone tissue engineering aims to mimic the natural process of bone formation by delivering a source of cells and/or growth factors in a scaffold matrix to induce cellular attachment, migration, proliferation, and osteoblastic differentiation. In bone tissue engineering, collagen scaffolds play an essential role in supporting bone regeneration. Green *et al*. (2003) reported the fiber skeleton of natural marine sponge and suggested its application for tissue-engineered bone. The skeletons of Poriferans appear to have unique properties that make them appealing as potential bioscaffolds for cell-based bone tissue engineering. These properties include the fibrous skeleton, the collagenous composition, the ability to hydrate to a high degree, and the possession of open, interconnected channels created by a porous structured network. Therefore, Green *et al*. (2003) demonstrated that Poriferans are able to induce osteoblast attachment, proliferation, migration, and differentiation *in vitro*.

Another bioengineering application of collagen is in artificial blood vessels and valves. Induced immune response using jellyfish-derived collagen was comparable to that caused by bovine collagen. The feasibility of using jellyfish collagen for tissue-engineered grafts in a pulsatile perfusion bioreactor was also studied. Collagen generally has a relatively low mechanical strength for application in blood vessels. In order to improve the mechanical properties of porous collagen scaffolds, fabricated tissue engineered scaffolds for blood vessels composed of porous jellyfish collagen tube and reinforced by biodegradable polymers such as polylactide-co-glycolide (PGA) fibers. It was shown that the jellyfish collagen/PL.GA scaffolds had good mechanical properties, were durable under the mechano-active system, and possessed tissue compatibility with smooth muscle and endothelial cells for vascular tissue-engineering applications

Collagen derived from chum salmon (*Oncorhynchus keta*) was crosslinked with 1-ethyl-3-(3-dimethylaminopropyl)-carbodiamide (EDC) during collagen fibrillogenesis and evaluated its potential use as a scaffold. Collagen is also in demand as a skin replacement and collagen-based implants have been widely used as vehicles for the transportation of cultured skin cells or skin replacement and burn wounds.Reconstituted drug carriers for type-1 collagen is suitable for use as a skin replacement and in treatment of burn wounds because of its mechanical strength and biocompatibility (Rao, 1995).

Food and Nutraceutical Applications

Naturally occurring bioactive extracts or single compounds of collagen have spawned an important and dynamic new area of research, resulting in substantial advances in nutritional knowledge. The marine environment represents a relatively untapped source of functional ingredients with application to various aspects of food processing, storage, and fortification and marine-derived compounds, including collagen. It has immense potential use as functional food

ingredients for health maintenance and the prevention of chronic diseases (Shigemura *et al*., 2003).

To Improve the Textural Properties

Collagen is considered as a quantitatively major constituent of the muscle connective tissue of multicellular animals. It has been reported to have an important food function in developing meat texture. A close relationship has been reported between the texture and the collagen content of muscles of many aquatic species (Mizuta *et al*., 1994). Moreover, enzymatic degradation of collagen has been revealed to be responsible for the post-mortem tenderization of fish meat during chilled storage (Sato *et al*., 1997). Collagens in the muscle of marine animals also play a key role in maintaining meat texture. Sivakumar *et al*. (2000) also reported the same application after a comparative analysis of abdominal and leg muscle collagens of the marine crab (*Scylla serrata*). The most prevalent marine proteins used in foods are collagen, gelatin, and albumin, all of which can be extracted from fish and seafood byproducts.

To improve the Sensory Properties

Collagen derived from marine sources is used to improve sensory properties such as the texture, juiciness, mouth feel, elasticity, consistency, and stability of different types of food. Fish-derived collagens are used widely in foods, in the manufacture of glues, and in several otherindustrial applications and are used as a food additive to improve the texture, water-holding capacity and stability of several food products (Rustad, 2003).

As Packaging Materials

Collagen has been utilized to produce edible casings for the meat-processing industries, films, and coatings. They have been used as a delivery system for antioxidants in poultry and applied directly to poultry meat surfaces and processed meats to prevent microbial growth, salt rust, grease bleeding, handling abuse, water transfer, moisture loss, and oil adsorption during frying. Consumers are demanding that food packaging materials be more natural, disposable, potentially biodegradable, and recyclable (Lopez-Rubio *et al*., 2004). Currently, the meat industry uses collagen films in the processing of meat products such as sausages. When heated, intact collagen films can form an edible film that becomes an integral part of the meat product. These commercially available collagen caseins have been purported to reduce shrink loss, increase the permeability of smoke to the meat product, increase juiciness, allow for easy removal of nets after cookingor smoking, and absorb fluid exudates.

In Functional Foods

Fibrillar collagens form a metazoan-specific family, and are highly conserved from sponge to human. On the other hand, the increase in the jellyfish population has led us to consider this marine animal a natural product for food and medicine (Addad, 2011).Various

physiological and pharmacological effects are exerted through the oral intake of foods and beverages, such as the curing of joint diseases (e.g. osteoarthritis, chronic rheumatism), the alleviation of osteoporosis, the prevention of arteriosclerosis and hyper tension, the accelerated curing of wound sites, the curing of dermatological diseases (e.g. eczema, skin roughness, atopic dermatitis, pigment deposition), the improvement of moisture-retaining properties of the skin, the improvement of skin aging (e.g. wrinkles,pigmented spots, dullness, sag, keratinization), the prevention of hair loss, thinning hair, and the prevention of ulcers. Pei *et al*. (2010) demonstrated that dietary administration of marine-collagen protein could facilitate acquisition of spatial learning and increase the passive avoidance ability. In other words, the protection of marine-collage protein against oxidative stress to the brain may have application in ameliorating impairments to learning and memory.

Table 1: Yield of collagen from different sources (marine origin)

Species	**% yield**	
	Acid soluble collagen	**Pepsin soluble collagen**
Japanese sea bass	51.4	36.4
Flat fish	57.3	85.5
Deep sea red fish	47.5	92.2
Ocellate puffer	10.7	44.7
Brown backed toadfish	-	54.3
Pagrus major	2	-
Odonusniger	46.48	70.94
Balloon fish	4	19.5
Brown banded bamboo shark	1.04	9.59
Blacktip shark	1.27	10.3
Black drum	2.3	15.8
Cuttle fish	33	35
Skipjack tuna	42.3	-
Scallop mantel	-	0.4
Bull head shark	50.1	-
Atlantic salmon	23.7	70.5
Purple sea urchin	35	-
Jelly fish	-	46.4
Horse mackerel	43.5	-
Chub mackerel	49.8	-

Table 2: Commercially available collagen based wound dressings

Product/ company	Key components	Collagen source/type	Clinical conditions
Apligraf	Neonantal fibroblasts	Bovine type I	Diabetic foot ulcer
Alloderm	Acellular dermal matrix derived from cadaveric skin	Human	Hernia, abdominal wall repair
Biogbrane	Silicone film with nylon fabric embedded within it	Porcine, type I	Burns
Cellerate RX	Activated hydrolysis collagen fragment	Bovine type I	Diabetic pressure ulcer
Colavite	Collagen/ sodium alginate	Porcine USP grade	Advanced wounds
Dermagraft	Cryopreserved neonatal human dermal fibroblast	Human	Full thickness diabetes foot ulcer
EZDerm	Xenograft with collagen crosslinked with an aldehyde	Porcine	Partial thickness wound

Conclusion:

Marine-derived collagen (MDC) has good biocompatibility and biodegradability and it has made extensive exploration in food emulsions and biomedical applications. MDC can be extracted from fish waste products, which are an economical and sustainable source of collagen and can be used as an alternative to land-based collagen. Land-based collagen carries the risk of transmission of zoonotic diseases, but pig-derived collagen cannot be used in some foods. MDC protein has a very important application in food and can be used as a food emulsion to encapsulate fish oil for protection. It has guiding significance for the formulation of low-fat meat products and is beneficial to improve food safety and nutritional value. Asnutraceutical and pharmaceutical products, MDC might serve as potential antioxidants, even can inhibit the development of tumors.Similar to materials such as polyhydroxyalkanoate (PHA), MDC is widely used in medical tissue, especially in bone tissue engineering, cartilage tissue engineering and functional repair of skin tissue. Good biocompatibility enables the efficiency of collagen in some specific environments, so as to produce specific functions. In addition, due to its biodegradability, MDC can be a good drug encapsulation and sustained-release system to improve the effectiveness of drug delivery. Cross-linking can also improve the mechanical strength and degradation characteristics of collagen membrane. Based on this chapter, collagen with excellent physical and chemical properties has been widely recognized and attracted more and more attention from clinical, medicine, food and other fields. Table 1&2 highlights the yield and products of collagen available commercially.

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EVALUATION OF AGRO-WASTES FOR CULTIVATION AND LONG TERM PRESERVATION OF AGRICULTURALLY IMPORTANT FUNGI

Sandeep Garg[1] and Lal Sahab Yadav[2]*

[1]Department of Zoology,

[2]Department of Botany,

Smt. Chandibai Himathmal Mansukhani College, Ulhasnagar,

Mumbai University, Maharashtra, India

*Corresponding author E-mail: lalsahablal@gmail.com

Abstract:

Several fungi are very much useful in organic agricultural practices as biofertilizers and to control the insect pest for getting good quality products. The mass cultivation of such fungi on synthetic media is very expensive. In this research various agricultural wastes were utilized and evaluate for mass multiplication of agriculturally important fungi. Agro-waste groundnut pericarp, pea pericarp, arahar pericarp and subabhul pericarp were utilized to cultivate agriculturally important fungi T*richoderm viride*, *Chaetomium globosum*, *Aspergillus japonicus*, *Metarhizium anisopliae* and *Paecilomyces lilacinus*. The agro-waste groundnut pericarp and arhar pericarp were found very excellent carrier material for all tested fungi. Maintaining the efficacy of these important fungi for long time is very much essential. There are several effective methods of preservation of fungi are known, mostly cryopreservation in liquid nitrogen and storage in glycerol and mineral oils are used for long term preservation. In this work five different species of different genus of agriculturally important fungi are preserved at room temperature as well as at 4°C on agricultural wastes in the glass bottles. All tested fungi were found 100% viable and very active upto 12 months of preservation at room temperature and 18 months at 4°C under refrigerator. This study helps to preserve and maintain efficacy of some important fungi with easy and low cost for long term period.

Keywords: Mass multiplication, preservation, efficacy, agriculturally important fungi.

Introduction:

Organic forming is gaining popularity in agriculture, in order to get good and quality products, bio fertilizers and bio-insecticides, pesticides are being used (Abbey *et al.,* 2020). Fungi play very important role to improve nutrient uptake and soil health and can prevent from

diseases. Many microbes have been used as bio fertilizers and some are also very much effective to control insect pest (Pirttilä *et al.,* 2021).Fungal genus *Trichoderma, Metarrhizium, Paecilomyces, Chaetomium* and *Aspergillus* are very much useful in agriculture. Some of these fungi are mycoparasites secrete antimicrobial secondary metabolites and phytohormones, some are very good degraders of organic waste and mobilize plant nutrients for better growth and yields (Shores *et al*., 2010). Now a day's several microbial based products developed and marketed as biopesticides, biofertilizers, growth and yield enhancers as well as nutrient solubilizers and organic matter decomposers (Woo *et al.,* 2014). Production of such fungal product in bulk adequate and good quality inoculums is essential. The mass cultivation of fungi using synthetic culture media is very expensive. Therefore, the current need to search cost effective and nutrient rich source to cultivate these agriculturally important fungi for mass cultivation. Several agricultural and agro industrial wastes viz, saw dust, wheat brawn, husk of rice, coffee, paddy straw, grains, oil cakes, fruits peals etc. have been used to cultivate fungi (Yadav, 2012; Saranraj *et al.,* 2017; Naeimi Shahram, 2020). Agro wastes are rich with carbon, nitrogen and other nutrients and also very good in moisture contents, which are suitable for growth of fungi (Akharaiyi, 2016**)**.The present study was aimed to find out the suitability of various agricultural residues for biomass production of selected fungal isolates.

Maintaining the efficacy of these fungi in products like bio fertilizers, bio insecticide-pesticides and as degraders, it is must to ensure their optimal viability and genetic stability. It is only possible by preserving them under good condition in pure form. There are several methods of Microbial preservation. Some of them are very effective for long term preservation like liquid nitrogen preservation, preservation in glycerol and mineral oils, on silica gel etc (Hwang, 1966, 1968; Hwang and Howells, 1968; Corbery and Le Tacon, 1997; Espinel- Ingroff *et al*., 2004; Borman *et al*., 2006). In several laboratories short term preservation of fungal cultures are being practised by serial transfer on fresh slant, this method is very simple and easy to maintain cultures upto 6 months to 8 months (less than 1 year) Although this method is a time consuming and labour intensive. There are some pros and cons of all such preservation methods reported by several workers (Stalpers *et al*., 1987; Ryan *et al*., 2000). In this research very simple, easy to perform, environmental friendly and low cast method of preservation is tried by utilizing agricultural wastes for highly used fungal isolates in agriculture.

Materials and Methods:

Collection and pre-treatment of agricultural wastes

Various commonly and locally available agri-wastes like pericarp of groundnut, Groundnut, Arhar and Subabhul were collected and sun dried. The sun dried agri wastes were pulverized (2 mm Mesh size) and stored at room temperature for further testing.

Physicochemical characteristics of selected agri-wastes

Some important physical and chemical characteristics of wastes were analyse as per standard procedures.

Moisture percentage

Moisture content of the samples was determined by air oven (Gallenkamp) method at 105^0C

Water holding capacity

Water holding capacity was determined by using 10 gm oven died pulverized material of respective wastes. A Whatman no1 filter paper disc was placed in a funnel over a conical flask. 100 ml water was added gradually in the funnel containing powdered waste. The water that drained out through powder was measured and subtracted from the initial volume. The water holding capacity was expressed in percentage.

The organic matter content was determined by method describe by Bhardwaj and Sharma (1991) and total nitrogen content by using micro-kjeldahl process as describe by Sadasivam and Manikam 1992.

Cultivation of fungi on agri-wastes

The agriculturally important fungi *Chaetomium globosum*, *Trichoderma viride*, *Metarhizium anisoplae*, *Paecilomyces lilacinus* and *Aspergillus japonicus* were isolated from various sources and pure culture were authenticated from Agharkar Research Institute and also deposited in ARI-NFCC Pune, India. Fifty gram Powder of agri-waste was dispensed in glass bottles (100ml capacity) separately in triplicate and maintained 60% moisture level in powder by adding basal salts broth media in the bottles. The bottles were plugged with cotton and autoclaved twice on one day interval at 15lbs for 20 minutes. The autoclaved bottles were inoculated with 1 ml of spore suspension ($1X10^7$) of the respective fungi. All inoculated bottles were incubated at room temperature for growth on substrate. The visible growth pattern of selected fungus on respective substrates was recorded in terms of vegetative growth (mycellial) and spore formation by making slide every days. The extent of growth was expressed as, -,+,++,+++ indicating no growth, poor growth, moderate and rich growth respectively.

Preservation of fungi on Agri-wastes

All selected fungi after full growth on various agri-waste were under taken for colony farming unit count separately by pour plate method and initial cfu count were recorded. Approximately 5 gm of inoculums from each bottle was transferred aseptically in sterile borosil vial for study the longevity of fungi on substrate (agri-wastes) and stored safely at room temperature as well as at 4^{0C} in fridge. A total 50 sets of vial were maintained separately for each culture on various substrates. Fungal cultures are regularly tests for its regeneration capability in every three months,

Viability test of fungi on agri-waste

Viability test of fungal culture on various substrates were continuously performed in every three month upto 36 months on PDA, PCA and MEA media using one gram of substrates from stored vail by using serial dilution method.

Results and Discussion:

Culture media contains mainly carbon sources and nitrogen along with some mineral salts which support the growth of fungi. Mostly fungi are grown in laboratory on many synthetic media like Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), Sabouraud's Dextrose Agar(SDA), Corn Meal Agar(CMA) etc for characterization. The use of commercially available media for mass cultivation of fungi is not feasible because they are very costly. Many alternative source as culture media have been used for cultivation of fungi (Okonkwo *et al.,* 2006; Tharmila *et al.,* 2011; Ravimannan *et al.,* 2014).

In this study pericarp of four different leguminous fruits were utilized to cultivate and preserve the agriculturally impotant fungi. The phisico chemical characteristics like texture, moisture content, water holding capacity, organic carbon and total nitrogen contents in the selected agri-wastes were analysed as per standard procedure and given in table1. Moisture play very important role to grow fungi on any substrate. The selected substrates showed good moisture content and water holding capacity. The water holding capacity of Groundnut pericap and Arhar pericarp was above 45% which make them suitable for fungal growth and also for long term preservation. Fungi need carbon as source of energy, nitrogen for reproduction and spore formation, vitamins, growth factor and mineral salts (Ruth *et al*., 2012). The organic carbon and nitrogen contents in the selected agri-wastes were good enough to support the fungal growth. The organic carbon in all substrates was above 36% whereas nitrogen content in the

substrate was above 1%. They possessed appropriate organic carbon nitrogen ratio to support the fungal growth.

Table 1: Physical and chemical characteristics of agri-wastes

Parameters	Groundnut Pericarp (GP)	Pea Pericarp (PP)	Arhar Pericarp (AP)	Subabul Pericarp (SP)
Colour	Pale Brown	Pale Yellow	Brownish Black	Brown
Texture	Fine powder	Fine powder	Fine powder	Fine powder
Particle size	1-2mm	1-2mm	1-2 mm	1-2mm
Moisture %	8.7%	4.25%	4.77%	5.4
Water Holding Capasity	49%	32%	48%	31%
Organic Matter	70.1	66.12	72.40	62.5
Organic Carbon	38%	38.5%	42%	36%
Nitrogen	1,65%	1.2%	1.53%	1.0%

The growth pattern of selected fungi on agri-wastes at room temperature was observed regularly and recorded as -, +, ++,+++ indicating no growth, poor growth, moderate and rich growth respectively (Table 2). The visible mycellial growth on the substrates was observed on third day after the inoculation. The substrate colonization rate (growth rate) of fungi was expressed +,++,+++ based on mycellial spread on the substrates in the bottle and at the same time slides were prepared to check any spore formation by fungi on substrate. *Trichoderma viride*, *Aspergillus japonicus*, *Paecilomyces lilacinus* and *Chaetomium globosum* exhibited very good growth as well as spore formation on all selected substrates. The growth of *Metarhizium anisoplae* on pea pericarp and subabhul pericarp was slow in compare to groundnut pericarp and arhar pericarp. The spore formation of *Metarhizium* was recorded on ninth day after inoculation. All fungi colonized entire substrate in the bottle within 15 days.

After 15 days of growth on the substrates, colony forming unit count of fungi on each agri-waste was recorded by taking one gram of substrates from the bottle by pour plate method on PDA media (Table 3). All fungi exhibited excellent cfu count on each substrate. The cfu count of *Trichoderma viride*, *Aspergillus japonicus* and *Paecilomyces lilacinus* was excellent on almost all substrates. The highest cfu count of *Trichoderma viride* occurred in Groundnut Pericarp and Arahar Pericarp ($4.3X10^{11}$ and $4.1X10^{11}$) respecivily. The cfu count of *Paecilomyces lilacinus* and *Aspergillus japonicus* were also highest on Groundnut and Arahar Pericarp ($3.0X10^{11}$, $3.0X10^{11}$ &$3.5X10^{11}$, $3.1X10^{11}$). *Chaetomium globosum* showed almost similar count on all substrates. Cfu count of all selected fungi showed comparatively low on Pea pericarp and Subhabul pericarp.

