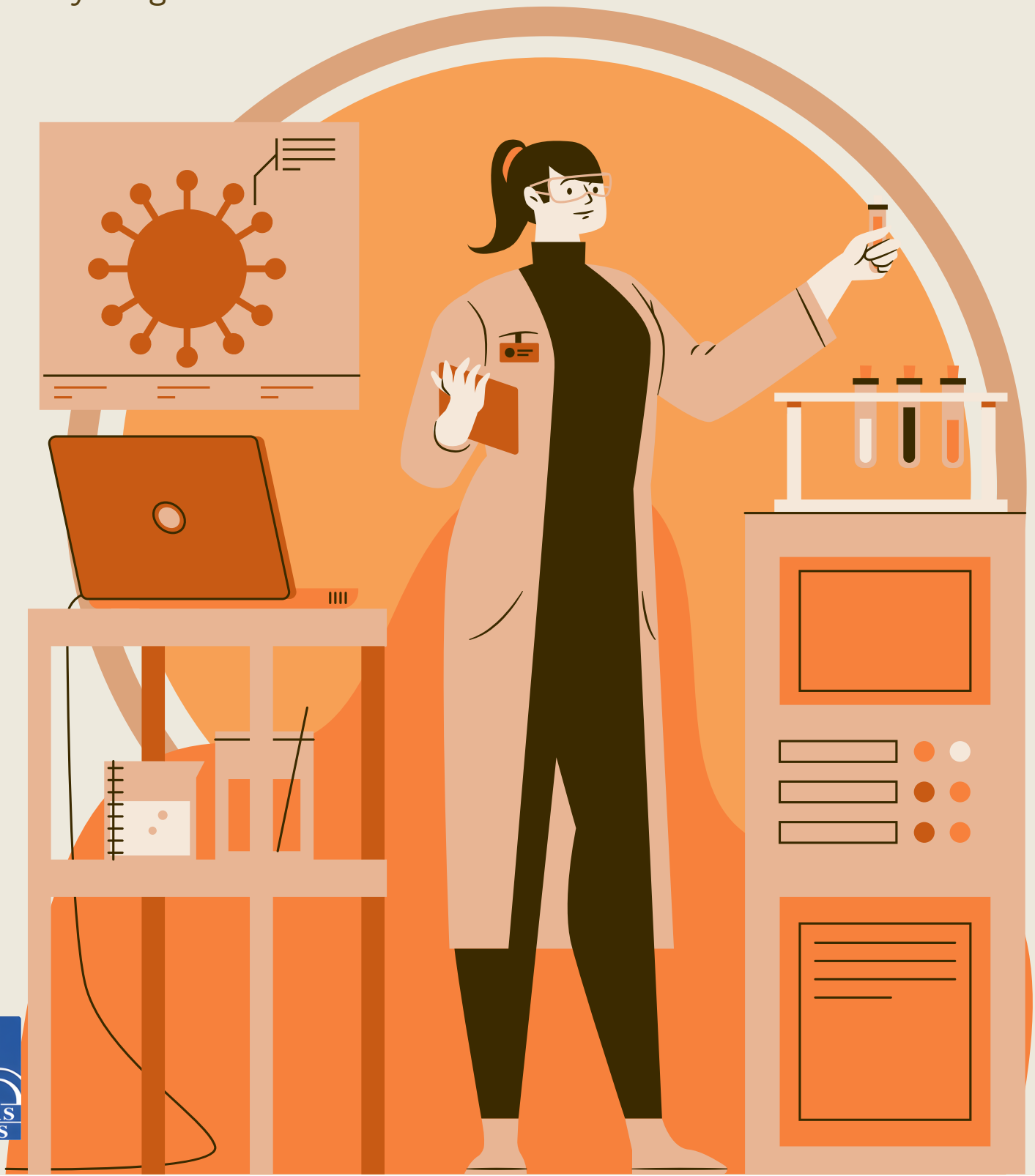
EMERGING TRENDS IN BIOTECHNOLOGY

Dr. Manish Soni
Dr. Ruby Varghese



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Published by: Alexis Press, LLC, Jersey City, USA
www.alexispress.us

First Published 2022

A catalogue record for this publication is available from the British Library

Library of Congress Cataloguing in Publication Data

Includes bibliographical references and index.

Emerging Trends in Biotechnology by *Dr. Manish Soni, Dr. Ruby Varghese*

ISBN 978-1-64532-378-5

CONTENTS

CHAPTER 1

ANALYSIS OF GENOMIC DNA ISOLATED FROM HUMAN SAMPLE WITH PCR -RFLP METHOD

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ABSTRACT:

Extraction and isolation of DNA from the human samples such as urine, Cheek swab buccal area samples, and plasma blood were performed in this research study followed by analyzingit with the help ofMolecular photocopying also known as Polymerase chain reaction (PCR) followed byRestriction fragment length polymorphism (RFLP). The use of the 'phenol-chloroform method PSI was employed for isolating DNA from different sample sources. The 1030 base pair (bp) region of the mitochondrial D- loop was amplified. Restriction digestion with the use of molecular marker ALU-I was evaluated for the analysis of PCR product. The quantity and quality of isolated DNA were tested by the use of the spectrophotometer. Cheek cell swab (Buccal cells) samples were processed to DNA isolation, directly or later to refrigeration (4–6°C) for almost3 days. Therefore, DNA extracted from buccal swabs and urine samples can be used for forensic as well as research analysis. The present study also includes protocols and techniques for the storage of biospecimens for longer intervals of time with the use of temperature and chemicals.

KEYWORDS:

Genomic DNA, Human Sample, Molecular Marker, Mitochondrial DNA (mtDNA), Polymerase Chain Reaction (PCR), Restriction Fragment Length Polymorphism (RFLP).

1. INTRODUCTION

Researchers are gradually experimenting with complementing experimental recordsper ecological data, comprising DNA. Plasma has been an alternative for producing chromosomal DNA for almost all significant research investigations[1]. Although, these research results may require an alternative source when the study subjects refuse to offer a plasma sample or when simply an individual collection method is operationally or commercially achievable. Originally, by now, blood continues to be a commonly used source of human nucleic acid for studying and carrying out various research studies and developing preventive methods for genetic disorders. Nevertheless, plasma has quite a fewrestrictions, such as the necessity of a trainedworkforce, apparatus, and work arrangement, in that wayobstruct sample collection in anadditional epidemiological background.

Moreover, definite groups of individuals including toddlers arehesitant to provideplasma. About the specimen alone, plasmatoocomprisessomeintrusive amino acid chains and molecular photocopying suppressers thatsignifies aninfectivedanger for acquired immunodeficiency syndrome and other microbes[2].Hence, furthernew sources of nucleic acids can be more suitable. Other studies also report that saliva has previously stayed employedas a valued, substituteused asa molecular label. Here, a cheek swab sample seems to be a good choice to be

included in the research study as it can be easily performed by a simple mouth wash, is also cost-effective, and requires low sample volume. Urine samples can also be used for extracting DNA, since it deteriorates quickly, the handling needs to be fast The present study suggests storage methods for storing biological samples like urine with the use of different chemicals and temperature conditions[3]. However, it can be stored for a longer period under freezing conditions.

2. LITERATURE REVIEW

Souvik Ghatak et al. demonstrated an easy protocol for the isolation of chromosomal DNA from Human specimens for molecular photocopying and restriction fragment length polymorphismStudy.The research was accomplished to estimate the amount plus the extent of DNA isolated from a few readily accessible specimens besides determining the period required for performing PCR amplification. The Authors successfully displayed that hair as well as urine specimens could be an alternativemeant for safely producing an even smaller quantity of PCR-ready DNA. The authors introduced a fast, economically- feasible, and reliable procedure of specimenassortment and easy chromosomal DNA isolation from a cheek swab, urine, plus hair with the use of traditional PCI protocol. Cheek swabs specimenswere processed for isolation of DNA, right after otherwise can be used once refrigerated at '4–6°C' for 3 consecutive days. Also, the current study states that hair specimens demonstrated can contribute to becoming an important origin of G- DNA for molecular photocopying assays. DNA isolated from the hair samples could further be employed in the analysis of genetic diseasesconcerningcriminologicalinvestigationas an outcome of the easy specimenassortment in a 'noninvasive'method, lesser specimen capacity usage, along with great storingability[1].

Latifa El Bali et al. A Comparison research investigation with 'seven' market available testing kits intended for extracting DNA from human urine specimens which are also appropriate for 'DNA biomarker'established Community-related well-being researches.The study, which involves several samples as examples, requires a quick, reliable, and definitive extraction procedure. Furthermore, for feasibility, it is necessary to collect urine at a time that is distinct in comparison withthe initial void plus should bestocked properly till the testing. In this current study,the authors have collated 'seven' commercialized kits choosing the utmost suitable method for isolation of DNA from human urine samplesmeant for research investigations. The yield of isolated DNA was investigated with the use of various evaluation protocols. Incorporation of genotyping was feasible with the use of isolated DNA from sample urine preserved at '−20°C' over a while, with this a satisfactory quantity can also be aggregated with the collection of urine at contrasting times throughout the day, that is especially essential for communitywell-being investigations[2].