Table 2: Growth pattern of selected fungus on agro-waste in laboratory

Fungi	Agro-wastes	Growth in terms of Mycellial/ Spore formation on agro-wastes					
		Day-3	Day-5	Day-7	Day-9	Day-11	Day-13
C. globosum	GP	++/-	+++/+	+++/++	+++/+++	+++/+++	+++/+++
	PP	++/-	+++/+	+++/++	+++/+++	+++/+++	+++/+++
	AP	++/-	+++/+	+++/++	+++/+++	+++/+++	+++/+++
	SP	+/-	+/-	++/-	++/-	++/+	++/++
A. japonicus	GP	++/+	+++/++	+++/+++	+++/+++	+++/+++	+++/+++
	PP	++/+	+++/++	+++/+++	+++/+++	+++/+++	+++/+++
	AP	++/+	+++/++	+++/+++	+++/+++	+++/+++	+++/+++
	SP	++/+	+++/++	+++/+++	+++/+++	+++/+++	+++/+++
M. anisople	GP	++/-	++/-	+++/+	+++/++	+++/++	+++/++
	PP	+/-	++/-	++/-	++/+	++/++	+++/++
	AP	++/-	++/-	++/-	++/+	++/++	+++/++
	SP	+/-	+/-	+/-	++/-	++/+	++/+
P. lilacinus	GP	+/-	++/+	+++/++	+++/++	+++/+++	+++/+++
	PP	++/+	+++/++	+++/+++	+++/+++	+++/+++	+++/+++
	AP	++/+	+++/++	+++/+++	+++/+++	+++/+++	+++/+++
	SP	++/+	+++/++	+++/+++	+++/+++	+++/+++	+++/+++
T. viride	GP	++/+	+++/++	+++/+++	+++/+++	+++/+++	+++/+++
	PP	++/+	+++/++	+++/+++	+++/+++	+++/+++	+++/+++
	AP	++/+	+++/++	+++/+++	+++/+++	+++/+++	+++/+++
	SP	++/+	+++/++	+++/+++	+++/+++	+++/+++	+++/+++

Growth pattern Mycellial development/Spore formation +, poor; ++, Moderate; +++, rich(full growth and spores formation); - no sporulation

Table 3: C.F.U count of fungi on agro-waste after 15th day and percentage of available moisture at the time of preservation.

Agro-waste	Moisture %	C.F.U				
		C. globosum	***A. japonicus***	***M. anisople***	***P. lilacinus***	***T. viride***
Groundnut Pericarp	60%	$1.8X10^{9}$	$3.5X10^{11}$	$1.1X10^{9}$	$3.0X10^{11}$	$4.3X10^{11}$
Pea Pericarp	58%	$1.56X10^{8}$	$2.0X10^{9}$	$1.5X10^{10}$	$2.6X10^{9}$	$3.2.0X10^{10}$
Arhar Pericarp	60%	$1.8X10^{8}$	$3.1X10^{11}$	$2.0X10^{9}$	$3.0X10^{11}$	$4.1X10^{11}$
Subabul Pericarp	60%	$1.25X10^{8}$	$3.0X10^{10}$	$1.6X10^{7}$	$2.0X10^{10}$	$3.8X10^{10}$

Viability and Shelf Life

Commercial formulation of biological products such as bio fertilizers, bio-insecticide pesticides is basically depending on potential of used organisms and their longevity in the formulation. Several commercial available agricultural biological products has been formulated in liquid as well as in powder form, in which active organisms have been blended with carrier material like talcam powder and in some liquid base organic chemicals. In this research longevity study of the selected fungi on various agri- wastes were performed and result was recorded (Table 4).

Table 4: Viability test of isolates on agri-wastes at room temperature and 4^0C

Fungi	Agro-wastes	CFU Count after intervals of six months stored at room temperature				CFU Count after intervals of six months stored at 4^0C temperature			
		3M	6M	12M	18M	6M	12M	18M	24M
C. globosum	Groundnut pericarp	√	√	√	√	√	√	√	√
	Pea Pericarp	√	√	√	X	√	√	√	X
	Arhar Pericarp	√	√	√	√	√	√	√	X
	Subabul Pericarp	√	√	√	X	√	√	√	X
A. japonicus	Groundnut pericarp	√	√	√	X	√	√	√	√
	Pea Pericarp	√	√	√	X	√	√	√	X
	Arhar Pericarp	√	√	√	√	√	√	√	√
	Subabul Pericarp	√	√	√	X	√	√	√	X
M. anisople	Groundnut Pericarp	√	√	√	X	√	√	√	X
	Pea Pericarp	√	√	√	X	√	√	X	X
	Arhar Pericarp	√	√	√	X	√	√	X	X
	Subabul Pericarp	√	√	X	X	√	X	X	X
P. lilacinus	Groundnut Pericarp	√	√	√	√	√	√	√	√
	Pea Pericarp	√	√	√	√	√	√	X	X
	Arhar Pericarp	√	√	√	√	√	√	√	√
	Subabul Pericarp	√	√	√	X	√	√	X	X
T. viride	Groundnut Pericarp	√	√	√	√	√	√	√	√
	Pea Pericarp	√	√	√	X	√	√	√	√
	Arhar Pericarp	√	√	√	√	√	√	√	√
	Subabul Pericarp	√	√	√	X	√	√	√	X

Note:’ √’ viable, X’ no growth not viable

Agri-wastes contain several reusable substances of high value of nutrients. The presence of sugars (carbon), proteins (nitrogen), minerals and water make these agri- wastes a suitable environment for the development of microorganisms, mainly fungal strains, which are able to quickly grow in these wastes.

After 15 days of growth at room temperature, the population of fungi on the substrates were recorded in terms of cfu count and further compared the cfu after certain period of intervals upto 18 months and 24 months. It was recorded that over the period of time at room temperature in all four substrates the number of CFUs reduced which was mainly due to reduction in moisture contents in the substrates. The pattern of population at 4^{0C} was almost stable upto six months and further slow decline was observed. A good population of the biocontrol fungus *Trichoderma viride, Paecilomyces lilacinus* and phosphate solubilizer *Aspergillus japonicus* recorded on substrate groundnut pericarp and arahar pericarp even after 18 months of storage at room temperature and upto 24 months at 4^0C.

The population dynamics of all selected isolates on substrate Pea pericarp and Subabhul pericarp after 6 months of storage were low and further very rapid decline not a single isolates showed viability after 12 months of storage at room temperature. It may be due to complete loss of moisture content from the substrate and compaction. Isolate *Trichoderma viride*, *Chaetomium globosum, Aspergillus japonicus* and *Paecilomyces lilacinus* showed excellent viability after 18 months of storage at 4^0C on all substrates. Isolate *Metarrhizium anisoplae* showed viability on Peapericarp and Subabhul pericarp upto 12 months of storage.

Conclusions:

Agricultural wastes were evaluated for mass multiplication of agriculturally important fungi and its shelf life was assessed for 24 months at two temperatures room temperature and at 4^0C. The present result shows that Groundnut pericarp, Arhar pericarp, Pea pericarp and subabhul pericarp substrates can be used to ensure the quantity and quality of all selected agriculturally important fungi inoculums at a low cost. However, the viability of isolates is influenced by the physical and chemical characteristics of the substrate. Groundnut pericarp and Arhar pericarp substrates retain the viability of all isolates for a long period. The decline in viability of isolates at room temperature was due to decline in moisture contents in the substrate. All isolates survived better at room temperature over a period of 12 months and at 4^0C upto 18 months. This study suggesting that a feasible, environmental friendly and low cost procedure of

using agro-industrial wastes for the biomass production of agriculturally important fungal agents and also for preservation for long term in natural state.

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A REVIEW ON FLY ASH AS A POTENTIAL SOURCE OF SOIL AMENDMENT IN AGRICULTURE

Deepa Singh*1, Devendra Mani Tripathi[1] and Smriti Tripathi[2]

[1]Department of Microbiology,

[2]Department of Environment and Developmental Studies,

Bundelkhand University, Jhansi

*Corresponding author E-mail: deepajhs.singh90@gmail.com

Abstract:

Globally, thermal power plants used coal for the generation of 80% of electrical energy resulted generation of massive quantities of coal combustion by-products particularly, FA (fly ash) which is being regarded as a pollutant. Over the past four decades, utilization of FA as an ameliorant of quality of soil received a great attention all over the world. The properties of FA make it suitable for the soil such as favourable pH, silt-sized particles, low bulk density, water holding capacity (WHC), and presence of nutrients. The constitute of FA suggest that ash can be used as an agent capable of improving physical and chemical parameters of degraded soils. The findings focused on the generalized conclusion on the FA impact on physic-chemical and biological properties of soil such as enzymatic activities and microbial biomass carbon which are proved to be an appropriate indicator because of their relationship to the soil biology and rapid response to nutrient management changes. The microbial activity in soil increases due to addition of FA in combination with other amendments consequently provided sufficient nutrition for the proliferation of microorganisms. The natural agricultural ecosystem largely depends on the beneficial microbes that are present in soil rhizosphere responsible for maintaining growth and sustain productivity. However, continuous research is needed to monitor the long term impact of FA on the quality of the soil.

Keywords: Fly ash, enzymatic activities, microbial activity, amendment, microorganisms

Introduction:

The progress industries have put large pressure on our natural resources such as air, water and soil. During the progress of cultural evolution natural resources brutally damaged by man and environment in this earth is spoiled rapidly by growing urbanization, agricultural practices and lack of planned industrialization. In last few decades, rapidly increasing industrialization and population growth has severe crisis in environment by polluting water, soil and air. The

generation of solid wastes increases, especially from industrial activity, those are alarming proportions for the environmental pollution.

Global energy depend on the coal based thermal power plant

Worldwide energy demand is largely (>30%) met by coal fired power plants.Production of coal worldwide is bout $3.5x10^9$ t/annum. Coal will remain occupy an important position as energy source worldwide because of its high reserves in comparison to the decreasing reserves of both natural gas and petroleum.InIndia, to meet the requirement of energy about 70% generated from coal combustion in thermal power plant. FA generated by coal during the combustion for production of energy is a by-product of industries which recognized as a pollutant. The huge total amount of FA produced globally, which is estimated to 750 million t/annum, but only less than 50% of global production of FA is used (Izquierdo and Querol, 2012).According to the latest report by central electricity authority (CEA), available data for year 2018-19 about 169 million tonnes of FA released by thermal power plant in India.The utilization of FA needs to be increased to reduce the pollution and disposal problem of FA.

In India, FA is utilized in various sectors such as cement, lime brick, lime gypsum blocks, building tiles, aggregate in building blocks and road, agriculture. The FA used by cement industry for manufacturing cement is 2.45 million ton of FA was used in the year 1998-1999 while it increases to about 59 million tonne by 2019-20. The self-hardening properties of FA make it suitable for manufacturing of roads in high ways. About 10 million tonne of FA used in 2019-20 for construction. In agriculture less than 2% FA was used (CEA, 2019-20). There is an urgent need to enhance the utilization of FA in every sector. FA as a soil amendment has a wide scope but still the utilization of FA in this sector is very least. In fact, there is a great opportunity to use in degraded soil or available land in the world, which require to recover with appropriate amendment therefore, huge land area required for the storage of FA, that may be recover by various plant species (Pandey, 2012).

Physical property of coal fly ash

FA show a variation in their mineralogical and physico-chemical properties, it depend on the residue of parent coal, combustion condition, type of emission control device, handling and storage methods (Jala and Goyal, 2006). The release of FA is of two types i.e. fly ash and bottom ash. Fly ash consist of 80% of total ash release in combustion of coal, because it is lighter fraction, it transfer away from flue gasses up into the chimney and collected by a mechanical processes, filter bag, fabric filters. Particles of FA are very fine and glasslike, which range 0.01 to 100 µm (Davison *et al.,* 1974). They are spherical in shape than bottom ash. Bottom ash fraction is heavier and coarse (>100µm) that drop by flow of air to the furnace bottom. Bottom

ash particles are unevenly, angular in shape and sand to gravel sized (Meawad*et al.,* 2010) commonly both fly ash and bottom ash mixture know as pond ash, as a slurry it is disposed by a pipeline to pond ash, when it is not used for industrial purpose. Usually the FA color varies from tan to grey and black; depend upon the residue of unburned carbon present in the ash (Kassim and Williams, 2005). The heterogenous properties of FA contain glassy particles such as mullite, quartz and various iron oxides. The majority of elements contain in FA along with significant amount of trace elements such as mercury, chromium and cobalt, these elements are accumulate in the smaller ash particle (Adriano *et al.*, 1980). Coal combustion is a potential source of various trace elements including emission of heavy metal into the atmosphere. Collectively heavy metal is a general term which is used to the group of metals and metalloids having an atomic density greater than 4 g/cm^3.

General properties of FA vary with the quality and source of coal combustion process and age of ash. FA mainly contain silica (SiO_2), calcium oxide (Cao), alumina (Al_2O_3), iron oxide (Fe_2O_3), sodium oxide (Na_2O), Magnesium oxide (MgO), unburned carbon, Potassium oxide (K_2O) and sulfate (SO_4) (Singh *et al.*, 2011). Refuse ash consist of high Fe_2O_3 and SiO_2 but very low in SO_3. Anthracite ash contains high Al_2O_3, SiO_2 with small amount of K_2O. Being a heterogenous mixture of amorphous and crystalline phase FA have a lower bulk density, high specific area, higher electricity conductivity and moisture retention capacity and lower cation exchange capacity. The pH of FA is linked with the Cao and SO_4 ratio (Mattigod*et al.,* 1990) may varies from 4.5 to 12.0 depend on sulphur content in the parent coal and type of coal used for combustion affect the content of sulphur in FA while those FA produced worldwide including India are alkaline in nature. According to ASTEM C618 FA are classified into class C and Class F. The concentration of Si, Ca, Fe, and Mg oxides, reactive water soluble and amorphous phase gives a differentiation in both the classes, to explain the uses of FA. In class C, lignite and sub-bituminous coal are in class C with pozzolanic property and high degree of own hardening capacity. This class contains 20% lime (CaO) whereas bituminous and sub-bituminous coal gives class F ash. This class contains 10% lime (CaO) with pozzolanic property.

Impact of fly ash on quality of soil:

Soil Physico-chemical properties

The impact of FA on the physical properties of soil is primarily due to changes in texture of soil. Alternation in the texture of soil is directly related with porosity, water holding capacity, bulk density and hydraulic conductivity, which may affect the plant growth and on nutrient accumulation and biological activity of the soil (Jayasinghe and Tokashiki, 2012). Direct amendment of FA modified the soil texture from sandy loam to silt loam and sandy clay to loamy, not only improving their physico-chemical characteristics (Truter *et al.*, 2005) but also

increases the pH and EC of the soil and improved the soil nutritional status by increasing the availability of phosphorus over time (Schonegger *et al.*, 2018). Kalra *et al.* (2000) found the mixture of FA-soil was observed under the incorporation of the different dose of FA in the sandy-clay-loam sandy-loam and sandy soils, moisture stored at field capacity enhanced with the FA doses while reverse pattern was noticed for the clayed soil. Moisture content held at the wilting point increased for all the soils with the FA doses. The pH of soil-ash mixtures reduced with FA content for clayey, sandy-loam and sandy soils while the reverse pattern was noted for sandy clay loam soil. The EC was increased of the mixture for all the soils with the FA dose. The OC improved with the FA dose for the sandy and sandy-loam soils while for clayey and sandy-clay-loam soils reduced. Increased level of FA lead to improve soil properties, nutrient availability and nutrient uptake of rice (Mulla *et al.*, 2000). The application of FA in combination with other amendments like FYM, sewage sludge, lime mud and paper mill sludge at different doses increased the pH of various acidic soils (Ram *et al.*, 2007).

The amendment of FA with industrial waste such as paper factory sludge and rice husk ash increases organic carbon, pH, available nutrients while decreasing bulk density (Karmakar*et al.*, 2009).Similarly, on pot experiment, leguminous crop *Cajanus cajan L*, grown in garden soil under various combination of FA, increase the value of pH, porosity, particle density and water holding capacity whereas bulk density decreased (Pandey *et al.*, 2009). As the dose of FA increases the bulk density of the soil decreased. Under the combined amendment of organic source bulk density was noticeably decreased. The reduction in bulk density was due to lower bulk density of FA. The significant raised in soil physico-chemical parameters such as pH, EC, moisture content, OC, OM but N, K and P of the soil decreased in higher percentage of FA applied while the heavy metal such as Cu, Zn, Fe and Ni were increased at higher percentage of FA amendment (Dash *et al.*, 2015). The different amendment of FA summarized in Table 1.

Heavy metal status of soil

As we all know that FA has both soil amending and nutrient-rich properties that improve the growth and yield of crop but FA contains some heavy metals that may hinder the normal metabolism of microorganisms (Tripathi *et al.*, 2020). Translocation of metals from FA amended soil in the plant *Sesbania cannabina* L. and noticed that toxic metals level such as Cr, Cd content was below detection level in all the treatments. The level of metals such as Fe, Mn, Zn, Pb and Ni was observed higher in FA than in garden soil except Cu. Among all the metals, Fe was found to accumulate higher while Ni accumulation was lower among all the metals in various part of the plant. The result indicated that 100% FA, metal accumulation was found in order Fe>Mn> Zn> Cu =Pb> Ni after the exposure of 90 day (Sinha and Gupta, 2005).

The heavy metal accumulation in *Vigna radiata* increased with increasing application of FA and was found greater in shoots than roots (except for Cu and Mn) and seeds (except Mn). Hence, the antioxidants like carotenoids, non-protein thiol, and free proline and ascorbic acid observed that these raise more in plants grown in 10% FA as compared to grown in garden soil. The increase in amendment of FA from 10 to 25 % FA leads to increase cysteine and malondialdehyde (MDA) as compared to garden soil (Gupta and Sinha, 2009). Accumulation of biomass in the stems and roots of *Populus saplings* enhanced with increases the dose FA upto 20% whereas in leaves and in s saplings increased with application of FA upto 10% only. Concentration of micronutrients such as Fe, Zn and Mn in leaves and stem of *Populus deltoides* shows higher values upto 10% FA application and then declined by 78, 71 and 62 % respectively (Sudha and Dinesh, 2010). FA contains many essential elements and toxic metals of the soils. The crop grown in three different soils (peatland, swampland and irrigated-rice soils) applied with 20 t ha^{-1} FA. The amendment of FA increased the number of rice tiller and dried–rice straw wt. in peatland soils and production of rice in swampland and peatland soil. Although, the concentration of Al, Pb, Ni, Cd, and Cr in the rice straw and grain of peatland soil were not affected by the application of FA (Priatmadi *et al.,* 2019). Amendments of FA with N, P, and K no change on the concentration of Zn, Fe, Cu, Mn, Cd and Cr with 10% FA amendment while 100% FA increase all the metal contents on the soil than other treatments (Nayak *et al.,* 2015).

Soil microbes take part in almost all the soil biochemical reactions, which plays an essential function in soil organic matter production and its decomposition, biochemical cycles and soil structure formation. The metal and metalloids in the FA may alter the enzymatic activities, degradation of proteins, nucleic acid, membrane and chlorophylls ultimately it may increase peroxidation of lipids due to raised free radicals (Dietz *et al.,*1999). Some heavy metalseffect the growth of soil microbes (Gulser and Erdogan, 2008). The different amendment of FA summarized in Table 1.

Effect of fly ash on biological properties of the soil

An experiment was conducted on various doses of fly ash amended (0, 2.5, 5, 10, 15, 25 and 50 % w/w) in the soil with presence and absence of earthworm and result indicated that fly ash exerted little or no inhibition on enzymatic activity (dehydrogenase (DHA), amylase and protease) and soil respiration up to 2.5% fly ash application while further addition of fly ash all enzymatic activities and soil respiration were significantly decreased. The inhibition was significantly enhanced in the presence of earthworm with fly ash upto 5 % observed rise microbial activity and it is concluded that amendment of fly ash at lower dose may beneficial for restore fertility status of acidic soil especially when earthworm is present (Pati and Sahu, 2004).Field experiment was conducted in an Alfisols soil with maize crop to examine the effect

of fly ash and soil amendments such as lime and gypsum with or without farm yard manure on the urease and dehydrogenase activity. Addition of lime with fly ash and FYM resulted 66% more dehydrogenase activity as compare to control (16.54 µg TPF/g soil /h) whereas FYM with lime, gypsum andfly ash resulted higher dehydrogenase activity as compared to alone application. Urease activity in lime is 72.5 µg urea /g soil /h or fly ash (70.74µg urea/g soil/h) treatment was at par, but higher than gypsum or control. Amendment of lime only or with farmyard manure recorded 20-23% more microbial biomass carbon (MBC) over control. When lime was applied the yield of Maize grain was enhanced as compared to control (38. 6 q/ha) by 27% (Chandrakar and Jena, 2016).