M. Stoneking et al. Determining the DNA quantity and sterility using extraction from cheek swabs. The researchers developed a case-control study that will estimate if particular genetic polymorphisms can revise the outcome of drugs used for high blood pressure. Further given the vast pharmacogenomics data, the authors managed to carry out a transversional investigation to produce DNA quantity with sterility.Volunteers had registered from the population-based pharmaco-morbidity record linkage system (PHARMO). As per the result sex, age, water pills, and research laboratory person have to be necessarily factored into consideration during the collection of cheek swabs. To obtain excellent DNA concentration and high DNA sterility, it is mandatory to have a competent research labstaff[3]. theexamination of the mtDNA D-loop

section is selected for the analysis as the major intergenic region of the human 'mtDNA' genome which also contains the point of replication of the 'one strand D-loop region' and in cooperationwith the point of transcription[4].

The above research studies contain various procedures and protocols for the isolation of genomic DNA from various biospecimens however the present study contains an economically feasible yet reliable protocol for the investigation of the biological samples utilizing the PCR- RFLP protocol. The research studies reported above involve the application of commercial testing kits whereas the protocol reported in this research study does not report any kit-based method for analysis instead it accounts for the amalgamation of traditional as well as modern techniques.

Research Questions:

- How to isolate DNA from Human Sample?
- What are the uses of PCR-RFLP methods for analyzing DNA Samples?

3. METHODOLOGY

3.1.Research Design:

DNA extraction is a technique used for the study and investigation of a crime scene or a genetic disorder. The assay helps the researchers, and scholars find out the cause of a particular disease prevailing in molecular or clinical biology leading to the development of relevant diagnostics techniques for their identification and differentiation. When coupled with Polymerase chain reaction 'PCR' an enzymatic assay technique that helps in generating millions of copies of Deoxyribonucleic acid 'DNA'. Thus resolving the issue of less sample quantity for generating results. PCR is employed to amplify a specific region of (DNA) leading to rapid generation of DNA copies. The assay depends on the use of primers, deoxynucleoside triphosphate (dNTP), and Thermus aquatics (Taq polymerase) enzyme, a thermal stable DNA polymerase I. 'Restriction length polymorphism' 'RFLP' is a biochemistry application used for examining any contamination in DNA isolated from a particular source. This research displays that urine samples can also turn out to bean alternative source of securelyobtaining sample quantities of PCR-equipped DNA. The research effectively creates a quick, economical also a'non-invasive'procedure of specimen assembly plus a safe DNA isolation from cheek swabs and urine samples withthe use of PCI protocol [1], [5].

3.2.Sample and Instrumentation:

The present study comprises, three fit mature participants who were asked to participate in the research study demographic bracket, 21–35 age, and data comprising health status, gender, genealogy, the texture of hair, along with hair medication data were gathered. Every participantemployed was requested to wash their mouths with normal water, for 25 s in advance of sampling oral swabs, for avoiding any contaminants because of the accumulation of foodstuff.For every single volunteer, a cotton swab was swabbed from both the sides of the buccal mucosa for 20 s, along with it whole three specimens were assembled in '450 µl 9 M Tris-HCl, 9mM EDTA, 1% SDS, containing 1.0-ml'micro centrifuged tubes.Isolated DNA obtained from cotton buds was analyzedvia (vide infra). Every employed participant in the study was thoroughly explained about the research and was instructed to collect urine. Urine samples were gathered in a sterilized specimen bottle followed by mixing it mildly with overturningdesigned

for 35 min before performing the dispensation. To elude impurity which is an outcome because of frequent selectionof the samples in addition to understanding the influence of storage consequenceson the specimen reliability, Urine specimen from every sample was aliquoted '4 ml' inside suitable bottles. Phosphate buffered saline PBS '450 µl' was introduced in a '0.5 ml' urine specimen comprising in 1.5-ml micro centrifuged tube with the addition of 0.5 M Ethylenediaminetetraacetic acid EDTA of (8.0 pH) concluding a volume of 9 mM EDTA for preventingany likely nuclease activity in the sample urine. The tubes were further subjected to vortex systematically for 1 min. It is advisable to freeze the urine solutions at (−20°C) or use them immediately[6].

3.3.Data Collection:

3.3.1. DNA Isolation from cheek swabs:

Cheek swab specimens were disolved in 450 µl lyse buffer '9 mM Tris (8.0 pH), 9 mM EDTA, with 1.0% Sodium dodecyl sulphate', and '40 µl of 9% SDS, plus with the addition of 4–9 µl 10 mg/ml proteinase K'. Further specimens were put through to incubation for 1–3.5 h at 56°C unless the tissues completely dissolves. Isolation of DNA from every individual sample was carried out with the addition ofan equivalent amount of phenol:chloroform: isoamyl in an solution of alcohol of (25:24:1) followed by softly mixing the tubes through reversing for 3 min approximately. The specimens were subjected to centrifugation at (4°C), for 10 min at 10,000 g. later transferring the top liquid sheet to a new, and cleanmicro centrifuged tube. RNase A (9 µl of 9 mg/ml; Qiagen) was introduced, and the solution was set for incubation for 30 min at 37°C. An equivalent amount of chloroform: isoamyl alcohol suspension was infused and centrifuged, again subjecting it to 10,000 g for 10 min at (4°C). Further, removal of the top liquid sheet wasshifted in a clean sanitizedmicro centrifuged tube, additionally, twice the volume of ice-cold isopropyl alcohol was added, alongside the one-fifth volume of 1 M sodium acetate, followed by chilling for 1h at −20°C prior preparation for precipitation. Post an hour, the specimens were centrifuged at (4°C), for 10 min at 10,000 g. Afterward, removal of the supernatant, 200 µl from 70% ethanol was performed, next, the sediment at the bottom of the tube was suspended; the suspension was further centrifuged for 10 min at 10,000 rpm, then decanting of the supernatant was done carefully. Air drying of the pellet was completed under laminar airflow, the dry pellet was resuspended in 40 µl nuclease-free water and stored at −19°C or −85°C.

3.3.2. DNA Isolation from Urine:

Frozen urine specimens were defrosted at 25°C and were transferred instantly to the ice prior to performing the DNA isolation. The urine sample was upturned within a sample beaker for creating a homogenized mixture of cells. 1-milliliter sample was transported to a microcentrifuge tube and centrifugation was performed at (4°C) for 10 minutes at 10,000 g. The top layer was discarded, and the dried pellet comprising of cells were frosted for 18 min at −19°C. Lyse buffer '450 µl; 9 mM Tris, 1.0 mM EDTA, 9% Sodium dodecyl sulphate, pH9.0 was introduced to the dried sediment, followed by a specimen vortex for pellet resuspension. Proteinase K was added (15 µl of 15 mg/ml; Merck), and the tube further was set for incubation inside a water bath(2-2.5 h at 56°C). Sodium acetate '50 µl of 2 M' along with the addition of 0.3 ml chilled isopropanol, was blended properly and froze at −20°C for 1h, proceeded by centrifugation for 18 minutes at 10,000g (5°C). Discarded supernatant, and 200 µl of 70% ethanol was introduced, followed by gently tapping the pellet. The supernatant was successfully discarded by using Centrifugation for

10 min at 10,000 rpm. Air-drying of the pellet inside a laminar airflow with resuspension in 40 µl nuclease-free water and freezing at −19°C or −85°C for longer storage[1]–[3].

3.3.3. DNA Isolation from Plasma:

White blood cells from the blood were removed utilizing lysis on the erythrocyteswith the use of 'ammonium bicarbonate and ammonium chloride' a hypotonic bufferthat provides a negligible lysed influence on lymphocytes. Lysis buffer of RBC up to three volumes was introduced to the blood specimen and blended thoroughly by using a vortex. Followed by performing up-side-down motion for a complete 4 min with centrifugationfor 10 min at 20, 00 g.The top layer was regularly cast-off, providing the remaining ∼1 ml to avert damage to cells. For the pellet, add3 volumes of erythrocytes lyse buffer**,** then vortex, invert, repeating the centrifugation steps for1 - 3intervalsunless a clean upper layerand a rich pellet with snowy textureis acquired**.** Post performing the lastrinse**,** the top most layer wasscraped off entirely, and resuspension ofthe sediment i.e. pelletwas done in a 400 µlPhosphate buffered saline, proceeding furtherwith the addition of cell lysis buffer 300µl'9 mM Tris-HCl, 9 mM EDTA, 40 mM NaCl, 10% SDS, pH 7.5and 9 µl proteinase K (10 mg/ml stock)'.The specimens were subjectedto vortexing in order to completelyresolve the sediment and laterincubated at 56°Cfor 2.5 h in a water bath for lysing.An equivalent amount of phenol **(**pH 8**,**balanced with Tris**)** was successively included inthe testing tube and blended nicelyby turning the tube upside downforalmost 1 min**.** Centrifuging the testing tube at 10,000 g (4°C),for 10 minthe upper aqueous layer wasremoved and was moved to anew sterile tube comprising the identical amount of phenol '1:1'andchloroform: isoamyl alcohol '24:1'.Later,the testing tubewa**s** invertedintended for proper mixing for about 1 min and centrifugation for 10 minat 10,000 g (4°C). transferring the upper aqueous layerto anew sterile tube, with the addition of'9 µl of 9 mg/ml RNase A'.