An experiment was conducted to study the effect of different doses of fly ash amendment (0. 5, 10, 20, 30 % FA) to the soil. The result showed that the bacterial number, dehydrogenase activity and microbial biomass responded in a parabolic manner to the increasing dose of application (R^2 = 0.62, 0.54 and 0.49 respectively). The result indicates that soil microbial biomass, an enhanced in 143% was observed from 148 µg g^{-1} soil in the treatment receiving no fly ash. Application to 359.3 µg g^{-1} soil in soil amendment with 10% fly ash as the rate of fly ash application was increased to 30%, the soil microbial biomass decreased to 110.7 µg TPF g^{-1} soil day^{-1} (Kohli and Goyal, 2010). Nayak *et al.* (2013) evaluated that addition of different doses of fly ash affect microbial biomass carbon in agricultural soils. The soil respiration and microbial activities were not declined upto 2.5% of fly ash amendment but these activities declined significantly @ 10% and 20% of fly ash amendment. Thus, amendment of 10% and 20% fly ash treated soils found a decreasing trend of microbial biomass carbon in soil with time and decrease was showed throughout the incubation period. The study concluded that amendment of fly ash upto 2.5 % can be used safely without harmful effect on soil biological activity and improve nutrient cycle in agricultural soils. However, at 20% fly ash amendment increase in all the metals concentration except Zn and Cr as compared to control. The microbial activity measured in term of Fluorescein Diacetate (FDA) assay and denitrifiers increased upto 40% fly ash while alkaline and acid phosphatase were decline as the dose of fly ash increase. Amendment of fly ash at lower dose (10% to 20% on soil vol.basis) in soil increase micronutrients, microbial activities and yield of crop (Nayak *et al.*, 2015).The different amendment of FA summarized in Table 1.

Impact of fly ash on crop productivity

Application of fly ash in combination with inorganic and organic fertilizer show better resulted in soil health and crop growth.An amendment of fly ash with different growing crop summarized in Table 2.

However fly ash was more suitable when applied with other sources of nutrients. In sesame, the application of FA @ 30 t ha^{-1} along with FYM (farm yard manure) show maximum uptake of N, P, K, number of capsule $plant^{-1}$, number of seed $capsule^{-1}$, 1000 seed weight, grain yield and yield parameters (Thanunathan *et al.,* 2001). The application of FA @ 10 t ha^{-1} mixed with organic sources such as paper factory sludge (PFS), FYM, crop residue (CR) and chemical fertilizers (CF). The increased in grains yield of rice, peanut pod yield and equivalent yield of both the crops by 31, 24 and 26% respectively, as compared to CF only. This indicated that utilization and careful disposal of fly ash and organic wastes in agriculture for solving the soil productivity problem. Although, it reduces the costly uses of chemical fertilizer, can bring a greater economy in cultivation and reduces the environmental issues (Mittra*et al.,* 2005). In a long term field experiment, the application of fly ash or pond ash @ 30-40 t ha^{-1} with the recommended dose of NPK fertilizer alone or with FYM @20 t ha^{-1} was used for sunflower cultivation and maize crop. The result indicated that the yield of 35.7q ha^{-1} was observed in treatment receiving pond ash @ 40 t ha^{-1} with FYM @ 20 t ha^{-1} followed by fly ash @30 t ha^{-1} the yield increased 53.30 and 45.00 % respectively (Yeladalli *et al.,* 2008).

In sandy loam acid lateritic soil, the field experiment was conducted for two years. The study was conducted under the amendment of fly ash, organic wastes and chemical fertilizer on rice and their influence on mustard grown in sequence. The yield of rice was higher under the amendment of fly ash, organic wastes and chemical fertilizer used in an integrated manner as compared to application of chemical fertilizer alone. The fly ash effect on mean rice equivalent yield of rice and mustard cropping sequences was maximum upto 14% when applied in combination with organic wastes and chemical fertilizers. When fly ash applied alone results minimum yield advantage 3% (Rautaray *et al.,* 2003). A pot experiment was conducted to assess the impact of coal fly ash on growth and yield of chickpea. Different doses of fly ash such as 0, 10, 20 and 40% were applied with the amendment of uniform dose of NPK fertilizers. Growth and yield of chickpea were observed to be increased due to thermal power plant waste water and application of fly ash. FA @ 10% resulted better response over control (Ahmad I, 2017). Growth and metal accumulation were studied in two varieties of *Cicer arietinum* L. varieties (var. C-235 and var. CSG-8962) grows in different amendments such as 100% FA, 100%GS, 100% PM or 100% SD mixed with FA 50%, 75% or 90% GS, PM or SD respectively. At 100% fly ash amendment the growth of both the varieties was extremely low while addition of either GS or PM drastically increased the growth performance with 5-10 times more biomass as compared to fly ash only (Gupta *et al.,* 2007).

Table1: Impact of fly ash on the physico-chemical, heavy metal as well as biological properties of soil

Amendments	**Crop**	**Parameters**	**Result (increase/decrease)**	**References**
Physico-chemical properties				
Soil+ FA (10%, 20%, 30%, 40%)		pH clayey, sandy loam, sandy soil Sand clay loam soil	Decrease Increase	Kalra *et al.,* 2000
		Moisture content	Increase	
		OC Sandy, sandy-loam soil Sandy-clay-loam soil	 Increase Decreased	
GS+FA(0%,25%,50%,100 %w/w)	*Cajanus cajan*	pH porosity particle density WHC Bulk density	Increase Increase Increase Increase Decrease	Pandey *et al.,* 2009
FA (5%, 10%, 15%, 20%)	*Capsicum annuum*	Chl.a,b,total chl., carotenoid, pH,EC,moisture content, OC,OM, N,K,P Cu, Zn,Fe,Ni	Higher @5%FA Increase Decrease at higher FA applied Increase at higher FA applied	Dash *et al.,* 2015
FA@2%+ soil	Wheat	pH, EC, P	Increase	Schoneggar *et al.,* 2018
FA+sludge+rice husk	Rice	pH, available nutrient, OC Bulk density	Increase Decrease	Karmakar *et al.,* 2009

Heavy metal content				
FA 100%	*Sesbaniacannabina L*	Fe, Mn, Zn, Pb and Ni was observed higher in FA	Fe>Mn> Zn> Cu =Pb> Ni	Sinha and Gupta, 2005
Higher FA dose	*Vignaradiate*		Greater in shoots than roots (except for Cu and Mn) and seeds (except Mn)	Gupta and Sinha,2009
FA 10%	*Populus saplings*	Fe, Zn and Mn	Increases	Sudha and Dinesh, 2010
FA 20 t ha^{-1}	Rice	Al, Pb, Ni, Cd, Cr	Not affected	Priatmadi *et al.,* 2019
FA 10%	Rice	Zn, Fe, Cu, Mn, Cd, Cr	Not affected	Nayak *et al.,* 2015
Biological properties				
FA@2.5%+earthworm		DHA,amylase,protease, soil respiration	Maximum	Pati and Sahu, 2004
FYM+ lime + gypsum+ FA	Maize	Enzymatic activities (urease, DHA), MBC	Increase	Chandrakar and Jena, 2016
FA@ 10%		Bacterial number, DHA, and microbial biomass	Maximum	Kohli and Goyal, 2010
FA (lower dose) upto40% **FA@2.5%**	Rice	FDA, alkaline and acid phosphatase MBC, Soil respiration	Increased upto 40% FA Maximum	Nakay *et al.,* 2015 Nayak *et al.,* 2013

Table 2: Impact of fly ash on various growing crops

Amendment	Soil type	Crop	Yield increased	References
FA@30t/ha **FA@30t/ha +FYM@12.5t/ha**	Sandy clay loam	Sesame	28.6% 63.8%	Thanunanathan *et al.*, 2001
FA+GS+PM+SD		Chickpea(var.C-235and var. CSG-8962)	5-10times increased biomass	Gupta *et al.*, 2007
FA+organicwaste +fertilizer **FA alone**	Sandy loam acid lateritic	Rice- Mustard	14% 3%	Rautaray *et al.*, 2003
FA@10tha^{-1}+ PFS+FYM+CR+CF	Acid laterite soil	Rice Peanut pod Equivalent yield of both	31% 24% 26%	Mittra *et al.*, 2005
FA@10%		Chickpea(*Cicerarietinum L*	Increase	Ahmad I, 2017
FA@40 t ha^{-1}+ FYM 20 t ha^{-1} **FA @30 t ha^{-1}**		Sunflower and Maize	53.30% 45%	Yeladalli *et al.*, 2008

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Conclusion:

The finding conclusively propose that soil amended with fly ash proved to be a suitable for physicochemical and biological properties of soil and also for maintaining soil fertility which ultimately improved the yield of crop productivity. Therefore, several studies suggest that the potential use of fly ash in soil not only improve nutrient supplement for degraded soils but also solving the disposal problem of fly ash to some extent.

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FLORISTIC DIVERSITY, ANTHROPOGENIC ACTIVITY AND CONSERVATION STATUS OF NAGABANAS OF ULAYIBETTU, MANGALURU TALUK, D.K, KARNATAKA

Ramya Rai P. D.[1] and Manohara Acharya*[2]

[1]Department of Botany, Alva's College, Moodabidire, Karnataka

[2]Department of Botany, Canara College, Mangaluru, 575 003 Karnataka

* Corresponding author E-mail: achrya.manohara@gmail.com

Abstract:

Conservation of nature and natural resources has been an integral part of diverse cultures in different ways. The traditional practices show the relation of human beings with nature. People all over the world lived inharmony with nature and conserved its valuable biodiversity.Traditional beliefsand practices play a crucial role in the conservation of the environment. In Dakshina Kannada districts where small patches of vegetation with different gods and goddessare called banas. Nagabana is one of the types of sacred groves found in DakskinaKannada. These are the patches of virgin forest with high plant diversity. Floristic survey of 15 Nagabanas (groves) of Ulayibettu of Mangaluru Taluk, Dakshina Kannada district, Karnataka, Indiahas revealed that Nagabanas are patch of vegetation which has idols of Nagadevathe (God of Serpent) and other co-deities like Rakteshwari, Guliga, Bairava, Kallurti. Total 155 Plant species were identified which belongs to 132 genera and 51 families. Members of Fabceae and Moraceae are predominantly found in these Nagabanas. Among the 155 species, 22 are herbs, 47 are shrubs, 58 are trees, and 24 are climbers and 1 epiphyte. Out of 155 species 3 species are under Lowest Risk or Least Concern category according to IUCN and 40 are medicinal plants. The species of plant occurring in the *banas*are diverse, and they are strikingly different from those of that occur in their surrounding areas.

Keywords: Deities, Nagabana, Floristic survey, IUCN, traditional, biodiversity ‘

Introduction:

Conservation of nature and natural resources is an important aspect of life (Singh *et al.*, 2017). There are different methods of conservation of plants and natural resources. One among them is the sacred groves in India. In ndia worshiping nature dates back to the Vedic period.

Biological studies of sacred grooves of India were initiated by Gadgil and Vatak (Rajasri ray *et al.,* 2012).The IUCN treats sacred groves as natural religious places.

Sacred Groves help to improve soil quality, replenish water resources and are pivotal for biodiversity conservation of plants and animals including rare, endemic, threatened, vulnerable species and ethnobotanical species. Most of India's sacred groves are associated with a deity or a spiritual being, who protects the grove and local people. The vegetation cover of these Nagabanashas traditional ethnobotanical value, especially in the field of ethnomedicine, which accounts for the conservation of groves over the years (Sharma *et al.,* 2021).

Dakshina Kannada is one of the districts of Karnataka which has rich vegetation of Western Ghats. Here Sacred Grovesare commonly called Bana (Vana). People of this place also worship different gods and goddesses. There are well-constructed temples for a few gods and other gods are worshiped in nature. These natural worshipping places are commonly called banas.Most of these banas are with different gods and deities. God of a serpent called Nagadevathe is the maindeity worshipped inthis bana so these groves are called Nagabana. Along with the Nagadevathe other deities, Bhutas or Daivas such as Guliga, Kallurti, Bhairava, Raktheshwari, Kallurti, Kshetrapala, are also worshiped together as a cluster. Rituals and other religious practices are conducted yearly in the nagabanas for health and prosperity of the people and their land. (Bhandari and Chandrasekhar, 2003).

Sacred groves are segment of landscape has its own geographical feature and conserved by village people under the belief of serpent God and keep them undisturbed indicating a divine relationship with nature and human (Murugan *et al.,* 2008).

It's observed that almost in all the houses of the present study area there is minimum of one Bana. Area of the nagabanas ranges from few cents to an Acre. God of serpent nagadevate is considered the most powerful deity in Dakshina Kannada district because Nagadevathe is considered as the God for heath and wealth. Serpent worship is not only found in Dakshina Kannada but also in most of the states of India in different forms. Hence serpent worship is considered as Pre-Dravidian culture of 3000 years old (Chandrashekara and Sankar, 1998).

Nagabanas are an assemblage of floristically rich vegetation with strikingly different plant species from those of that occur in their surrounding areas and mostly rich in endemic species. Various socio-economic factors such as conversion of lands for agricultural purposes, fragmentation of the grove-owning families and the modern outlook of the younger generation on the superstitious beliefs have resulted in the depletion and disappearance of the Nagabanas.

Nowadays in the place of virgin Nagabanas symbolic worship by stones or by constructing concrete shrines and idols has become common (Bhandari and Chandrashekhar, 2003).

Materials and Methods:

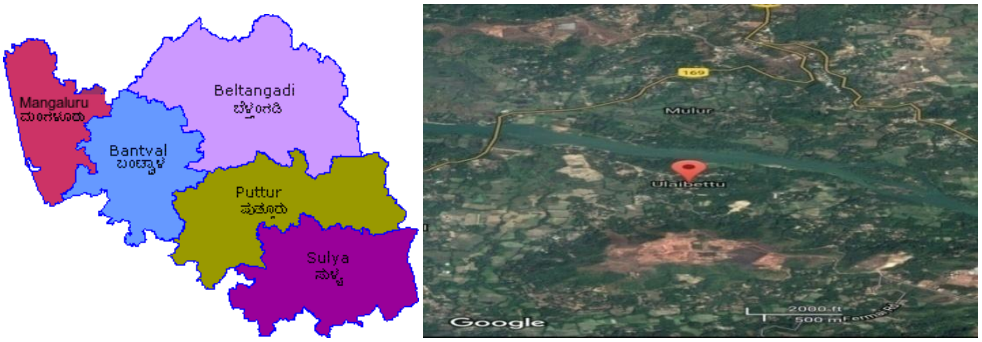
Methodology:

Field survey was conducted to gather the information related to these nagabanas withspecial interest on socio-cultural back ground of these areas. A preliminary survey was conducted in these areas. Information about these nagabanas was collected by consulting the elderly people of the villages, panchayath agencies, after receiving prior consent. The data collected included the general information regarding the nagabanasand the associated deity, nearest human habitation, access to them, and their floral diversity. Information regarding the socio-economic status and the occasion during which rituals are carried out was gathered by using the semi-structured questionnaires (Martin, 2008).

Specimens of flowering plants were collected and identified with the aid of different regional floras (Bhat, 2014) and voucher specimen are pressed and deposited in the Department of Botany Alva's college, Moodubidre. It was made possible to have a comprehensive and exhaustive study of the vegetation of the entire area which resulted in the documentation of the floristic diversity of this area.

Study area:

We have selected Ulaibettu Grama Panchayath of Mangaluru Taluk, Dakshina Kannada district, Karnataka, India which is situated 12^0 9233^l N,$74^0 9296^l$ E and is 8km away from Mangaluru city. It covers an area of 567.17 Hectares and Panchayath is constituted by 4 wards. The Palguni is the lifeline river of this village arising from the Western Ghats on the east. Flows through this village to join the Arabian Sea in the west. This area has tropical rain forest as it forms foothills of Western Ghats. Most of the area is covered by areca plantation, paddy cultivations, and patches of vegetable cultivations. The Ulayibettu word originated from local language called Tulu which means that the area which is hilly region but placed in a remote place of Mangaluru city. The annual average temperature is 20^0-35^OC and annual average Rain fall is 3972 mm and humidity 65-70%. Population as per 2011 census is 4162. Literacy rate is 76%. This region is culturally well known for Yakshaghana and Bhootaradhane. People commonly communicate through Tulu and Kannada languages. During field visit according to local informants we located the nagabanas by using GPS. Randomly identified the floral component with the help of literature and mean while we maintained digital herbarium.



Google Map of Ulayibettu Grama Panchayath

Results:

Floristic composition was studied by the regular field visits. Total of 155 plant species were identified and given in the table 1.

Table 1: List of plants

Sr. No.	Plant Name	Family
1.	*Abutilon indicum* (Lam.) Wight & Arn.	Malvaceae
2.	*Acacia mangium* Wild.	Fabaceae
3.	*Acacia melanoxylon* R.Br.	Fabaceae
4.	*Acacia nilotica* (L.) Delile	Fabaceae
5.	*Achiranthus aspera* L.	Amaranthaceae
6.	*Acmella paniculata* (Wall. ex DC.) R.K.Jansen	Asteraceae
7.	*Adenanthera pavonina* L.**	Fabaceae
8.	*Agave americana* L.	Asparagaceae
9.	*Albizia saman* (Jacq.) F. Muell.	Fabaceae
10.	*Alstonia scholaris* (L.)R.Br.**	Apocynaceae
11.	*Alternanthera sessilis* (L.) R.Br.ex.DC.	Amaranthaceae
12.	*Anamirta cocculus* (L.) Wight &Arn.	Menispermaceae
13.	*Antidesma menasu* (Tul.) Miq. Ex Muell.-Arg.**	Phyllanthaceae
14.	*Apamasili quosa* Lam.**	Aristolochiaceae
15.	*Aporosa lindleyana* (Wight) Baill.	Phyllanthaceae
16.	*Artocarpus heterophyllus* Lam.	Moraceae
17.	*Artocarpus hirsutus* Lam.	Moraceae
18.	*Azadirachta indica* A. Juss.**	Meliaceae
19.	*Baliospermum montanum* (Willd.)Müll.Arg.**	Euphorbiaceae

20.	*Bombax ceiba* L.	Malvaceae
21.	*Borassus flabellifer* L.**	Arecaceae
22.	*Borreria verticellata* L.	Rubiaceae
23.	*Breyniavitis-idaea* (Burm.f.) C.E.C.Fisch.**	Phyllanthaceae
24.	*Bridelia retusa* (L.) A.Juss.	Phyllanthaceae
25.	*Bryophyllum pinnatum* (Lam.) Oken	Crassulaceae
26.	*Calophyllumino phylum* L.*	Calophyllaceae **
27.	*Calotropis gigantea* (L.) W. T. Aiton **	Apocynaceae
28.	*Canthium dicoccum* (Gaertn.) Merr.	Rubiaceae
29.	*Carallia brachiata* (Lour.) Merr.	Rhizophoraceae
30.	*Careya arborea*Roxb.	Lecythidaceae
31.	*Carissa carandas*L.	Apocynaceae
32.	*Caryo taurens* L.	Arecaceae
33.	*Cassia alata* (L.) Roxb.	Fabaceae
34.	*Centranthera sp*	Orobanchaceae
35.	*Chassalia curviflora* (Wall.) Thwaites	Rubiaceae
36.	*Cheilocostus speciosus* (J.Koenig) C.D. Specht	Costaceae
37.	*Chionanthus mala-elengi* (Dennst.) P.S.Green	Oleaceae
38.	*Chromolaena odorata* (L.) R.M.King&H.Rob.**	Asteraceae
39.	*Cinnamomum malabatrum* (Burm. F.) Presl	Lauraceae
40.	*Cinnamonum verum*J.S.Presl **	Laureaceae
41.	*Citrus maxima* (Burm. f.) Osbeck	Rutaceae
42.	*Cleome viscisa* L.	Cleomaceae
43.	*Clerodendrum infortunatum* L.	Lamiaceae
44.	*Combretum razianum* K.G.Bhat	Combretaceae
45.	*Combretum latifolium*Bl.	Combretaceae
46.	*Crotolaria pallid* Aiton	Fabaceae
47.	*Cyclea peltata* Hook. f. &Thoms.**	Menispermaceae
48.	*Derris sp*	Fabaceae
49.	*Desmodium trichostachyum* Benth.	Fabaceae
50.	*Vernonia elaeagnifolia DC* L.	Dioscoreaceae
51.	*Diospyro sebenum* J.Koenig ex Retz	Ebenaceae