Incubating the specimens for 30 min at 37°C prior to the addition of an equivalent amountof chloroform: isoamyl alcohol '24:1' overturning the tube for a minute and performing centrifugation for 10 min 4°C at 10,000 g.The upper aqueous layer was scrappedand shifted to a sterile tube, with the addition of double the amount of 100% alcohol was subsequently added then upturned carefully time and again. Followed by chilling at −20°C and centrifuging for 20 min (4°C) at 10,000 g. The top aqueous layer was removed, and 240 µl of70% isopropyl ethanolwasfurtherintroduced**,** besides this pellet out gently tap, and centrifuged for 10 min at 10,000 rpm, followed by decanting of the supernatantwas carefully performed. Air-drying the pellet under the laminar airflow, and resuspending in 1× TE buffer or40 µl nuclease-free water and freeze at −20°C/ −80°C for storage[1], [7].

3.4.Data Analysis:

3.4.1. DNA Purity and Concentration Analysis:

A quantitative determination of spectrophotometry assay for DNA was achieved by using a UV-Vis spectrophotometer (Thermo Fisher). Absorbance was recorded at 260 and 280 wavelengths (A260 and A280, separately) nm. The absorbance quotient (OD260/280) offers an approximation of the purity of DNA. The absorbance quotient value of 1.8 greater than ratio 2 was contemplated to be acceptable, pure DNA. A ratio greater than 2 an indicative of RNA impurity. A ratio of lesser than 1.8 is an indication of protein decontamination, however, a ratio greater than 2.0 an indicative of RNA impurity.

3.4.2. The integrity of DNA:

The integrity of the genomic DNA was estimated by resolving DNA extracts on a 0.8% agarose gel by electrophoresis, succeeding by staining with ethidium bromide (EtBr) for visualizing the DNA bands. Individually the DNA specimen was classified, conferring to the electrophoretic shift of the sample DNA when associated with an identified molecular weight marker[8].