52.	*Diospyro scandolleana Wight*	Ebenaceae
53.	*Dipterocarpus indicus* Bedd.	Dipterocarpaceae
54.	*Eclipta alba* (L.) Hassk.**	Asteraceae
55.	*Euphorbia neriifolia* L.	Euphorbiaceae
56.	*Ficus sp*	Moraceae
57.	*Ficus benghalensis* L.	Moraceae
58.	*Ficus hispida* L. f.	Moraceae
59.	*Ficus microcarpa* L. f.	Moraceae
60.	*Ficus racemosa* L.**	Moraceae
61.	*Ficus religiosa*L.**	Moraceae
62.	*Ficus retusa*L.	Moraceae
63.	*Flacourtia montana* G. graham **	Salicaceae
64.	*Flueggea leucopyrus* willd **	Phyllanthaceae
65.	*Garcinia xanthochymus* J .Hk. Ex Anders.**	Clusiaceae
66.	*Gardenia resinifera* Roth **	Rubiaceae
67.	*Getonia floribunda*Roxb.	Combretaceae
68.	*Gliricidia sepium* (Jacq.) Walp	Fabiaceae
69.	*Glycosmispenta phylla* (Retz.) Correa**	Rutaceae
70.	*Gnetu mula* Brongn.*	Gnetaceae
71.	*Grewia nervosa* (Lour) G. panigrahi	Malvaceae
72.	*Heliotropium indicum* L.	Boraginaceae
73.	*Hemidesmus indicus* (L.) R. Br.**	Apocynaceae
74.	*Hibiscus furactus* Wall.	Malvaceae
75.	*Hibiscus mutabilis* L.	Malvaceae
76.	*Holarrhena pubescens* Wall. Ex G.Don**	Apocynaceae
77.	*Holigarna arnottiana* Wall. Ex Hook. f.	Anacardiaceae
78.	*Holigarna ferruginea* March.	Anacardiaceae
79.	*Hopea ponga* (Dennst.) D.J.Mabberley**	Dipterocarpaceae
80.	*Hydnocarpus pentandra*(Buch.-Ham.) Oken**	Achariaceae
81.	*Hyptissua veolens* (L.) Poit.	Lamiaceae
82.	*Ichnocarpus frutescens* (L.) W. T. Aiton	Apocynaceae
83.	*Ixora brachiata* Roxb.**	Rubiacece

84.	*Ixora coccinea* L.	Rubiaceae
85.	*Jasminum malabaricum* Wight	Oleaceae
86.	*Justicia gendarussa* Burm. fil.**	Acanthaceae
87.	*Justicia procumbens* L.	Acanthaceae
88.	*Justicia ligustrina*Vahl	Acanthaceae
89.	*Kirganelia reticulate* (Poir.) Baill.**	Phyllanthaceae
90.	*Kyllinga nemoralis* (J.R.Forst. &G.Forst.) Dandy ex Hutch. &Dalziel	Cyperaceae
91.	*Lantana camara L.*	Verbenaceae
92.	*Lophopetalum wightianum* Arn.*	Celastraceae
93.	*Loranthus parasiticus* (L.)	Loranthaceae
94.	*Ludwigia parviflora* Roxb.	Onagraceae
95.	*Lygodium palmatum* (Bernh.) Sw.	Lygodiaceae
96.	*Macaranga peltata* (Roxb.) Mull.Arg.	Euphorbiaceae
97.	*Marsilea quadrifolia* L	Marsileaceae
98.	*Melastomam alabathricum* L.**	Melastomataceae
99.	*Memecylonma labaricum* Cogn.	Melastomataceae
100.	*Memecylo nedule* Roxb.	Melastomataceae
101.	*Memecylon umbellatum*Burm. f.	Melastomataceae
102.	*Merremia tridentate* (L.) Hall. Fil.	Convolvulaceae
103.	*Michelia champaca* L.	Magnoliaceae
104.	*Mikaniami crantha* Kunth	Asteraceae
105.	*Mimosa pudica* L.	Fabaceae
106.	*Mimuso pselangi* L.**	Sapotaceae
107.	*Morindaci trifolia* L. , nom. cons.**	Rubiaceae
108.	*Mukiama deraspatana* (L.) M.Roem.	Cucurbitaceae
109.	*Muntinigia calabura* L.	Muntingiaceae
110.	*Murraya koenigii* (L.) spreng**	Rutaceae
111.	*Nerium indicum*Mill.	Apocynaceae
112.	*Nothopegia beddomei* Gamble	Anacardiaceae
113.	*Oleadioica* Roxb.	Oleaceae
114.	*Pandanus sp.*	Pandanaceae

115.	*Pandanus foetidus*Roxb.	Pandanaceae
116.	*Passiflora foetida*L.	Passifloraceae
117.	*Pennisetum pedicellatum*Trin.	Poaceae
118.	*Phyllanthus niruri* L.**	Phyllanthaceae
119.	*Piper sp*	Piperaceae
120.	*Plumeria alba* L.	Apocynaceae
121.	*Polyalthia longifolia* (Sonn.) Thwaites	Annonaceae
122.	*Pongamia pinnata* (L.) pierre	Fabaceae
123.	*Pothos scandens* L.	Araceae
124.	*Psychotria flavida* Talbot.	Rubiaceae
125.	*Pterospermum acerifolium* (L.) Willd.	Malvaceae
126.	*Saccharum sp.*	Poaceae
127.	*Sapindusem arginatus* L.	Sapindaceae
128.	*Scoparia dulcis* L.	Palantaginaceae
129.	*Sida acuta* Burm. F.	Malvaceae
130.	*Sida rhombifolia* L.	Malvaceae
131.	*Smilax zeylanica* L.	Smilaceae
132.	*Solanum xanthocarpum*Schrad. & J.C Wendl.	Solanaceae
133.	*Spatholobuspar viflorous*(DC.) Kuntz	Fabaceae
134.	*Strobilanthussp*	Acanthaceae
135.	*Strychnosnux-vomica* L.**	Loganiaceae
136.	*Swieteniama hagoni* (L.) Jacq.	Meliaceae
137.	*Synedrellano diflora* (L.) Gaertn.	Asteraceae
138.	*Syzygiumcaryo phyllatum* (L.) Alston	Myrtaceae
139.	*Syzygium cumini* (L,)**	Myrtaceae
140.	*Tabernaemontana divaricata* (L.) R.Br.exRoem.&schult	Apocynaceae
141.	*Tabernaemontana heyneana* Wall.**	Apocynaceae
142.	*Tamarindus indicus* L.**	Fabaceae
143.	*Terminalia catappa* L.	Combretaceae
144.	*Terminalia paniculata* Roth	Combretaceae
145.	*Toddalia asiatica* (L.) Lam.	Rutaceae
146.	*Tremao rientalis* (L.) Bl.	Cannabaceae

147.	*Tridax procumbens* L.	Asteraceae
148.	*Uvaria narum* Wall.	Annonaceae
149.	*Vateria indica* L.**	Dipterocarpaceae
150.	*Vernonia cinerea* (L.) Less.	Asteraceae
151.	*Vernonia elaeagnifolia* DC	Asteraceae
152.	*Vitex negundo* L.**	Lamiaceae
153.	*Wattakaka volubilis* (L.f.) Stapf.**	Asclepidaceae
154.	*Zizipus oenoplia* (L.) Miller	Rhamnaceae
155.	*Zizipus rugosa* Lam.	Rhamnaceae

*Lowest risk or Least concern, **Medicinal Plants,

The different nagabanas are also documented. Along with this the deities and the occasion during which these are worshipped also surveyed .

Table 2: List of Nagabanas

Sr. No.	Nagabana Address and Age	Deities worshipped	Area covered	Occasions
1	Battaramane, 2nd Ward, Ulaibettu. **500 years old**	Nagadevathe. Rakteshwari&Guliga.	5cents	Nagarapanchami, Ashleshapooja.
2	Kadapu, 2nd Ward, Ulaibettu. **350 years old**	Nagadevathe. & Rakteshwari.	10 cents	Nagarapanchami, Ashleshapooja&Varshikotsava.
3	Majale,2nd Ward, Ulaibettu. **500 years of old**	Nagadevathe.	10 Cents	Nagarapanchami.
4	Sallaje hattira,2nd Ward,Ulaibettu. **200 years old**	Nagabhramha&Rakteshwari.	400 Sq.ft	TambilaPooja, Ashleshapooja, Nagarapanchami.
5	Aranthakodi, Ulaibettu.	Nagabhrahma,Nagameenu, Raktheshwari,Kshethrapala,Nandikona	1Acre	Nagarapanchami &Ashleshapooje

6	Patrakodi,permanki, Ulaibettu. **25 years old**	Nagadevathe.	25 Cents	Naga thambila, Nagarapanchjami , Varshikotsava&Nagamandala
7	Hosmane , Permanki,Ulaibettu. **300 years old**	Nagadevathe.	500sq.ft	Nagarapanchami
8	Gutthu,Permanki, Ulaibettu . **100 years old**	Nagadevathe.	500sq.ft	Nagarapanchami& Varshikotsava
9	Bontemar,Permanki, Ulaibettu . **200 years old**	Nagadevathe.	50 Cents	Nagarapanchami& Varshikotsava
10	Ullipadu, Permanki, Ulaibettu. **100 years old**	Nagadevathe. &Rakteshwari	15-20 Cents	Nagarapanchami& Tambila.
11	AnantharamaBhattaraNagabana,Ulaibettu. **700 years old**	Nagadevathe.	---	Nagarapanchami& Tambila.
12	Mudjappu, Mr.Satishshetty . **100-150 years old.**	Nagadevathe.	---	Tambila
13	Mudjappu, Gopalmaster . **65years old.**	Nagadevathe.	---	Tambila&Ashlesha pooja
14	Kolakebailu, Mr.AshwinShetty. **100 years old.**	Nagadevathe.	---	Tambila
15	Kansanadyuna Mudjappu . **200 years old**	Nagadevathe.	---	Tambila&Ngarapanchami

Discussion:

During the study period we could able to locate 15 nagabanas in the different villages of Ulaiyebettu.These15 Nagabanas of different Villages of UlaibettuGramaPanchayath were surveyed and the floristic composition of these were studied.We have found that Most of the Nagabanas belong to the regional families and rest of them come under temple administration. According to the local informants some of the Nagabanas are approximately 200-700 years old and were surrounded by acres of vegetation (Table-2). But study revealed that individual Nagabana are now covered by small patches of vegetation (few cents to rarely an acre) rich in herbs, shrubs, climbers and very less with tree species. Total 155 species were identified belonging to134 genera under 51 families. Among these members of the family Fabaceae and Moraceae were found to be dominant. Among the 155 species, 22 are herbs, 47 are shrubs, 58 are trees, and 24 are climbers and 1 epiphyte.

By referring the literature we could conclude ,Out of 155 species 3 species *Calophyllum inophyllum, Gnetu mula,* and *Lopopetalum wightianum* are under Lowest risk or Least concern category according to IUCN and out of 40 medicinal plants *Alternanthera sessilis, Apama siliquosa, Balliosperm montanum, Azadirachta indica, Breyniavitis-idea, Calophyllum inophyllum, Calotropis gigantea, Cyclea peltata, Eclipta alba, Ficus religiosa, Holorrhena pubescens, Ixora coccinea, Justicia gendurusa, Kirginelia reticulate, Melastoma malabathricum, Mimusopselengi, Syzygium cumini, Vateria indica, Vitex nigundo* and *Wattakakavo lubilis* are included.

It's known that the virgin nagabanas are usually rich in plants like *Mimusop selengi, Vateria indica, Aporosa linleyana, Caryota urens, Antidesma menasu, Combretum latifolium, Ficus hispida, Getonia floribunda, Ixor abrachiata, Oleadioica,* and *Psychotria flavida*. These are rarely found in these nagabanas. *Combretum razianum, Pterospermum acerifolium* and *Diospyro sebenum* species arerarely found.

Species like *Acacia mangium, Acacia melanoxylon, Albizzia saman, Glicidia sepium, Hibiscus mutabilis, Mecaran gapeltata, Muntingia calbura, Neerium indicum, Plumaria alba, Swietenia mahogany* and *Tabernaemontana divaricatum* arefound frequently that indicates disturbances (Human activity) found in almost all the nagabanas. *Anamirta cocculus, Combretum razianum, Combretum atifolium, Cyclea peltata, Derris sp, Getonia floribunda, Gnetumula, Hibisus furcatus, Ichno carpus frutescence, Jasminum malabaricum, Lygodium palmatum, Mikinia micrantha, Mukiamaer aspatana, Smilax zeylanica, Spatholobus parviflorus, Uvaria narum* and *Wottakakav olubilis* are the climbers found in most of the Nagabanas that increase the beauty (Table-1). Data of floristic survey highlighting the high density of herbs and

shrubs but less density of tree species found in each Nagabanas; that indicates that big patches of vegetation are reduced into small bushes. The change in the vegetation is due to the the anthropogenic activites.this has contributed a lot for the loss of virgin forest from these areas.

Conclusion:

Nagabanas are considered the floristic rich areas and also considered the naturally protected areas in the name of different deities**.** TheNagabanas were conserved mainly because of the believes of people on Nagadevathe (God of Serpent) and other Co-deities such as Rakteshwari, Guliga, Nandikona, Kshetrapala, Nandikona, Kallurti. In ancient time people were afraid to enter Nagabanas because Nagabanas had inhabited with live serpents.as the snakes prefer to live under cool, shady environment.The people used to visit these Nagabana only on rare occasions especially during Nagarapanchami,or any annual rituals or for offerings. Atpresent theseNagabanas have sophisticated concrete shrines in the name of development (Jeernoddhara) meanwhile area covered by the nagabana are reduced. Thick vegetation turned into thin due to different socio-economic reasons like extension of shrine area, development of agricultural lands, fragmentation of the grove-owning families and loosing belief of the younger generation on the deities. These Nagabanas are the last shelters for the different kind of wild animals and also act as a water reservoir to the village for summer season. Due to the depletion of vegetation of Nagabana some important endemic plant species are lost, land becoming dry and some familiar wild animals are disappearing from the villages.It is also observed in the present study that that the human interference in the name of priests for different kinds of offerings have polluted the area with non-biodegradable materials. Present newly constructed nagabanasare not suitablehabitat for the serpents. That's the main reason for the disappearance of live snakes from these places. At present the actual concept of conservation of vegetation of nagabanas are lost. As a result, we have selected Ulayibettu Grama panchayath of Mangalurutaluk for study of nagabanas. Sacred groves have a direct and perpetual pious position, and they help to sustain society's social fabric. The Sacred groves serve a critical role in ensuring that ecosystem services such as air, soil, and water conservation, flora and fauna conservation, carbon capture and storage, temperature management, and traditional knowledge conservation run smoothly (Kandari *et al.,* 2014). It's the basic duty of all of us to create awareness among the people to protect this sacred groves.al least in the name of gods, or dieties the vegetation can be saved and can be passed over to the next generation. Conservation of these nagabanas is easier

comparetonew other projects because we can reduce the anthropogenic activities easily at these places.

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PLANTS USED IN KODAVA RITUALS

C. Jagath Thimmaiah[1], Devamma A. A.[1] and Manohara Acharya[2*]

[1]Department of Botany, FMKMC College Madikeri, Karnataka, India 571201

[2]Department of Botany, Canara College, Mangaluru, Karnataka, India 575003

*Corresponding author E-mail: acharya.manohara@gmail.com

Abstract:

Biodiversity conservation is critical for preserving ecological equilibrium. Religious beliefs among the local population are an important tool for environmental preservation. The Kodavas have their own ritual, which is performed by the people and their neighbours. The chapter lists a total of 36 plants that were utilised in Kodava rituals.

Keywords: Rituals, Biodiversity conservation, ecological equilibrium etc.

Introduction:

Kodagu is well known in the world for coffee and its "brave warriors". The people of Kodagu are called Kodavas. The Kodavas were the earliest inhabitants, agriculturists and martial race community lived there for centuries. Kodava is one of the largest and culturally dominant communities, giving the district its distinct image. Kodavasspeak a language called Kodavathakk (language). The Kodava language is of Dravidian dialect which is a mixture of Malayalam, Kannada and Tamil languages [3].

Kodavas worship the elements of nature. They worship the sacred river Kaveri as Kuladevate. Interesting example of nature worship is their devakad (sacred groves). Kodagu is believed to have the highest density of sacred groves in the world – one or more grove for each village. Ancient belief is that the gods hunt in these forests. Hunting and felling trees in a devakad is therefore forbidden, and the trees grow tall and dense, making these groves bio-diversity hot-spots. Traditionally, each devakad is managed by a local committee, with the thakkame (responsibility) given to a particular okka (Family) [4].

Kodava culture is unique and strikingly different from that of the neighbouring (Hindu) cultures in their dress, language, social structure, ancestral homes, religious practices, customs, festivals, songs and dances. The main festivals of the community include Puttari, Kailpould and Kaveri Sankramana. Puttari is a festival related to rice harvest, the entire family gets together to celebrate the festival. The Kailpouldis solely dedicated to the worship of guns and agricultural implements. Kaveri Sankramana means worship of the River Kaveri, for this festival, thousands of devotees gather and takes a dip in the holy water [5]

Kodava community follow some ritualswhere a number of plant species are used. In this article we have documented the rituals followed and plants used.

Study area:

Kodagu is located on the eastern slopes of the Western Ghats. The district is bordered by Dakshina Kannada district to the northwest, Hassan district to the north, Mysore district to the east, Kasaragod district of Kerala in west and Kannur district of Kerala to the southwest, and Wayanad district of Kerala to the south ie N 12° 20. 2496' E 75° 48. 4145'. It has a geographical area of 4,102 km2 (1,584 sq mi). The district is divided into the three administrative taluksieMadikeri, Virajpet and Somwarpet. Madikeri is the headquarters of Kodagu [1].

Kodagu is considered rich with wildlife and has three wildlife sanctuaries and one national park i. e. the Brahmagiri, Talakaveri, and Pushpagiri Wildlife Sanctuaries, and the Nagarhole National Park. The main river in Kodagu is the Kaveri (Cauvery), which originates at Talakaveri, located on the eastern side of the Western Ghats, and with its tributaries, drains the greater part of Kodagu [2]

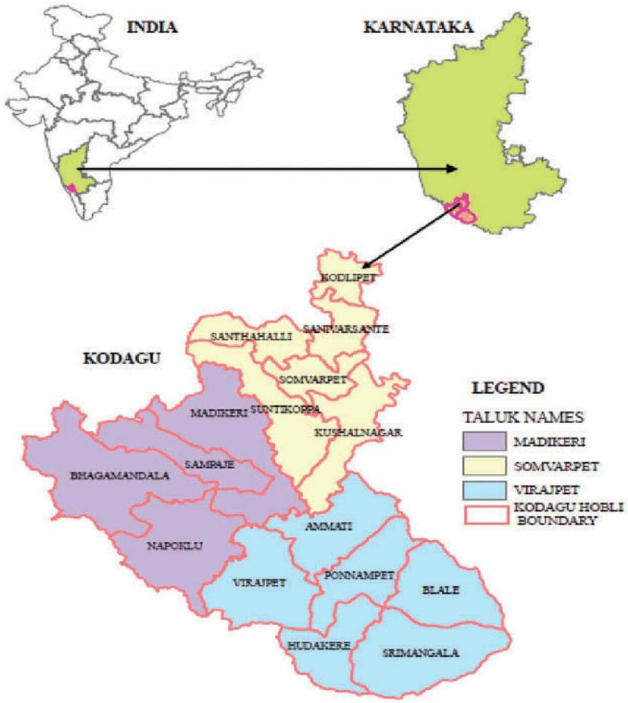

Figure 1: Study area

Results:

Kodava festivals, rituals and plants used

Kodavas celebrate three major festivals- Kailpould, Kaverysankramana and Puthariand other rituals that includes birth, marriage, death and agricultural practices.

1. Kailpould:

This festival is celebrated on 3rdSeptember every year, and it marks the end of paddy transplantation. On this festival the weapons and the agricultural implements are decorated with flowers called as thokpuv (*Gloriosasuperba)* and worshipped.

2. Kavery Sankaramana:

It is the celebration of birth of river Kaveri on 17th or 18th of October. A day before this festival the stumps of *Schefflera venulosa* locally called as botthu is planted in front of the house, paddy fields and aimadas.

On top of this stump a small piece of the climber *Gnetumula* (Botthuballi) is fashioned into a ring and fixed. Dosa and pumpkin (Cucurbita *pepo*) curry is kept on this bothu. It is believed that these offerings keep the birds which damage the paddy crop away. Some even say that bothu indicates the ownership of land by Kodavas.

3. Puthari:

Celebrated when the full moon falls on Rohini nakshatra in the month of November or December, it signifies the beginning of rice harvesting. On this day leaves of mango tree (*Mangifera indica*) are used for decorating (Throrana).

Banana (*Musa paradisiaca*) is used for preparing a dish locally called as thambuttuusing roasted rice flour. It is eaten with Gingely (*Sesmaumindicum*) & grated coconut. Steamed yam (*Dioscoreaalata*) is eaten with syrup of jaggery. On this occasion one leaf each from *Gmelina arboria*, *Ficus religiosa*, *Holigarna grahamii* and *Cardiospermum helicacabum* is used to make the leaf rolls traditionally called as 'nere' for inserting new ears of paddy. The leaf rolls are tied using the fibre of *Grewia serrulata.*

Bitter guard (*Momordica charantia*) pieces are dipped in a mixture of rice flour and water. It is pressed against door frames and the front door of the ain house to leave an impression to mark the celebration

4. Kakkada 18:

Celebrated on 18th day of kakkada month in kodavacalender (Kakkada is a month in Kodavacalender beginning in mid-July and ending in mid-August). On this occasion an extract of the plant locally called as Maddhthoppu *(Justicia wayanadensis)* is used for preparing payasam and pudding. It is said that the plant contains 18 varieties of medicines and keeps the body warm in the cold rainy season. The leaves and stems of this plant are soaked overnight in water or boiled with water to extract a deep purple coloured juice. This juice is used in the preparation of payasam& pudding.

5. Wedding Rituals:

Banana plant (*Musa paradisiaca)* is decorated with flowers of *Euphorbia pulcherrima* (Kanipuv) which are cut on wedding day. On the day prior to the wedding day a pendal is put in

front of the house using banana plants with its bunches, leaves of jack (*Artocarpos heterophyllus)* and mango (*Mangifera indica).*

6. Death Rituals:

Banana (*Musa paradisiaca*) mucilage is used to stick gold coin on the forehead of the deceased. Tulsi (*Ocimum sanctum)* leaves are used to perform last rituals of offering water to the deceased. The palm leaf (*Caryotaurens)* is used as fan to keep away the insects. Sandalwood *(santalum album*) pieces are used to fumigate the area.

Garike grass (*Cyanadon dactylon)* is fashioned into a small ring around the ring finger of close relative to perform certain traditional practices. Mango (*Mangifera indica)* leaves are used for sprinkling water from mud pot. The deceased will be carried to the cemetery in a chair that is used as a bier. A semicircular arch is built with bamboo (*Bambusa arundinacea*) and attached to the bier to which a red silk cloth is tied. Asa part of final rites at the cemetery the body is anointed with a mixture of coconut oil and turmeric (*Curcuma longa*) powder.

On this day a tender banana bunch is cut in the afternoon (Forbidden otherwise) andpalya is prepared. Next day morning close relatives of the deceased go to the cremation ground, the spot is cleaned with water. Paddy (*Oryza sativa*) and mustard (*Brassica nigra*) seeds are sown at that spot. If seeds sprout it is believed that the ground has cooled down and the soul of deceased is at peace

7. Postpartum Care:

Certain practices are followed to protect the mother in postpartum period and also as a part of childcare. Some of the commonly used plants are mentioned below:

After the childbirth mother is given a decoction of Gaalimadh (*Artemesianila giriaca),*for 3 days. A mixture of *Kari jeerige* (*Vernonia anthelmintica*), Nanjanbeeja, turmeric (*Curcuma longa*), garlic (*Allium sativum*) with little salt is given for 12 days. Mother eats pepper (*Piper nigrum*) along with honey and ghee till 40th day. Betel leaf (*Piper betle*) is eaten with areca nut after food. It is believed that mother in postpartum period has a tender body and these health practices protect her from infection. After 40 days she is fed with payasam prepared from urad dal(*Vigna mungo*) for three days and fenugreek (*Trigonella foenum -graecum*) seeds for three days. After sixty days of childbirth she is given a sweet prepared from gingely (*Sesamum indicum*) called as elladige in local language.

The newborn baby is given a juice of different leaves for 5-6 days. Some of the commonly used plants are *Solanumindicum, Physalis angulata, Solanum americanum, Momordica charantia* and *Drymaria cordata* [6, 7 and 8].

Table 1: List of Plants used in Kodava rituals

Sr. No.	Plant Name	Family	Kodava Name	Part Used	IUCN Status	Kodava Rituals
1.	*Allium sativum* L	Amaryllidaceae	Bellulli	Bulb		Postpartum Care
2.	*Artemisia nilagirica*(C.B.Clarke) Pamp	Compositae	Gaalimadh	Leaf	NE	Postpartum Care
3.	*Artocarpus heterophyllus*Lam.	Moraceae	Halasu	Leaf	-	Wedding Rituals
4.	*Bambusa bambos* (L.) Voss	Poaceae	Nell	Stem	NE	Death Rituals
5.	*Brassica nigra*(L.) K.Koch	Brassicaceae	Kadu	Seed	LC	Death Rituals
6.	*Cardiospermum halicacabum*L.	Sapindaceae	Irnoli	Leaf	LC	Puthari
7.	*Caryo taurens*L.	Arecaceae	Pane	Leaf	LC	Death Rituals
8.	*Cucurbita pepo* L.	Cucurbitaceae	Kumbala	Fruit	LC	KaverySankaramana
9.	*Curcuma longa* L.	Zingiberaceae	Manja	Rhizome	DD	Death Rituals,Postpartum Care
10.	*Cynodon dactylon* (L.) Pers.	Poaceae	Garike pill	Arial part	NE	Death Rituals
11.	*Dioscorea alata* L.	Dioscoreaceae	Puttarrikalnji	Tuber	NE	Puthari
12.	*Drymaria cordata* (L.) Willd. exSchult.	Caryophyllaceae	Panathoppu	Leaf	NE	Postpartum Care
13.	*Euphorbia pulcherrima*Willd. ex Klotzsch	Euphorbiaceae	Kanipuv	Flower	LC	Wedding Rituals
14.	*Ficus religiosa* L.	Moraceae	Aala	Leaf	NE	Puthari
15.	*Gloriosa superba* L	Colchicaceae	Thokpuv	Flower	LC	Kailpould
16.	*Gmelina arborea* Roxb.	Lamiaceae	Kumbali	Leaf	LC	Puthari
17.	*Gnetu mula* Brongn.	**Gnetaceae**	Botthuballi	Twig	LC	KaverySankaramana
18.	*Grewia serrulata* DC.	Malvaceae	Achinaar	Stem fibre	NE	Puthari
19.	*Holigarna grahamii* (Wight) Kurz	Anacardiaceae	Kakeyele	Leaf	NE	Puthari
20.	*Justicia wynaadensis* B.Heyne	Acanthaceae	Maddutoppu	Leaf and	-	Kakkada 18

				Stem		
21.	*Mangifera indica* L.	Anacardiaceae	Maange	Leaf	DD	Puthari,WeddingRituals,Death Rituals
22.	*Momordica charantia* L.	Cucurbitaceae	Kaipakke	Leaf	NE	Puthari,Postpartum Care
23.	*Musa paradisiaca* L.	Musaceae	Baale	Whole plant	NE	Puthari , Wedding Rituals, Death Rituals
24.	*Ocimum tenuiflorum* L.	Lamiaceae	Tulasi	Leaf	NE	Death Rituals
25.	*Oryza sativa* L.	Poaceae	Nell	Fruit	NE	Death Rituals
26.	*Physalis angulata* L.	Solanaceae	Gummate\Pottute	Leaf	LC	Postpartum Care
27.	*Piper betle* L.	Piperaceae	Kodiyele	Leaf	NE	Postpartum Care
28.	*Piper nigrum* L.	Piperaceae	Nallamalu	Fruits	NE	Postpartum Care
29.	*Santalum album* L.	Santalaceae	Chaand	Stem	-	Death Rituals
30.	*Schefflera venulosa* (Wight &Arn.) Harms	Araliaceae	Botth	Stump	NE	KaverySankaramana
31.	*Sesamum indicum* L.	Pedaliaceae	Ellu	Seed	NE	Puthari ,Postpartum Care
32.	*Solanum americanum* Mill.	Solanaceae	Kaaki	Leaf	NE	
33.	*Solanum indicum* L.	Solanaceae	Kachunde	Leaf	NE	Postpartum Care
34. `	*Trigonella foenum-graecum*L.	Leguminosae	Menthe	Seed	NE	Postpartum Care
35.	*Vernonia anthelmintica* (L.) Willd	Compositae	Kari jeerige	Seed	NE	Postpartum Care
36.	*Vigna mungo* (L.) Hepper	Leguminosae	Udd	Seed	NE	Postpartum Care

NE=Not evaluated, LC=Least concern and DD=Data deficient

Conclusion:

Kodavas originate from an area which is known for its diverse vegetation. Many of the plants are a part of their culture and some plants are very much required in various stages of life. Kodava rituals are unique and different from that of other community of Hindu religion and are developed mainly based on the experiences of the ancestors regarding seasonal health issues and other traditional health practices and agriculture.

In this work an attempt has been made to document these plants and to a certain extent, their significance. Many of these plants are well protected in the sacred groves present in each village. These plants generally have some relevance as antibiotic, decorative, as agents of biological control, in agriculture. A total of 36 species of plants belonging to 29 families and 34 genus with their botanical name, local names, family, conservation status, description, flowering season, part used in rituals, and medicinalvalues are listed here.

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MICROBIOLOGICAL STUDY OF DHANEGAON RESERVOIR WATER AT DHANEGAON, DISTRICT OSMANABAD, MAHARASHTRA (INDIA)

M. V. Lokhande
Department of Zoology,
Indira Gandhi (Sr) College, CIDCO, Nanded
*Corresponding author E-mail: mv_lokhande@rediffmail.com

Abstract:

In the present investigation to study the microbiological count of Dhanegaon reservoir the minimum value of SPC count was found at spot A while maximum value was found at spot C. The minimum 139/ml at spot A while maximum 160 / ml at spot C. The study was carried out in the year 2003-2004 and 2004-2005. But the range of SPC was suitable for growth of fishes and other aquatic vegetation. The seasonal fluctuation of bacterial count is maximum in the monsoon season while minimum in summer season. In monsoon the bacterial count was high due to mixing of domestic sewage and surface runoff water in reservoir.

Keywords: Microbiological study, Dhanegaon reservoir

Introduction:

Bacteriological water analysis is a method of analysing water to estimate the numbers of bacteria present and, if needed, to find out what sort of bacteria they are. It represents one aspect of water quality. It is a microbiological analytical procedure which uses samples of water and from these samples determines the concentration of bacteria. It is then possible to draw inferences about the suitability of the water for use from these concentrations. This process is used, for example, to routinely confirm that water is safe for human consumption or that bathing and recreational waters are safe to use.

Microorganisms are widely distributed in nature and are found in most natural waters. The microbiological examination are routinely conducted to ensure the safety of potable water, monitor the water quality for recreational, industrial and agricultural uses and also to evaluate prospective water resources for drinking purposes. The bacterial quality is an important parameter from public health point of view since the play key role in water borne diseases. It is generally expressed in terms of the parameters like *E coli*. and total bacteria. Microorganism are common in surface waters and are usually absent in deep well waters. It is difficult to detect the

pathogenic bacteria in water. The faeces of man and animal contain millions of bacteria /gm of faeces. The majority of which is *Escherichia coli* a natural habitat of the intestine. Hence the absence of *E. coli* in water is a positive indication of the absence of pathogenic bacteria.

The microorganisms in water include several harmless bacteria which are also useful for various human purposes. However, contaminated water may harbor several bacteria capable of causing diseases such as typhoid, fever, dysentery, diarrhea, cholera etc. These organisms commonly called as pathogenic bacteria may be in domestic sewage and other polluted water. Microorganisms are found everywhere but mostly found in water bodies. Their abundance and diversity used to guide suitability of water for fish, animals or recreational and amenity purposes (African Technical review, 1986) With increasing urbanization and industrilation water sources have been adulterated with industries as well as animal and human wastes. As results, water has become a formidable factor in disease transmission. It is not practicable to test water for all these organisms this is because the isolation and identification of many of these is often extremely complicated and seldom quantitative (WHO, 1983)

Bacteriological tests have been used to detect the presence of bacteria that are presumed to have fecal origin and thus indicator of sewage contamination. When indicator of fecal pollution are found the in the water and it gets contaminated; it considered to be potential dangerous to health. According to Gopal and Ghosh (1984) the sources due to insanitation are primarily cause of water borne disease like typhoid, cholera, dysentery, diarrhea and stomach disorders.

Materials and Methods:

The standard plate count is universally used to determine the number of viable bacteria. A sample is plated on solid medium to allow single bacterial cells to develop in to microscopic colonies that can be counted suggested by APHA, 1985 and Trivedy and Goel 1985.

Separate water sample were collected from the reservoir in three different sterile plastic containers. Further the samples were immediately processed for (SPC) within 3 hours in laboratory. SPC agar plates were prepared as per above composition and adjusted to the pH 7.0 +-0.1. After sterilization serial dilution of samples were prepared in sterile distilled water. The spread plate method was used as it allows only surface colonies to grow. The plates were incubated at 37^0C temperatures for 24 hours. From the colony count the volume plating and the dilution, the number of viable bacteria in the sample were calculated. An effective number of colonies range from 30 to 250 per ml.

Results:

In the present investigation the number of bacterial count ranged between 140 to 155 /ml with mean value 173.75 /ml at spot A, 141 to 158 /ml with mean value 150.59 /ml at spot B and 140 to 160 /ml with mean value 150.67 /ml at spot C during the year 2003-2004. The monthly mean value of bacterial count is shown in table 1 and graphically represented in Fig. 1

While in 2004-2005 it ranged between 139 to 159 /ml with mean value 149.84 /ml at spot A, 141 to 159 /ml with mean value 150.42 /ml at spot B and 140 to 160 /ml with mean value 151.42 /ml at spot C . The monthly mean value of bacterial count is shown in table 2 and graphically represented in Fig. 2.

Table 1: Monthly mean value of bacterial count in Dhanegaon reservoir during the year of 2003-2004

Year	**June 2003- May 2004**		
	Spots		
Months	A	B	C
June	155	155	158
July	155	158	160
August	154	158	154
September	153	155	157
Octomber	151	154	151
November	149	151	155
December	145	145	144
January	151	154	151
Februrary	149	149	151
March	145	144	144
April	144	143	143
May	140	141	140

All values are expressed in per ml

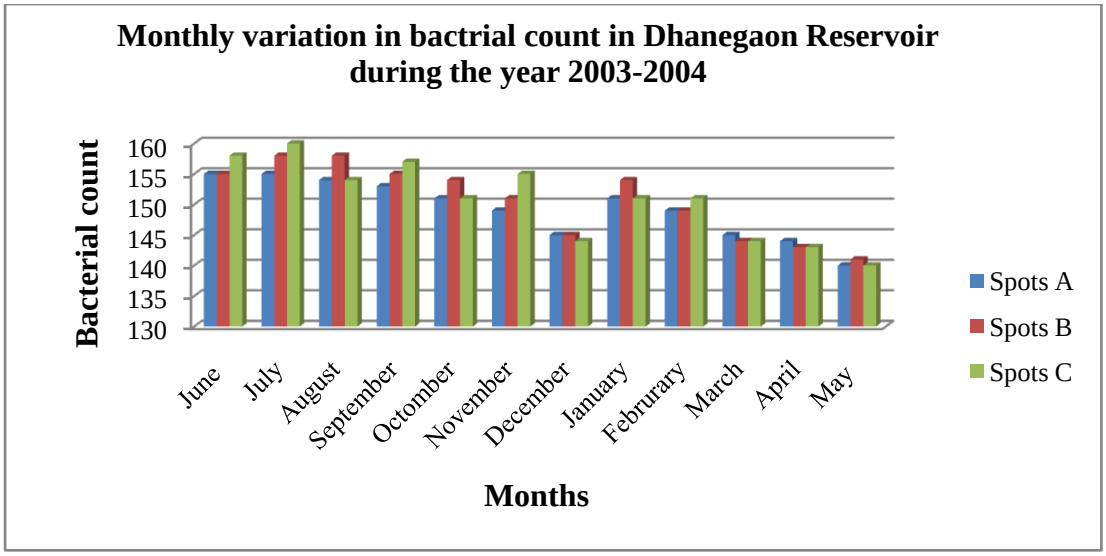

Figure 1: Monthly mean value of bacterial count in Dhanegaon Reservoir during the year 2003-2004

Table 2: Monthly mean value of bacterial count in Dhanegaon reservoir during the year of 2004-2005

Year	**June 2003- May 2004**		
	Spots		
Months	A	B	C
June	159	159	160
July	155	155	158
August	159	159	150
September	155	154	154
Octomber	149	149	159
November	153	151	153
December	149	149	145
January	144	150	153
Februrary	151	154	151
March	143	143	151
April	142	141	143
May	139	141	140

All values are expressed in per ml

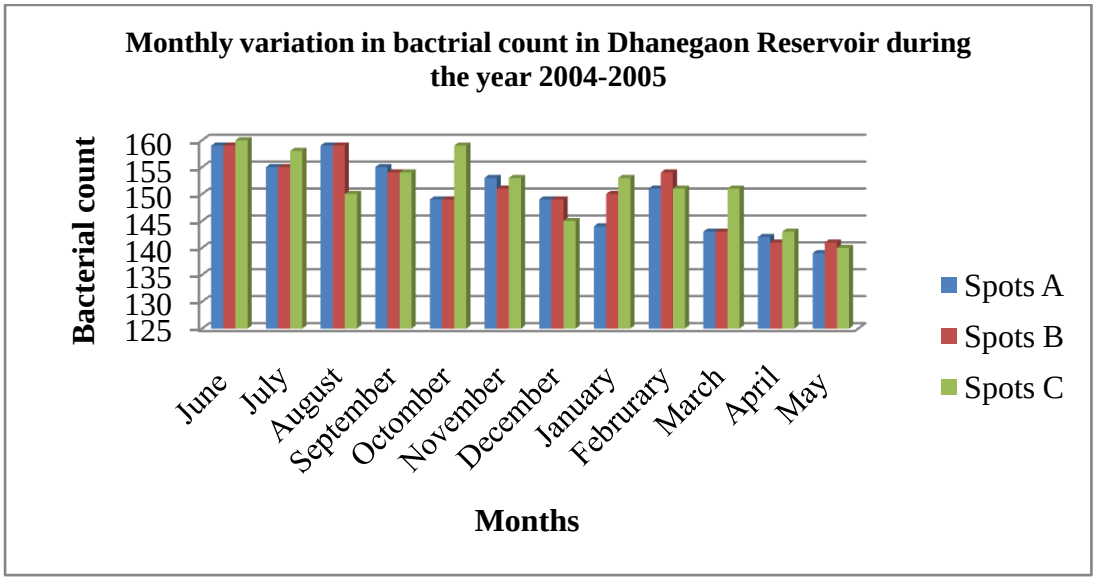

Figure 2: Monthly mean value of bacterial count in Dhanegaon Reservoir during the year 2004-2005

Disscussion:

Narayana and Rao (1981) studied on the bacteriological quality assessment from 40 wells and 62 bore wells in Warangal town. It was observed than 100% of the open well showed very poor quality water while 40% showed poor quality. The poor qualities of borewell were due to negligence of sanitary protection. Surve *et al.* (2006) worked on microbiological study of two fresh water bodies, Barul and Kandhar dam in Nanded district, India and observed that the monthly population of *E. coli* ranges between 00 to 16000 / 100ml in Barul dam and 00 to 22000 /100ml in Kandhar dam water.