3.4.3. Mt-DNA D-loop PCR Amplification:

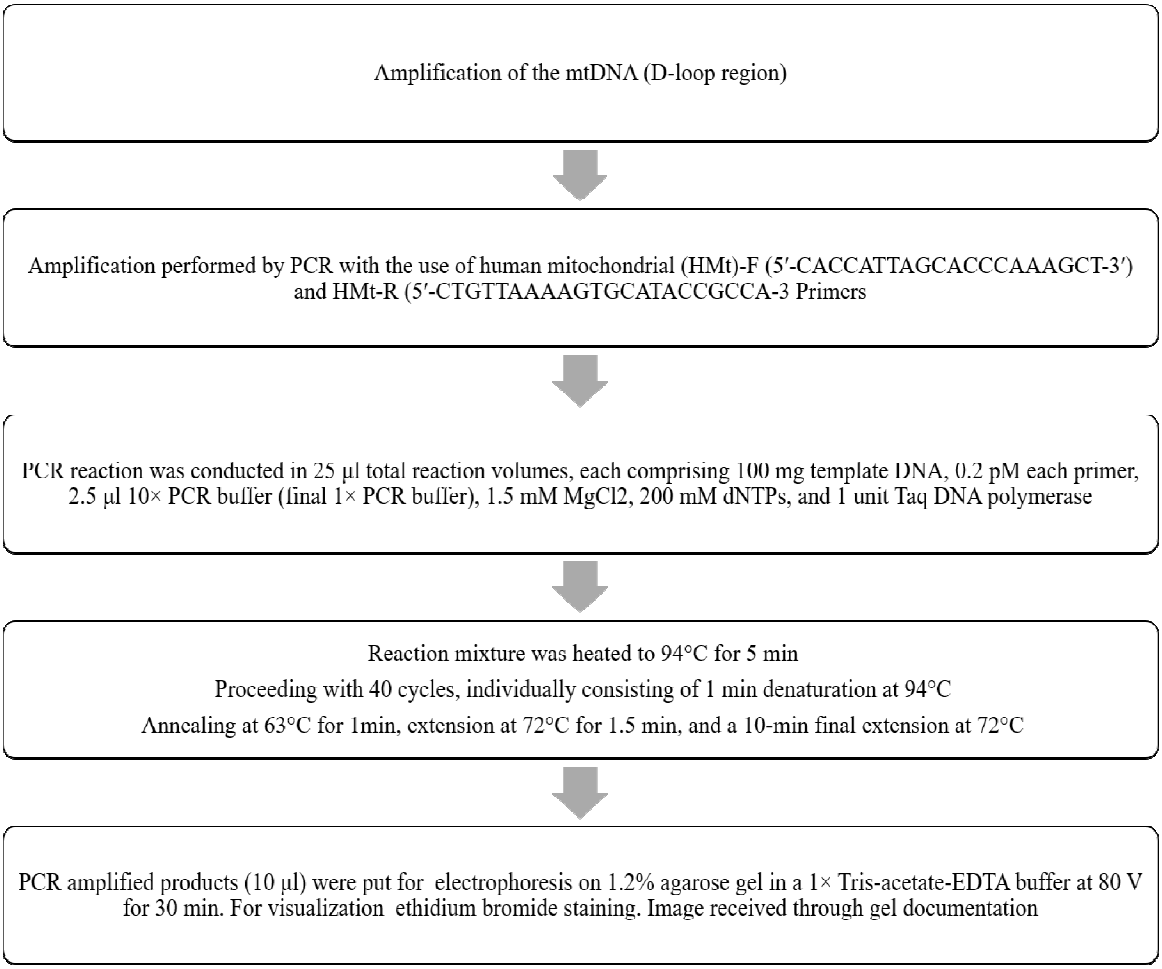

Figure 1: Illustrating the Procedure of the Research Followed

3.4.4. PCR product of Mitochondrial DNA D-Loop region with restriction digestion:

'RFLP'of the D-loop region of the mtDNA was executed in order to find out impurity in theextracted DNA as shown in the steps of Figure 1.Products obtained by PCR were subjected to digestion with restriction enzyme*AluI* within a complete measurement of 15 µl (reaction solution 7 µl, enzyme buffers 1 µl, enzymes 0.1 µl, and distilled water 6.9 µl) was kept for incubation for 4 h at 37°C. The restricted products were examined through gel electrophoresis with 2% agarose gel, and themolecular mass ofthe restricted fragment was evaluated with the help of a gel documentation system after staining it with EtBr[9].The current study demonstrates a quick, safe, and reliable technique for producing PCR-ready G-DNA sources such as human cheek samples and urine samples having a negligible specimen amount. For DNA isolation plasma was used as

a test sample. Revising the traditional PSI method, a reliable procedure was successfully developed and presented. The method is economical and greatly executed for DNA extraction with optimum volume and sterility.

4. RESULT AND DISCUSSION

4.1.DNA purity and Yield obtained:

The amount of the DNA extracted from three diverse specimen sources was estimated with the help of a "double-beam UV-visible spectrophotometer" along with anagarose gel electrophoresis assay (Figure2). 'Small-scale DNA isolation from cheek sample'(1.2 ml) led to '58–84 ng/µl G-DNA/isolation', '27–44 ng/µl in urine (4 ml)', and '58–96 ng/µl in plasma (48 µl) specimens'(Table 1). Likewise, the sterility of the isolated DNA from urine (1.43–1.56) and cheek cell samples (1.56–1.68) were less in comparison to blood (1.74–1.84) samples (Table 1). For DNA storage isolated from urine, blood, and the buccal swab was achieved by freezing them at −20°C for one month which also did not affect the PCR efficiency[1], [10], [11].

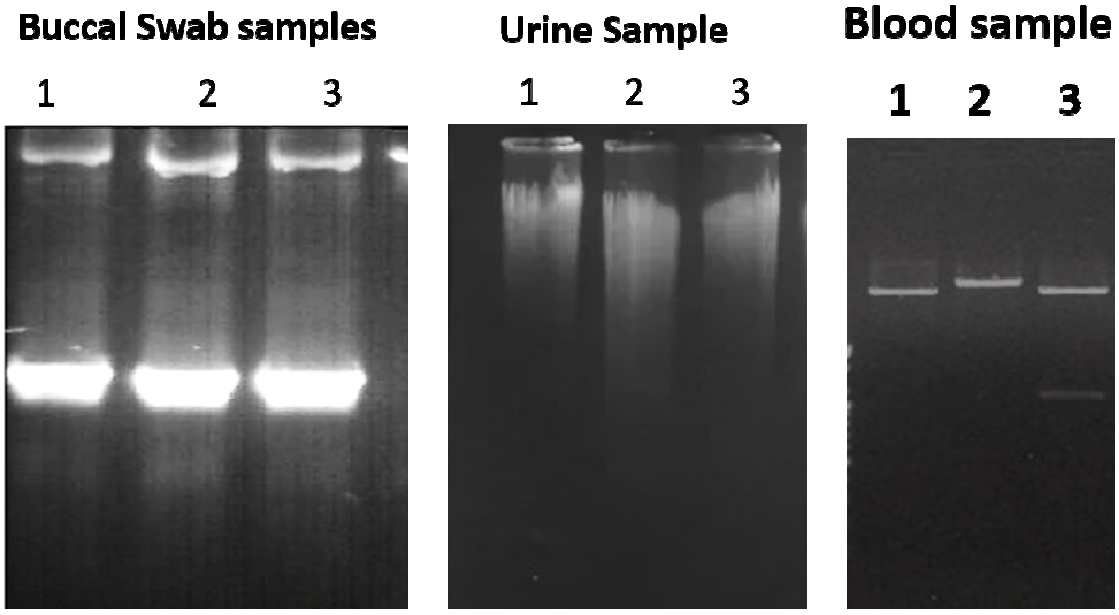

Figure2: Genomic mtDNA Isolated From The Buccal Swab, Urine, and Blood Samples (1 – 3 Three Distinct Copies)