Sundari *et al.* (2004) studied on bacteriological quality of drinking water of Chidambarum and observed that the range of standard plate count was 0 to 678 and the mean being 154 for Pre-monsoon, 0 to 673 with mean value 145 for Post- monsoon and for summer was 0 to 680 with mean value of 183. According to Mandal Debashis (1992) practical utility of incidence of coliphage and coliform bacteria along the sewage channel at Calcutta has been assessed. The levels of coliphage and coliform bacteria did not change much at various sites along a sewage channel. However, they found to increase in circular canal, Bughbabar and sewage channel near Vivekananda setu, Dakshineswar. They were more abundant in bathing ghats and their population showed rise during post monsoon months. Lomte and Samant (2005) worked on bacteriological analysis of drinking water of Kolhapur city and observed that the average value of standard plate count from 33 drinking water samples ranges from 52 to 1,46101

Spc/ml. Begum *et al.* (2004) worked on colilform bacteria in different sources of drinking water during different seasons and observed that the average total coliform ranges between 300.00 _+ 51.63 to1033.33 +- 125.61 per 100ml at river Brahmaputra, 333.33 +- 88.19 to 955.56 +- 167.59 per 100ml in well water. The maximum count was observed in monsoon and minimum in winter season. The average value of total colidorm in supply water ranges from 2.33 +- 1.37 per 100ml in winter season to 14.56+- 13..21 per 100ml in post monsoon season. In tubewell water ranges from 0.20 +- 0.20 per 100ml in winter to 3.56 +- 1.81 per 100ml in monsoon seasons. Pillai *et al.* (1999) studied on the drinking water of Durg municipality Madhya Pradesh. The MPN was recorded 79 pre 100ml of raw water, 2 to 79 per 100ml of tap water samples from different sites and the treated water showed absence of colifoms. Rai and Sharma (1995) noted the level of MPN from 4 to 40 per 100 ml in ground water of rural area of North West Utter Pradesh.

Adetunde and Glover (2010) stated that the human pathogens that present serious risk of disease whenever present in drinking water include Salmonella species, Shigella species, pathogenic Escherichia coli, Vibrio cholerae, Yersinia entercolitica, Campylobacter species, various viruses such as Hepatitis A, Hepatitis E, Rota virus and parasites such as Entamoeba histolytica and Giardia species.

Javed *et al.* (2011) working on the occurance of coliform bacteria at different school of district Peshwar and stated that the scanty hospital's data shows that many of the diseases treated are caused by water borne microbes indicating that a substantial proportion of morbidity in Pakistan is due to use of polluted water.

Malhotra *et al.* (2009) working on the bacteriological quality of water in different drinking water sources in schools of district Amritsar (India) and observed that the 39.8% of the samples were found to be unsatisfactory and unfit for human consumption. Monitoring the quality of water is very essential for environmental safety. WHO (1985) specified that potable drinking water should be devoid of total coliform in any given sample. Also, according to USEPA standards, water samples in which coliforms are detected should be considered unacceptable for drinking water as they are regarded as the principal indicators of water pollution.

Sidhu *et al.* (2016): studied on Bacteriological analysis of the drinking water from different schools in Northern India: A concern in developing countries and observed that the total water samples (903) taken from various water reservoirs of the schools (Govt. & Private), 360 (39.8%) samples were unsatisfactory for human consumption, 235(26.1%) were satisfactory and 308 (34.1%) were found to be excellent (Table-1). Among the total, 495 samples were taken from submersible pumps, out of which 189 were found to be unsatisfactory, 254 samples were

collected from piped supply/ taps (97 were unsatisfactory) followed by 83 samples taken from handpumps, of which 48 were reported to be unsatisfactory and of the 67 samples taken from tubewells, 24 were unfit for human consumption. Yasin *et al.* (2015) studied on physico-chemical and bacteriological quality of drinking water of different sources, Jimma zone, Southwest Ethiopia and observed that the all water samples collected from unprotected water sources were positive for total coliforms and fecal coliforms (FC). Accordingly, FC were detected in 80 % of the total samples with counts ranging between 0.67 and 266.67 CFU/100 ml although 66.67 % of tap water samples were negative for FC.

Conclusion:

In the present investigation the minimum value of SPC count was found at spot A while maximum value was found at spot C. The minimum 139/ml at spot A while maximum 160 / ml at spot C. The minimum and maximum specific bacterial count was found due to the fluctuation of temperature and the small human activities, mixing domestic sewage and washing of cattle's in the Dhanagaon reservoir. But the range of SPC was suitable for growth of fishes and other aquatic vegetation. The seasonal fluctuation of bacterial count is maximum in the monsoon season while minimum in summer season. In monsoon the bacterial count was high due to mixing of domestic sewage and surface runoff water in reservoir.

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EFFECT OF HEAVY METAL ON CENTRAL METABOLIC PATHWAY IN RELATION TO CHANGE IN PHYSIOLOGY AND BIOCHEMICAL ASSAY OF FRESH WATER FISH *OPHIOCEPHALUS STRIATUS*

U. E. Bais
Department of Zoology,
Indira Gandhi (SR) College CIDCO Nanded

Abstract:

Mining and industrial activity may cause toxic adverse substances including metals which pollute surface as well as groundwater resources. There is an increased public awareness regarding pesticides, fertilizers, and metals that might adverse effects on our indigenous fish populations and aquatic ecosystems. This is mainly because humans use these natural resources as food and water supplies and are therefore also exposed to products polluting these resources. Metals act as mutagenic compounds, interfere with xenobiotic metabolic pathways, and may also affect glycolysis, the Krebs cycle, oxidative phosphorylation, protein amino acid metabolism as well as carbohydrate and lipid metabolism. The present investigation the effect of Cadmium chlorides on few biochemical pathway in *ophiocephalus* (channa) *striatus* in relation to few biochemical analysis.

Keywords: Cadmium Chloride, *Ophiocephalus Stiatus*, Glycogen, Glucose

Introduction:

The metal which is relatively high density and toxic at low quantity is called as heavy metal, e.g., arsenic (As), lead (Pb), mercury (Hg), cadmium (Cd), chromium (Cr), thallium (Tl), etc. Some 'trace elements' are also known as heavy metals, e.g., copper (Cu), selenium (Se) and zinc (Zn). They are essential to maintaining the body metabolism, but they are toxic at higher concentrations. The heavy metals can enter the bodies to a small extent via food, drinking water and air. The excess quantities of heavy metals are detrimental as these destabilize the ecosystems because of their bioaccumulation in organisms, and elicit toxic effects on biota and even death in most living organisms (Gupta, 2013). The heavy metals are accumulated in living organisms when they are taken up, and stored faster than they are broken down (metabolized) or excreted. They enter into the water supply by industrial and consumer materials, or even from acidic rain breaking down soils and releasing heavy metals into streams, lakes, rivers, and groundwater. The

three most pollutant/environmental heavy metals have been reported include Pb, Hg and Cd (Gupta, 2013) but some other heavy metals can also badly affect the environment. 'Heavy metals toxicity' has been reported to be caused by different means; e.g., from contamination of drinking-water (Pb pipes), high ambient air concentrations near emission sources, or from the food chain. The heavy metals are poisonous since they bioaccumulation. The 'bioaccumulation' means an increase in the level of a chemical/toxicant in a biological organism over time, compared to the chemical/toxicant level in the environment (Gupta, 2013). Cadmium is released in the aquatic ecosystem from different natural and anthropogenic sources. Natural sources of cadmium are from the earth's crust and mantle by the volcanic eruption and weathering of rocks. Whereas anthropogenic sources include combustion of fossil fuels, fertilizers, agricultural waste and industrial use (plastic stabilizers, pigment, batteries, electroporating industries) which contaminate the water body (Järup, 2003) (Muntau and Baudo, 1992). The flora and fauna of water body uptake water soluble or sediment form of cadmium compounds, which indirectly enter into the fish body in course of the food chain. Whereas fishes uptake water dissolved free ionic form of cadmium directly through gill, gastrointestinal tract and skin (Li *et al.,* 2015; Perera *et al.,* 2015).

Cadmium is a non-essential element, with no known biological function (Viarengo, 1985). It can have severe toxic effects on aquatic organisms when present in excessive amounts (Holis *et al.,* 1999). Cadmium Pollution sources are diverse, but it is commonly accepted that electroplating plants are mainly responsible (Cuthbert, 1976). Agriculture (fertilizers and pesticides) and fossil fuel combustion, lead mining and zinc smelting that adds to cadmium pollution (Eaton, 1974; Cuthbert, 1976).

Materials and Methods:

Live and healthy fishes were collected from their habitat. Fishes were checked for injury & diseases & then washed in 1% $KMnO_4$ solution for 5 min. Then 10 groups of fishes is exposed to predetermined median lethal (LC_{50}) for 96 hrs. 10 group's fishes are used as a control in separate glass aquaria. During the experimental period fishes were dose not fed. There is no mortality is occurs in control groups of fishes during the experimental period.

After 96 hrs both control & an experimental (LC_{50}) fish is dissecting out carefully and Muscle, Gill, Liver, Kidney & Heart is used as a sample for estimating glycogen. The average length & Weight of fishes was 20-25 ± cm and 100-125 ± gm respectively and determine the Glycogen by Anthrone Method suggested by Hedge and Hofreiter (1962) and determination the glucose by Nelson-Somogy's method (Sood, 2006).

Results:

In the present investigation effect of cadmium chloride on the glycogen content in different tissues like in muscles12.45, in gill 10.70, in liver 51.0, in kidney 11.91 and in heart 8.50 mg/gm wet.wt. in control group while in experimental group in muscle 10.30, in gill 8.30, in liver 39.40, in kidney 8.90 and in heart 7.80 mg/gm wet.wt. The cadmium chloride significantly affects the physiology of fish. With respect to biochemical content all the tissues of experimental fish shows significant decrease in glycogen content which is shown in table no. 1. And table no. 2 show % decrease in each organ.

In the present investigation effect of cadmium chloride on the glucose content in different tissues like in muscles 19.12, in gill 10.10, in liver 62.10, in kidney 17.90 and in heart 15.10 mg/gm wet.wt. in control group while in experimental group in muscle 21.15, in gill 11.20, in liver 74.20, in kidney 21.10 and in heart 16.40 mg/gm wet.wt. In present study glucose level significantly increased in all the tissues of experimental fish which is shown in table no.3.In table no.4 shows % increase in glucose levels in each organs of experimental fish.

Table 1: Glycogen content in different organs of *Ophiocephalus (channa) striatus* exposed to median lethal (LC50) at 96 hrs. conc. of cadmium chloride ($CdCl_2$)

Sr. No.	Organ	Exposure	
		Control mg/gm wet.wt.	Expt. (LC50) at 96 hrs. mg/gm wet.wt.
1	Muscle	12.45 ± 1.03	10.30 ± 0.78*
2	Gill	10.70 ± 0.59	8.30 ± ± 1.19*
3	Liver	51.0 ± 3.37	39.40 ± 1.47***
4	Kidney	11.91 ± 0.65	8.90 ± 1.04***
5	Heart	8.50 ± 0.61	7.80 ± 0.56*

Values are mean ± SD of six replicates * P < 0.05, ** P < 0.01, *** P > 0.01,

Significant when student's test was applied between control & experimental groups.

Table: 2 Variation in the levels of Glycogen content in different organs in terms of % decrease (↓) over control in *Ophiocephalus (channa) striatus* exposed to median lethal (LC50) at 96 hrs. conc. of cadmium chloride ($CdCl_2$)

Parameter	Muscle % (↓)	Gill % (↓)	Liver % (↓)	Kidney % (↓)	Heart % (↓)
Glycogen	17.26	22.42	22.74	25.27	8.04

Table 3: Glucose content in different organs of *Ophicoephalus (channa) striatus exposed* to median lethal (LC50) at 96 hrs. conc. of cadmium chloride ($CdCl_2$)

Sr. No.	Organ	Exposure	
		Control mg/gm wet.wt.	Expt. (LC50) at 96 hrs. mg/gm wet.wt.
1	Muscle	19.12 ± 0.67	21.15 ± 1.28*
2	Gill	10.10 ± 0.96	11.20 ± 1.10*
3	Liver	62.10 ± 2.28	74.20 ± 1.10**
4	Kidney	17.90 ± 0.22	21.10 ± 1.11*
5	Heart	15.10 ± 0.15	16.40 ± 1.07*

Values are mean of ± SD (six replicates) mean, **P < 0.01, *P < 0.05, ***P > 0.01 significant when student's''t' test applied between control & experimental groups.

Table 4: Variation in the levels of glucose content in different organs in terms of % increase (↑) over control in *Ophicoephalus (channa) striatus* exposed to median lethal (LC50) at 96 hrs. conc. of cadmium chloride ($CdCl_2$)

Parameter	Muscle %(↑)	Gill %(↑)	Liver %(↑)	Kidney %(↑)	Heart %(↑)
Glucose	10.61	10.89	19.48	17.87	8.60

Discussion:

The decrease in glycogen level in all the experimental organs in present study probably the result of cadmium alters the activities of enzymes that work in glycolysis. Similar result has been mentioned (Cicik and Engin, 2003). In Present investigation glycogen level is highest decrease in Liver & Kidney. Liver is the chief organ of carbohydrate metabolism. Liver glycogen is concerned with storage & export of hexose units for maintenance of blood glucose & that of muscle glycogen is to act as a readily available source of hexose units for glycolysis within the muscle itself. A fall in glycogen level clearly indicates its rapid utilization to meet the enhanced energy demands in the toxicant exposed fish through glycolysis or Hexose monophosphate pathways probably due to increased respiration under stressfull condition of fishes. Similar kinds

of result were found by Yacoub and Gad (2012). Glycogen is also highest decrease in kidney in present study it is possibly due to kidney is major cadmium accumulating organs. Shah (2005) reported the highest accumulation of cadmium in the testes and kidney of *Tinca tinca*. Sobha *et al.* (2007) reported the same effect of cadmium on the muscle, gill, liver, heart and kidney glycogen reserve.

The increase in the glucose level of the different tissues of experimental fishes while decrement in tissue glycogen in different organs in experimental fishes probably the glycogen reserves is being used to meet the stress conditions. Similar kinds id results were reported by Sobha *et al.* (2007). The accumulation of heavy metals in the fish could affect directly the health conditions of the consumers living both in and outside the fishing site and consuming the fish on a daily basis. Therefore, the health risk assessment is essentially needed for fishes coming from contaminated resources. The health risk assessments, which are conducted based on the assumption of the most chemicals with noncancerous effects, exhibit a threshold response (Maura *et al.*, 2019).Same results was observed in fresh water bivalves *Lamellidens marginalis* by Shaikh (2019). In aquatic ecosystem, heavy metals are considered as the most important pollutants because of their toxicity and persistence. Heavy metal pollution possesses a great threat to fishes as they are non-biodegradable and once discharged into the water bodies get accumulated in the body of aquatic organisms especially fishes (Shaikh, 2019). Increased serum glucose levels in fish under stress were reported in *Cyprinus carpio* exposed to sub lethal concentration of cadmium. Same results were observed by Sobha *et al.* (2007). They found in fresh water fish *Catla catla* exposed to median lethal (LC_{50}) and sub lethal exposed to cadmium chloride.

The decreased glycogen reserve and subsequently increased glucose level in different organs. Glucose and glycogen levels significantly drop in *Channa punctatus* in muscle and liver reported by Mehjabeen (2015). Carbohydrates serve as the instant energy source during stress so during acute condition blood glucose level increases due to glycogenolysis but reduction can be correlated to utilization of stored glycogen to meet the energy demand or chronic exposure. In liver, glycogen mobilized to glucose whereas in muscle glycogen/glucose served as readily available source of energy, thus hypoglycemia was observed (Mehjabeen, 2015).

Conclusion:

Glycogen level is decreased in significantly in all the organs of experimental fishes. Highest decreased glycogen was observed in liver and kidney. Depletion of glycogen clearly indicate that

cadmium increases the rate of glycogenolysis which is evident that rapid utilization of glycogen for synthesis of glucose to meet the enhanced energy demanded under the stressful physiological condition .Highest reduction of glycogen observed in kidney and liver both are the target organs of cadmium chloride. At the same time present study observed that glucose content is increased significantly in all the organs of experimental fishes over the control. Highest increment of glucose is observed in liver and kidney. It is clear that highest reduction in liver and kidney glycogen is utilized for synthesis of glucose for fulfilling the extra energy demand under the stressful condition. It is clearly evident that under stressful physiological conditions in experimental fishes cadmium chloride significantly changes the physiology as well as metabolic pathway of toxicant induced fishes.

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SCIENTIFIC STUDIES ON WATER QUALITY WITH PHYSICO-CHEMICAL CHARACTERISTICS OF VISHNUPURI RESERVOIR, DISTRICT NANDED (M.S.)

R. G. Pawale

Department of Environmental Science,

Indira Gandhi (Sr) College, CIDCO, New Nanded

Abstract:

The purpose of present study is to understand the scientific knowledge about the physico-chemical status of Vishnupuri Reservoir, Nanded during the period of one year from June 2019 to May 2020. Monthly analyzed samples indicated that the water quality of Vishnupuri reservoir is quite suitable for aquacultural, agricultural and industrial purposes. To acquire the basic scientific knowledge about reservoir parameters are used temperature, total solids, pH, dissolved oxygen and Carbondioxide,

Keywords: Physico-chemical parameters, Water quality, Vishnupuri Reservoir.

Introduction:

Hydrobiology is the study of life in water whereas Limnology is the study of the physical, chemical, geological and of all natural fresh waters. Ecology relates mainly to the biological section of limnology but is different from fresh water biology including the feature of the fresh water environment as well (Forel and Leman, 1892). Fresh water habitats are lakes, ponds dams, reservoir are known as lentic (still) water habitats and rivers, mountain streams are running water known as lotic (flowing) water habitats. Limnology is the science, which deals with the study of structural and functional attributes of lentic environment and problems associated with them.It is a multidisciplinary science, including physics, chemistry, biology, and geology. It involves a great deal of detailed field and laboratory studies to understand the structural and functional aspect so freshwater environments. At present limnology plays an important role in the decision making processes for problems of dam construction, pollution control, fish enhancement and aquaculture practices. Applied limnology has great scope in healthy existence of natural and manmade water bodies and to harvest the natural resources at sustainable level

The physical properties of water in any aquatic system are largely regulated by the existing meteorological condition and chemical properties. The effect of physical forces such as light and heat are of great significance as they are solely responsible for certain phenomena, such

as thermal stratification, chemical stratification, diurnal and seasonal qualitative and quantitative variation in the plankton, micro and macroorganisms and also is the quality of water. The evening creasing population and rapid industrial growth in the present era is contributing to a maximum extent in influencing the physico-chemical properties of most water bodies (Miller, 1973).

Materials and Methods:

Study area:

The present study is an attempt to accumulate information pertaining to various above aspects of hydrobiology of standing water bodies of Vishnupuri Dam Vishnupuri Dam Constructed on the river Godavari; this is one of the largest lift irrigation projects in Asia. The project is situated near Asarjan village, at about 8 k.m. from Nanded city.

The project was completed in the year 1988. The back water covers 40 k.m. length of the river Godavari. Live storage of project is 80.79 Million cubic meters Out of which 43.95 Million cubic meters storage is reserved for drinking purpose for Nanded city and 10.26 Million cubic meters storage is reserved for Industrial purpose.

"*The idea of this project was put forward and pursued by former Chief Minister of Maharashtra late Shri. Shankarrao Chavan. Hence in his remembrance the Govt. of Maharashtra has named the water reservoir as theShankar SagarJalashaya"*

The water samples were collected from the two different selected sites (Sites I Upper site Site-II lower site) from the reservoir for analysis. The pH and temperature were recorded at the spot and rest of the parameters are analyzed in the laboratory on same day by using necessary preservatives' physico-chemical parameters are analyzed using various methods prescribed by APHA and IAAB for years i. e. June 2012-May 2013 results were recorded and compared with WHO standards regularly. The following physico- chemical parameters were studied.

Two sampling sites were selected as sites- I and II located in different places of the Vishnupuri dam Monthly water samples were collected from three selected sites form June 2019 to May 2020. The physico-chemical analysis of water samples were carried out by standard methods suggested by APHA and IAAB. The changes occurred in physico-chemical parameters such as atmospheric and water temperature, total solids, pH, dissolved oxygen, carbon dioxide, total hardness, biological oxygen demand and chemical oxygen demand were recorded at monthly interval.

Results and Disscussion:

The present study is aimed to investigate some of the important physical and chemical parameters along with the flora and fauna of the reservoir. Similarly by studying the phytoplankton and zooplankton quantitatively to find out what type of exotic fishes can be introduced in the reservoir in future so as to utilize the water body successfully for fish production. The monthly variation of physicochemical parameters are represented in table no. 1 and graphically represented in figure no. 1,2,3,4 and 5. Physico-chemical characteristics of an aquatic system reflect not only the quality of system but also the type and density of its biota. Analysis of such parameters generates information regarding pollution pattern and magnitude of pollutant loading of aquatic system.

Table 1: represented the monthly variation in Physico-chemical parameters

Months	Temp.		pH		Total Solids		Dissolved Oxygen		Carbondioxide	
	Site I	Site II	Site I	Site II	Site I	Site II	Site I	Site II	Site I	Site II
19-Jun	29.8	29.9	7.8	7.9	292	300	6.4	6.5	5.9	6
19-Jul	28.7	28.8	7.8	7.9	324	328	6	6.2	8.4	8.3
19-Aug	26.1	26.3	7.9	7.9	325	320	5.1	5.2	4.2	5
19-Sep	27.5	27.7	8.1	8.2	306	305	6.2	6.2	5	5
19-Oct	27.6	27.6	7.8	7.8	295	299	6.9	7	7.4	7.6
19-Nov	25.2	25.2	7.9	7.8	250	255	6.2	6	4.8	4.9
19-Dec	23.8	23.9	7.5	7.3	235	239	7.2	7.3	6.6	6.5
20-Jan	24.4	24.6	7.6	7.5	236	249	7.1	7.2	8.5	8.6
20-Feb	27.5	27.6	8.2	8.3	262	261	6.3	6.4	8	8.2
20-Mar	28.6	29	8.1	8.2	266	269	6.2	6.3	6.1	6.5
20-Apr	29.2	29.5	8.2	8.2	309	310	6.3	6.4	6.3	6.4
20-May	30.9	31	8.4	8.4	345	349	6.2	6.4	6.1	6.2

Water temperature

Water temperature is an important parameter, because it influences the biota n a water body by affecting activities such as behavior, respiration and metabolism. It is necessary to study temperature variations in water bodies, ecophysiological and toxicological aspects in animals

because, water density and oxygen content are temperature related and hence, temperature in directly affects osmoregulation and respiration of the animal (Manawar, 1970).

In the present investigation, the obtained values of Temperature from the water body of Vishnupuri Dam was recorded at site I the highest Temperature was recorded as 31^0C in the month of May 2020 and lowest was recorded as 23.6^0C in the month of December 2019 and at site II the maximum temperature was obtained as 300 C in the month of May 2013 and minimum 23.50 C in the month of December 2012. Water temperature followed pattern being higher in summer and lower in winter. The annual variation in temperature of a water body has a great be aring up on its productivity in general. At higher temperature, decomposition of bottom deposit result in fall in oxygen content and rise in carbondioxide content in pre-monsoon period (Manawar, 1970). Low temperature during winter season may be attributed to the shorter photoperiod and decreased atmospheric temperature (Kant and Raina, 1990). Similarly, result has been reported maximum in summer and minimum in winter (Anita *et al.*, 2005; Jawale *et al.*, 2009).

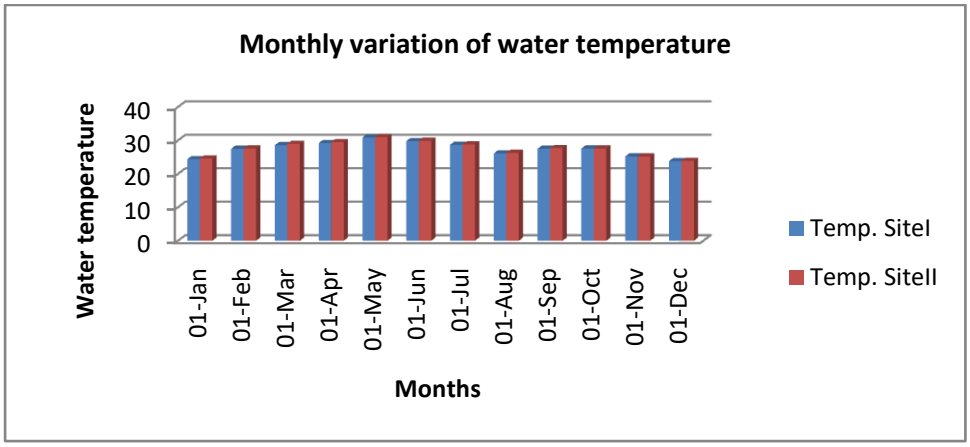

Figure 1: Monthly variation in water temperature

pH

In the present investigation, the obtained values of pH are shown from June 2012 to May 2013. During this study, at site I the highest pH value was recorded as 8.6 in the month of March 2013 and lowest was recorded as 7.5 in the month of December 2012 and at site II the maximum pH 8.6 was obtained in the month of March 2013 and minimum 7.3 in the month of December 2012. The highest values of pH during winter appeared to be influenced by low water level, number of phytoplankton and the highest value of oxygen (David *et al.* (1993) states that the low value of pH during monsoon might be due to high turbidity and elevated water temperature and also due to dilution caused by the rain water during monsoon (Rajashekhar *et al.*, 2007). According to Manawar (1970) the hydrogen ion concentration of natural water is an important

environmental factor the variation of which is linked with species composition. Chari observed that high pH value was related to heavy bloom of phytoplanktons.

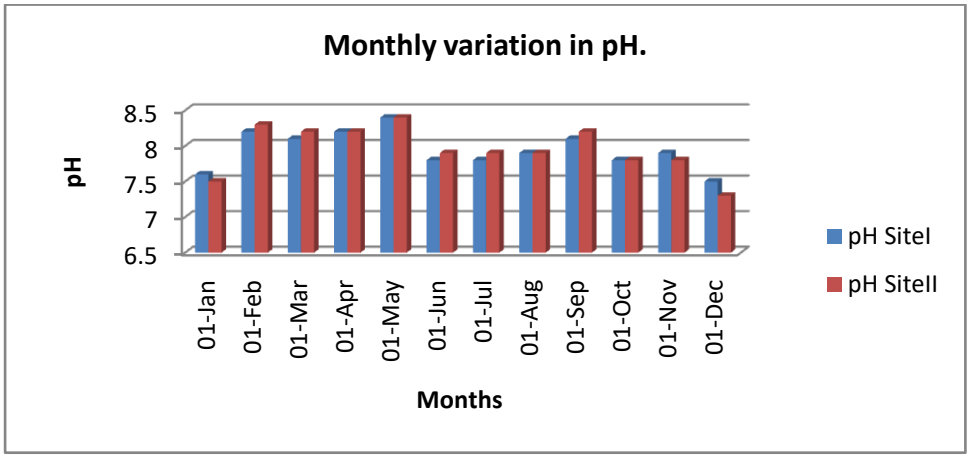

Figure 2: Monthly variation in water pH

Total Solids

In the present study the obtained values of Total solids are shown in Table-I and Table-II respectively for the period from June 2012 to May 2013. During this study, at site I the highest Total solids were recorded as 340 in the month of May2013 and lowest was recorded as 230 in the month of December 2012 and at site II the maximum Total solids was obtained as 340 in the month of May 2013 and minimum 235 in the month of December 2012. The total solid in water due to in organic substances organic matter suspended particles silt, clay and plankton, high total solids reduce the light penetration and effect water quality in directly & imbalance in aquatic life. Polluted water has high total solids (Ganpati, 1956). In water bodies total solid, total dissolved solids and total suspended solids are composed mainly of carbonate, bicarbonate and chlorides, sulphate, phosphates, nitrates, calcium, magnesium, sodium, potassium iron, manganese and organic matter, if their concentration increase beyond the normal limit the water becomes polluted.

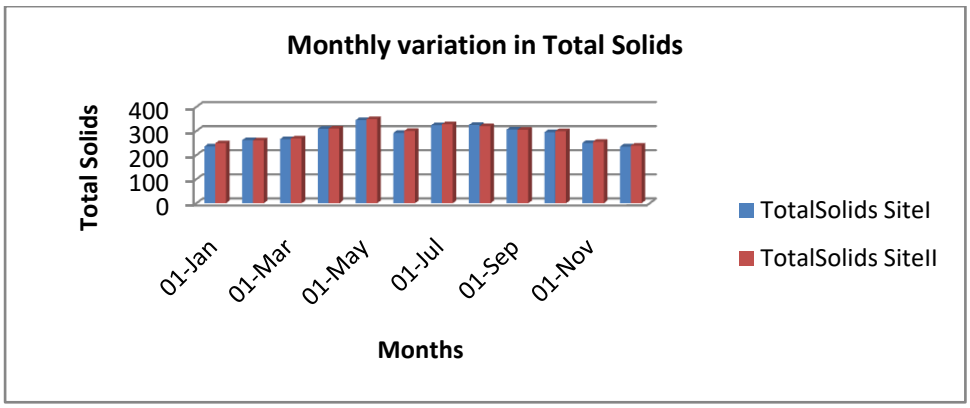

Figure 3: Monthly variation in Total Solids

Dissolved Oxygen

In the present study the obtained values of Dissolved oxygen was shown at site- I the highest dissolved oxygen was recorded as 7.2 in the month of December 2012 and lowest was recorded as 5.0 in the month of August 2019 and at site-II the maximum Dissolved oxygen was obtained as 7.6 in the month of December 2019 and minimum 5.4 in the month of August 2019. The dissolved oxygen is one of the important parameter in the water quality assessment. Its presence is essential in aquatic ecosystem in bringing various biochemical changes and its effect on metabolic activities of organisms. The dissolved oxygen was found higher in winter season and lower in summer season. It is essential for growth of algae and fish production. The dissolved oxygen did not show any definite annual pattern but its higher concentration during winter and early monsoon months are correlated by its inverse correlation with water temperature (Bath and Kaur, 1997). In winter due to circulation by cooling draw down of dissolved oxygen in water into Mar Lake represented by Udash and by Thapa in Kirtipur Pond. The higher value of dissolved oxygen during past monsoon might be due to lower temperature and photosynthesis activity of the phytoplankton and microphytes by Bohra (1990). The same result shown maximum DO in winter previous studies reported by Manwar and Gayathri and the minimum DO was shown in Monsoon.

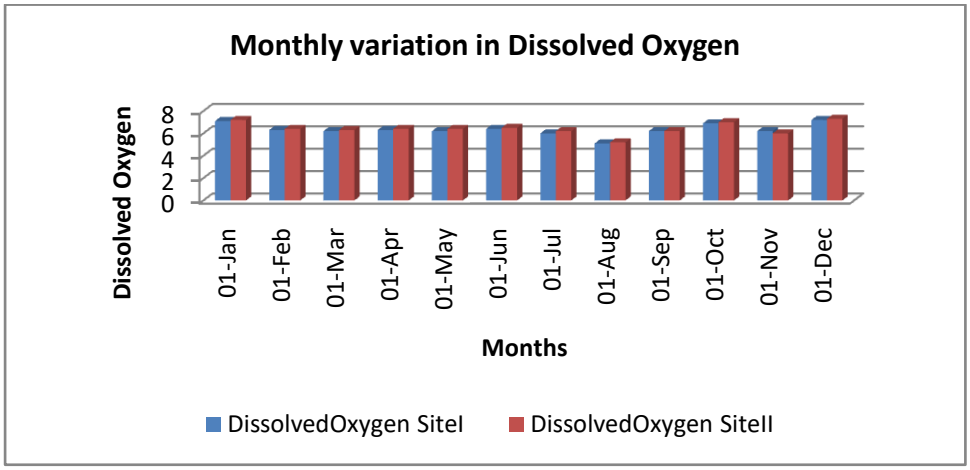

Figure 4: Monthly variation in Dissolved Oxygen

Free Carbondioxide

In the present study the obtained values of Free Carbondioxide are shown in Table-I and Table-II respectively for the period from June 2019 to May 2020. During this study, at site-I the highest Free carbon dioxide was recorded as 8.5 mg/l in the month of January 2013and lowest was recorded as 4.5mg/l in the month of November 2019 and at site-II the maximum Free carbondioxide was obtained as 8.4mg/l in the month of February 2020 and minimum 4.5 mg/l in

the month of November 2019. Free carbon dioxide and water temperature varied in dependently with low value of free carbondioxide when aquatic vegetation was more abundant and high value of free carbon dioxide when water inflow to the reservoir was greatest. The presence and absence of the free carbon dioxide in the surface water in mostly governed by its utilization by algae during photosynthesis &also through its diffusion from air.

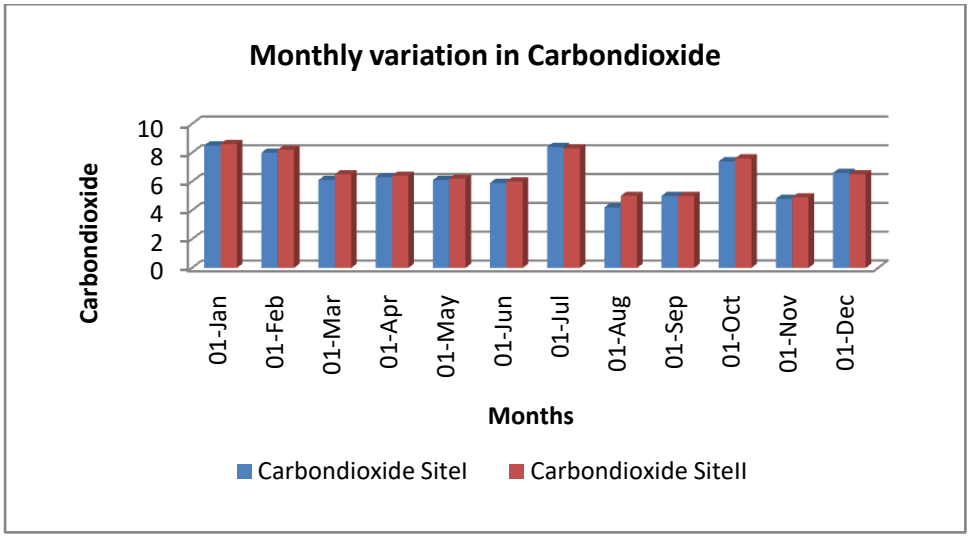

Figure 5: Monthly variation in Carbondioxide

Conclusion:

The present study is aimed to the water quality of Vishnupuri reservoir is useful for the drinking purpose and recreational activity is to be introduced in the reservoir. In the present investigation the physic-chemical parameters was analyzed and to obtain the range of the parameters are within the prescribed limit of WHO but it may be used for the drinking purpose it is necessary to the proper treatment before to use as drinking purpose. The range of physic-chemical parameter

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BOSWELLIA SERRATA: A NATURAL REMEDY FOR PAIN AND INFLAMMATION

Anshita Gupta*[1], Deependra Soni[2], Morari Radha Raman Dewangan[1], Suchita Wamankar[3] and Kaur Chanchal Deep[4]

[1]Shri Rawatpura Sarkar Institute of Pharmacy, Kumhari, Durg (C.G.), India

[2]Faculty of Pharmacy, Kalinga University, Naya Raipur, Raipur

[3]Rungta Institute of Pharmaceutical Sciences, Bhilai

[4]Rungta College of Pharmaceutical Sciences, Nandanvan Bhilai

*Corresponding author E-mail: anshita1912@gmail.com

Abstract:

Before the discovery of synthetic drugs peoples were dependent completely on the herbal medicinal plants for the prevention and treatment of various diseases or ailment. This is the era where a large number of synthetic drugs have been discovered for the treatment of various diseases and better health care of peoples suffering from various diseases, but herbal drugs are still the choice for the treatment of many diseases and preferred over synthetic drugs as these drugs are pharmacologically very active and having no side effects or low side effects when they used for the treatment of diseases. *Boswellia serrata* is a one of the medicinal plant amongst many other plants that are used traditionally for the treatment of various ailments or diseases. The medicinal plant has a long range of reported pharmacological activities viz. anti-inflammatory activity, anti-arthritic activity, anti-rheumatic, analgesic activity, antimicrobial activity anti-oxidant activity, anti-cancer activity, anti-tumor activity, anti-asthmatic activity, anti-diarrhoeal activity, immuno-modulatory activity, Hypo-lipidaemic and Hepatoprotective activity, anti-complementary activity, antifungal, anti-convulsant activity, anti-obesity, cardiotonic activity and anti-ulcer activity. The Oleo-gum-resin of the plant *Boswellia serrata* is used traditionally for the treatment of arthritis, osteoarthritis, gout, joint pain, skeletal muscle pain, back pain and diarrhoea. The plant is also used to treat bronchitis, asthma, jaundice, cough, bad throat and various types of intestinal infections. All the reported activities are suggested due to the presence of various secondary metabolites present in its chemical composition. This review paper provides information related to the phytochemical properties and pharmacological activities of *Boswellia serrata*.

Key words: anti-arthritic activity, anti-asthmatic activity, anti-rheumatic activity, immuno-modulatory activity, *Boswellia serrata*.

Introduction:

Nowadays herbal medicines have become as the most frequently used remedy for the treatment as well as a preventive measure against variety of diseases. Herbal medicines play an important role in traditional system of medicines as well as in modern system of medicines. *Boswellia serrata* Roxb belonging to the family Burseraceae, is commonly known as salai guggal, white guggal, loban, kundur, dhup and Indian olibanum or more commonly known as shallaki in Sanskrit, is the most important herbal medicines used to treat various ailments or diseases.[1][2][3]Seldom the plant is also called as "Gajabhakshya" a Sanskrit name used for *Boswellia serrata* describe that elephants enjoy this herb as a part of their diet.[4][5]It is also known as Indian Frankincense, Frankincense is a French word which means "pure incense".[6][7]The word olibanum is derived from the Arabic word "al- Luban" meaning the milk or white.[8]

B. serrata tree has been used in couple of countries in the traditional system of medicine for the treatment of variety of the diseases.[9] Beside *Boswellia serrata, Boswellia* genus is comprised of nearly 25 different species some of the important species are *Boswellia sacra, Boswellia carterii, Boswellia papyrifera, Boswellia neglecta, Boswellia frereana, Boswellia rivae, Boswellia ovalifoliolata.*[10][11][12][13]

The plant formulation is found useful when applied externally in conditions like stiffness of vessels, joint pain, inflammatory conditions, pain in legs, pus formation, and in various types of wound and stomach problems.The drug is also used in the treatment of cancer in eyes.[14]*B. serrata* have been also used in various diseases of eye, tooth, tongue and prevention of the contamination of the birth canal.[15] Many previous phytochemical study of Boswellia species shows presence of number of secondary metabolites like tannins, saponins, flavonoids, terpenoids, cardiac glycosides, reducing sugars, carbonyls, steroids, phenols but there is no alkaloid reported as their chemical composition which are responsible for the above mentioned pharmacological activities of the *Boswellia* species.[16] In this paper we review the chemical constituents and pharmacological activities of B. serrata or salai guggal.