Table 1: Complete DNA Quantity and Quality Through Instant Handling or Storing of Biospecimens

Spectrophotometry				
Biospecimen	**Direct handling**		**Storage processing**	
	Complete DNA quantity (ng/µl)	**Ratio of A_{260}/ A_{280}**	**Complete DNA quantity (ng/µl)**	**A_{260}:A_{280}**

	1	2	3	1	2	3	1	2	3	1	2	3
Blood sample	84	72	91	1.83	1.85	1.76	78	93	7	1.88	1.64	1.82
Buccal swab sample	82	60	83	1.56	1.63	1.53	56	52	56	1.63	1.66	1.63
Urine sample	30	24	37	1.47	1.43	1.56	24	38	38	1.47	1.47	1.47

4.2.The Outcome of Processed specimens:

To examine the DNA quality isolated on a small scale from cheek swabs, urine, and plasma specimens, amplification of the D-loop region of mtDNA was done with the help of the PCR technique. The D-loop region of mt-DNA was amplified effectively by every sample, regardless of the condition of the specimen, even if handled instantly post the collection or storage (Figure3 and 4). Hence, urine and buccal are a great substitute source, along with plasma specimen, whilst PCR-ready G-DNA is needed. Nevertheless, a substantial difference in quantity, in addition to the yield or the purity amongst all specimen kinds, is noticed. Therefore, a safe molecular photocopying assay could be executed even if less amount of DNA is isolated. PCR product concentrations were high for plasma DNA than in comparison to urine and cheek cell samples. The PCR- RFLP band arrangements were satisfactory in the plasma DNA case.

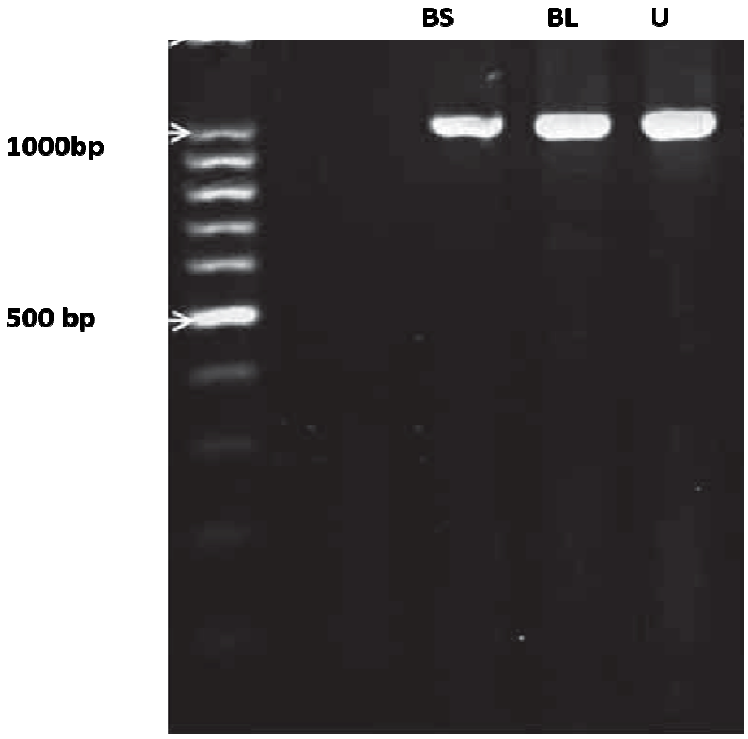

Figure 3: mtDNA D- Loop Region Amplified BS- Buccal Swab, BL- Blood DNA, U- Urine