Geographical source

B. serrata plant is commonly found in West Asia, South Africa, Southern Arabia Oman, and many part of India. In India *B. serrata* tree is found mainly in Rajasthan, Gujarat, Maharashtra, Madhya Pradesh, Orissa, Western Himalayas, and Bihar and in other dry hilly region of India.[17]

Morphology

B. serrata is a medium to large, deciduous tree usually with papery bark. Leaves are alternate, crowded at the ends of branches, imparipinnate with opposite leaflets and usually serrate. Flowers are hermaphrodite, small in size and white in axillary racemes. The flowers petals are 3-5 in number, deciduous and imbricated. Fruits are indehiscent containing 2, 5-pyrenes or pseudo capsular dehiscent rarely. Seeds are pendulous. Flowers grow in March- April and fruit grow in winter. The tree remains leafless during the entire period of flowering and fruiting.[18]

B. serrata is a medium to large, deciduous plant, up to 18 meter in height and 2.4 meter in girth. The bark of the plant is thin, greenish grey to yellow or red in colour which ultimately turn into ash colour. The bark peels off in smooth, exfoliate in papery flakes, blaze pinkish and exude small drops of resin. The oleo gum resin is obtained as exudate after an injury or natural crack in bark. The oleo gum resin obtained is fragrant, transparent and golden yellow in colour which turns into brownish yellow tears or drops and crusts.[19]

Vernacular name

Hindi - Kundur, Salai, Luban[20]
Kanada - Shallaki, Chitta, Gugul, Dhupa, Adimar, Tallaki, Maddi[21]
Tamil - Parangisambrani[21]
Telugu - Anduga, Kondagugi, Tamu[22]
Sanskrit - Ashwamuthri, Kunduru[23]
Urdu - Kundur[24]
Arabic - Luban, Kundur[25]
Persian - Kunduri[26]
Bengali - Kundur, Salai[27]
Gujarati - Dhup, Gugali[27]
Malayalam - Parangi, Saambraani[27]

Taxonomical Classification[28]

Kingdom - Plantae
Subkingdom - Tracheobionta
Super division - Spermatophyta
Division - Magnoliophyta
Order - Sapindales
Class - Angiosperms
Subclass - Eudicots

Family - Burseraceae

Genus - *Boswellia*

Species - *Serrata*

Phytochemistry and medicinal uses:

Phytochemistry

The phytochemical screening of various extracts of the plant *Bosewllia serrata* reveals the presence of various chemical constituents as major active chemical composition like essential oil, gum and resin. Essential oil is suggested to be a mixture of monoterpene, diterpene and sesquiterpenes. Gum portion on phytochemical screening suggested that it is consists of pentose and hexose sugar along with some digestive and oxidizing enzymes. Resin portion of *B. serrata* is mainly consists of pentacyclictriterpenic acid; Boswellic acid is the most active constituents of pentacyclic terpenes amongst other.[29]

The major phytochemicals of the resinous part is recognized as a monoterpenes (α-thujene), diterpenes (e.g. incensole, incensole oxide and iso- incensole oxide), a diterpene alcohol known as serratol, triterpenes (e.g. α- amyrin and β- amyrin), pentacyclictriterpenic acid (e.g. boswellic acids), tetracyclic triterpenic acids (e.g. tirucall-8, 24-dien-21-oic acids).[30][31] Gum resin of *B. serrata* is a mixture of pentacyclic triterpenoids and sugar moieties and approximately 200 other substances are also present along with them.[32] The major composition of the lipophilic portion of the oleo gum resin is pentacyclicterpenoidal derivatives e.g. Boswellic acids, major constituents of the plant *Boswellia serrata*, are considered as the pharmacological active constituents of the plant.[33]

The gum of *B. serrata* is reported to contain arabinose, rhamnose, glucose, galactose, Fructose, galacturonic acid and β sitosterol and the essential oil obtained from the gum is reported to contain phenol-o-cresol, m-cresol, p-cresol, thymol andcarvacrol and carboxylic acid-α-campholenic acid, 2,2,4-trimethylcyclopent-3-en-1-yl acetic acid and campholytic acid.[34][35][36][37]

The oil of the gum resin of the plant *B. serrata* reported to contain monoterpenes in high portion (97.3%) in which *E-β*-ocimene and limonene were reported as major constituents. The remaining 2.7% was accounted for sesquiterpenes, in which *E*-caryophyllene was reported as major constituents.[38]

The monoterpenes of the oil were identified as a 2-β-pinene, α-thujene, *E*-β-ocimene, 2,4(10)-thujadiene, camphene, sabinene, 1-β-pinene, myrecene, 2-carene, limonene, pinene, *Z*-β-ocimene, γ-terpinen, terpenoline, *p*-cymene, 1,4-cyclohexadiene, perillene, isopentyl-2-methyl

butanoate, isomylvalerate, 1,3,6-trimethylenecycloheptane, β-thujone, α-camphlene aldehyde, *allo*-ocimene, *trans*-pinocarveol, *p*-mentha-1,5-dien-8-ol, 4-terpineol, sabinyl acetate, myrtenal, verbenone, carvone, α-phellandrene epoxide and bornyl acetate.[38]

Sesquiterpenes were reported as α-cubebene, α-copaene, β-bourbonene, β-elemene, α-gurjunene, *E*-caryophyllene, α-humulene, *allo*-aromadendrene, α-amorphene, germacrene D, β-selinene, α-selinene, α-murolene, γ-cadinene, caryophyllene oxide and γ-murolene.[38]

Medicinal uses

Traditionally *B. serrata* gum resin is used as an antiseptic, antifungal and antimicrobial[39], anti-arthritic[40], anti-inflammatory[41], anti-obesity[42], anti-asthmatic[43], anticonvulsant[44] and as a cardiotonic[45].

B. serrata is traditionally used in the treatment of bronchitis, asthma, cough, bad throat and in the treatment of various intestinal problems.[46][47][48][49]

Traditionally *B. serrata* is used for the treatment of rheumatoid arthritis, osteoarthritis, gout, joint pain, skeletal muscle pain and back pain.[50]*B. serrata* is used for the treatment of various types of syphilitic and pulmonary disorders because it possess diaphoretic and astringent property. Boswellic acid, one of the most important active constituent of *B. serrata* has been reported to possess stomachic, diuretic, expectorant and stimulant properties. Boswellic acid is well known therapeutic agent for the treatment of hemorrhoids, dysentery, diarrhea and jaundice. The herbal formulation of the plant is used for the treatment of ulcer. The plant is used as a skin irritant for better flow of blood as well as for stimulation of menstruation.[51][52][53]

Pharmacological activities

B. serrata plant is known for its various pharmacological activities. Some of the activities are discussed below;

Anti-inflammatory activity

B. serrata is an ancient medicine, reported to possess potent anti-inflammatory activity and anti- atherosclerosis activity in numerous scientific studies.[54][54][56]

B. serrata reported to possess anti-inflammatory activity when tested on papaya latex model, the test showed a significant activity with mean 35% inhibition of inflammation. The activity reported is suggested due to the Boswellic acid, the most important chemical composition of *Boswellia serrata.*[57]

B. serrata reported topossess significant activity against ulcerative colitis, the activity is suggested because it blocks leukotriene biosynthesis in neutrophilic granulocytes, the activity being directed non- redox and non- competitive inhibition of 5- lipoxygenase.[58]

B. serrata reported to possess prominent anti-inflammatory activity without side effectsin 88% of patient with inflammatory diseases.[59]

B. serrata is used in traditional Ayurvedic medicine of India for the treatment of inflammatory diseases like chronic polyarthritis especially in the treatment of rheumatoid arthritis and osteoarthritis.[60]61][62][63]

B. serrata reported to possess anti-inflammatory and anti-arthritic activity when tested against the carrageenan induced paw edema adjuvant in rats. The inhibitory activity was reported as 39.75% and 65-73% administered orally in dose of 50-200 mg per kg and interaperitoneal in dose of 50-100 mg per kg respectively. The inhibitory activity is compare with phenylbutazone, used as a standard drug in the dose of 50mg per kg for the anti-inflammatory activity study of *Boswellia serrata*, which shows 47% inhibitory action.[64][65]

Analgesic activity

Gum resin of *B. serrata* reported to possess significant analgesic activity in experimental animal in addition to its sedative effect. The effect reported due to its reducing effect on spontaneous motor activity and cause Ptosis in rats.[66]

Anti-arthritic activity

B. serrata shown prominent anti-arthritic activity when anti-arthritic activity study was carried out on *Mycobacterium* adjuvant-induced poly-arthritis in rats. The inhibition of paw swelling was reported 34% and 49% in the dose of 50 mg/kg and 100 mg/kg respectively when compared with control.[67]

B. serrata exhibited marked anti-arthritic activity (45%-67%) in the dose rage of 50-100 mg/kg when chronic test of formaldehyde was performed. The drug was shown to be effective against both adjuvant arthritis (35-59%) and established arthritis (54-84%).[68]

Anti-asthmatic activity

Gum resin of *B. serrata* reported to possess vivid anti-asthmatic activity when it was tested by double-blind, placebo controlled study on 40 patients (23 males and 17 females) in the age range of 18-75 years having mean duration of bronchial asthma. The patients were treated with gum resin preparation of 300 mg thrice a day, daily for a period of 6 weeks. 70% patients were reported to show improvement of disease as evident by the disappearance of physical symptoms and sign like dysponea, rhonchi, number of attacks, increase in force expiratory volume (FEV) subset 1, forced vital capacity (FVC) and peak expiratory flow rate (PEFR) as well as reduction in eosinophilic count and ESR.[69]

Immuno-modulatory activity

Gum resin extract of the *B. serrata* was reported to possess a prominent immuno-modulatory activity when evaluated for anti-anaphylactic activity and mast cell stabilizing activity against passive paw anaphylaxis and compound 48/80 induced degranulation of the mast cell. The test was carried out in rats in dose dependent manner by using dexamethasone (0.27mg/kg) as control.[70][71]

Anticancer activity

Alcoholic extract of Oleo gum resin of *B. serrata* reported to possess marked anticancer activity when tested against the anti-carcinogenicity in mice with ehrlic ascites carcinoma and S-180 tumor, test result showed a prominent inhibition of tumor growth and the proposed mechanism of inhibition was inhibition of cell proliferation and cell growth caused by the interference with the biosynthesis of DNA, RNA and Proteins.[72]

Boswellia is reported as the most important and potent anticancer agent that occurs naturally. Methanolic extract of the gum resin of *B. serrata* contains β- Boswellic acid and its derivatives, which were reported to possess significant anti-carcinogenic, anti-tumor and anti-hyperlipidemic activities.[73]

B. serrata reported to possess inhibitory effects against the growth of the prostate cancer cells. The anti-cancer activity was reported due to the presence of the boswellic acids in its composition, boswellic acids are a pentacyclic triterpenoids. Amongst all boswellic acids, Acetyl-11-keto-β-Boswellic acid (AKBA) exerts inhibitory effect on prostate cancer by suppressing vascular endothelial growth factor receptor.[74]

Boswellic acids the most active chemical composition of the gum resin of the plant *B. serrata* reported to shown promising anti-cancer activity when prepared nanoparticles formulation of the boswellic acid was used in the treatment of prostate cancer. The proposed mechanism was that boswellic acid nanoparticles cause apoptosis and DNA fragmentation.[75][76]

Hypolipidemic and Hepatoprotective activity

Water soluble extract of the plant *B. serrata* reported to decrease total cholesterol (38-48%) and increase HDL (high density lipoprotein) in rats when fed on atherogenic diet, hence providing Hypolipidemic activity.[77]

Alcoholic extract of *B. serrata* reported cause hepatoprotection in galactosamine/ endotoxin induced liver damage in mice. The effect was reflected by reduced titre of Serum glutamic oxaloacetic transaminase (SGOT), Serum glutamic pyruvic transaminase (SGPT), aminotransferase and serum enzymes.[78]

Anti-ulcer activity

B. serrata reported to possess anti-ulcer activity when used in burn wounds and cold fissures with swine fat. It is found useful in all types of septic ulcers.[80]When the drug used with honey it showed a prominent effect on burn wounds.[80]

Antimicrobial activity

B. serrata had been reported to show a significant antimicrobial activity when tested against the microbial pathogens of oral cavity by using filter paper disc diffusion method. The maximum inhibitory concentration reported was 2-4 μg/ml. it showed concentration dependent bactericidal activity and also prevented the emergence of *S. mutant.* The antimicrobial study suggested that the drug can be used as antibacterial agent against oral pathogens as it has a great potential for use in mouthwash for the prevention and treatment of oral infections.[81]

Essential oil obtained from the bark of *B. serrata* plant reported to possess antibacterial activity against Gram positive and Gram negative. The essential oil was reported to show inhibitory activity against *S.aureus, E.coli* and *Proteus mirabilis* strands.[82]

Anti-diabetic activity

Oleo-gum-resin of *B. serrata* reported to possess significant anti-diabetic activity on non-insulin dependent diabetes mellitus. The test was carried out by using herbal formulation containing *B. serrata* oleo-gum-resin as one of the active ingredients in streptozocin induced diabetic rat model. There was noticeable reduction in blood glucose level comparable to that of phenformin used as control.[83]

Anti-diarrhoeal activity

B. serrata extract reported to possess promising anti-diarrhoeal activity and found effective in treating diarrhoea in patient with inflammatory bowel syndrome without constipation. It was found effective against the acetylcholine and barium chloride induced diarrhoea by inhibiting the contraction of the intestinal smooth muscles. The plant extract also reported to inhibit gastrointestinal transit in croton and castor oil induced diarrhoea in mice.[84]

In view of pharmacological activities of *Boswellia serrata*, number of works has done. Some of the reported pharmacological activities of the *B. serrata* are mentioned in Table 1.

Table 1: Pharmacological activities of *B. serrata* Roxb

S. No.	Pharmacological Activity	Plant Part	Test Model	References
1.	Anti-inflammatory activity	Gum resin	Carrageenan induced paw edema in rats	Atal *et al* (1980, 1981)
2.	Analgesic activity	Gum resin	Rats	Menon *et al* (1970)
3.	Anti-arthritic activity	Gum resin	*Mycobacterium* induced poly arthritis in rats	Vernon (1969)
4.	Anti-asthmatic activity	Gum resin	Double-blind, Placebo control study on 40 patients of 18-75 year old	Gupta *et al* (1998)
5.	Immuno-modulatory activity	Gum resin	Passive paw anaphylaxis and 48/80 compound degranulation of mast cell in rats	Pungle *et al* (2003), Upaganlawar (2009)
6.	Anticancer activity	Gum resin	Ahrlic ascites carcinoma and S-180 tumor in mice	Tsukada *et al* (1986)
7.	Hypolipidemic and Hepatoprotective activity	Gum resin	Galactosamin/endotoxin induced liver damage in mice	Zutsi *et al* (1986)
8.	Anti-ulcer activity	Gum resin	Burn wound	Deshpande *et al* (2016)
9.	Antimicrobial activity	Gum resin	Filter paper disc diffusion	Raja *et al* (2011)
10.	Anti-diabetic activity	Gum resin	Streptozocin induced diabetic rat	Al Awadi *et al* (1991)
11.	Anti-diarrhoeal activity	Gum resin	Acetylcholine, barium chloride, croton and castor oil induced diarrhoea in mice	Borrelli *et al*(2006)

Conclusion:

B. serrata is a deciduous plant, found mainly in the dry hilly region of India. The gum resin of *B. serrata* is mainly known for its medicinal uses and used traditionally for the treatment of various diseases. In Ayurveda and Unani System of medicine, *B. serrata* is used for the treatment of asthma, cough, inflammation, arthritis, osteoarthritis, rheumatoid arthritis,

hyperlipidemia, diarrhoea, fungal infection, obesity, convulsant and various types of cancers. The curative property of the plant is due to the presence of the various secondary metabolites; amongst boswellic acid derivatives are the most active. The boswellic acids, pentacyclic triterpenoids of *B. serrata* are the potent candidate against the inflammatory diseases, and the known mechanism by which they show anti-inflammatory activity is the inhibition of leukotriene biosynthesis and non-competitive inhibition of 5- lipoxygenase. Boswellic acid is a drug of choice for the patients with inflammatory and immunological problems. It is the better plant remedy as it has no or less toxicity and side effect in comparison with synthetic non-steroidal anti-inflammatory drugs.

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About Editors

Dr. M. Poornima is Principal Scientist, Aquatic Animal Health and Environment Division, ICAR-Central Institute of Brackishwater aquaculture, Chennai. She obtained Ph.D. in Veterinary Microbiology in 2007 from Sri Venkateswara Veterinary University (SVVU), Tirupati, Andhra Pradesh. She has 25 years of research experience. Her area of research includes development of diagnostics of emerging disease and therapeutics for aquatic animal health management. She is qualified NABL Laboratory Assessor since December 2019. She completed fifteen research projects and principal investigator of three projects funded by DBT. She has twenty research publications in peer reviewed journalsand presented research work in more thanfifty national, international conferences, seminars and workshops. She served as reviewer for many national and international journals.

Dr. Sunita R. Mukkawar is working as an Assistant Professor in Department of Microbiology, B. Raghunath ACS College, Parbhani, Maharashtra, India. She has completed B.Ed. and Ph.D. in Microbiology from Swami Ramanand Teerth Marathwada University, Nanded. She has teaching experience of 22 years and research experience of 11 years. Furthermore, she is a Food and Drug Administration (FDA) approved microbiologist for Microbiological analysis and testing. She is a recognized P.G. teacher of parent University. She is recipient of Best women Teacher Award from Avishkar Foundation, Solapur. In addition, she is one of the members of Board of Studies of Microbiology in Swami Ramanand Teerth Marathwada University, Nanded. She is also life member of Association of Microbiologists in India (AMI), Microbiologist society of India (MSI) and Indian Science Congress Association (ISCA). Moreover, her area of interests is applied, environmental microbiology and microbial technology. She has 13National and International research papers to her credit. She has expertise in analysis for antimicrobial activity of natural as well as chemically synthesized compounds. Major focus of her research is on biodegradation of lignocellulosic waste.

Dr. Kavita Chahal an Assistant Professor with 10 years of teaching experience in college, and 2 in Central School. She obtained a Ph.D. in Microbiology; and has cleared CSIR-NET in Life Sciences and ASRB-NET in Agricultural Microbiology. She is an Editorial Board Member of American Journal of Life Sciences; International Journal of Current Science Research and Review; International Journal of Scientific Research in Biological Science; Vidwan, an MHRD project (ID:175930), Active RMS member (ID: 116866) of International Journal of Creative Research Thoughts; and Member of Indian Academy of Sciences, Delhi, as a mentor for INSPIRE programs. She has to her credit 24 Research papers, 8 Book chapters,6 Books as an Editor, and 1 Book as an author. She has attended and presented papers atmore than 60 National and 30 International conferences. Presently, she is working in the Department of Botany, Government College Bichhua, Chhindwara, M.P. Her areas of research interest are Mycology and Pharmacology.

Dr. Sulochana Ramsing Rathod, M.Sc., B.Ed., Ph.D. (Botany) is presently working as Assistant Professor and Research Guide in Botany, Government College of Arts and Science, Aurangabad. Her area of research is Pathology and Fungal Biotechnology. Total 03 research students working under guidance for their doctoral degree. She participated and presented many papers in different national and international conferences. Dr. Rathod has published 16 research papers in national and international reputed journals. She also published 02 books and 05 chapters in book. She received awards from various organizations such as Bharat Ratan Dr. A.P.J. Abdul Kalam International Honor Award – 2021 for best teacher, International Award - The Iron Man of India 2021, Academic Award for best faculty organized by Universal Group of India.

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