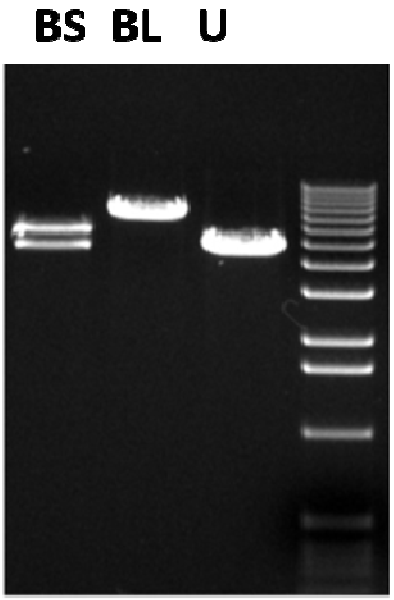

Figure 4:mtDNA D-loop PCR Product Region Restriction Digest by Marker AluI

The quantity of obtained DNA from cheek swabs as well as urine is greatly different depending upon the cotton swab or the nature of urine, how all participantswere swabbed, the method of swabbing, along with the complete count of cells in the urine specimen and on the swab. The predictable quantity employing particularly this procedure is 60–83 ng/µl/ for cheek swabs whereas with urine it is, 24–37 ng/µl/15 ml assembly (Table 1), that is, no less than two higher in comparison to the traditional protocols. The yield and purity of DNA extracted similarly depend upon the investigators' management technique. The reduction in the quality ofDNA and the amount was witnessed when the material was not employed instantaneously in lysis cell buffer with additional refinement. Degraded bands of DNA were detected in cheek cell swabs and urine specimens handled with delay in time, while degradation in plasma was not witnessed, perhaps as an effect of the consideration of the specimen along with the amount of nucleodepolymerase enzyme concentration within the specimen prior to the process of digestion. Though there was some extent of degradation in DNA with the specimen preserved in a colder temperature environment for almost 3 days, this research study does not display any important alteration among the DNA extracted and PCR amplified products from the cheek cells subsequently the specimenassortment or from the blood samples frozen at −20°C for 3 days. Furthermore, storage for 1 week, and chilling at 4°C or freezing at −20°C, similarly didn't influence the amount of DNA isolated or PCR amplified DNA. Further, in the case of plasma specimens, the superiorquality of DNA can be acquired intended for future usage,subsequentlyafter storage of the blood specimen inside an EDTA-coated vialat −20°C. For 4 months or above.

5. CONCLUSION

The fruitful specimenassortment followed by the isolation of G- DNA from cheek cell samples and urine is a non-invasive and safe alternative to the sharp intrusive plasma collection, equally for patients and phlebotomist 17-20. In this research paperan easy and innovative process of specimen collection and isolation of DNA is demonstrated. It is economical, simple to use, plus it is also quick, generating an adequate amount and good standard of DNA for the analysis of

PCR-RFLP. A collation of the isolation techniques displays that the easy PSI protocol is the best option for the isolation of DNA from cheek swabs, plasma specimens, and urine specimens. Underneath the right storing settings, isolated DNA from cheek swabs and urine could be effectively employed for the execution of PCR-based procedures. The DTT, anionic detergent solution, mtDNA isolation,high concentration of salt, and extraction protocol constructed in the research investigation signifies a fast and easy method that tops the amplification of mtDNAaccomplishment rate of aclassic glass-grinding/inorganicand extraction method for solvents whichis currently in use by variouspathological research labs. Comparatively less number of stages are employed in this procedurewhich facilitates lessintervaladdingto, effects reducing the possibility of impurity with a marginal loss of sample. The digestion protocol of DTT chemicals comprises chemicals, materials, as well as apparatuses that are easily accessible in any laboratory.

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CHAPTER 2

A COHERENT STUDY ON RECENT ADVANCES IN AUGMENTED REALITY TECHNOLOGY UTILIZED IN SURGICAL PROCEDURES

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ABSTRACT:

Three-dimensional augmented reality (AR) is a relatively recent innovation in the realm of minimally invasive surgery. Augmented reality (AR) is increasingly being used in healthcare settings. Therefore, a useful 3D planning data display that can be utilized to accurately compare distances or geographical connections during surgery would be required. The goal of this research is to determine whether or not augmented reality can improve surgical procedure results at the current time. According to recent research, doctors seem to be becoming more interested in using augmented reality during surgical procedures, which would improve the safety and efficacy of surgical treatments. Numerous studies concluded that newly created augmented reality apps operate as effectively as more traditional ways. Therefore, several problems must be overcome before augmented reality may be incorporated into routine practice.

KEYWORDS:

3D- Display, Augmented Reality (AR), Surgical Operations, Technology, Virtual Reality (VR).

1. INTRODUCTION

The newest medical technology, Augmented Reality (AR), is being utilized in surgery.This technique's main purpose is to correlate a model image to a video stream of the real environment. A 3D representation of the surgical area would be created as a result of this procedure. The surgeon would be able to see the operative area well now. They may then undertake a variety of surgical activities, including cutting, determining the drilling location, and detecting the tooth root or neural pathways as well as other valuable information [1].

There are now three distinct types of augmented reality-based surgical navigation that have been used in the healthcare industry.Projection-based systems, see-through, and video-based, are examples of augmented reality navigation systems. In regards to the identification approach, these categories may be broken down into two distinct groups: marker-based or marker-free. Because it eliminates the necessity for a cumbersome tracking system and has numerous advantages over the marker-based navigation system, this marker-free registration-based augmented reality-based navigation system is gaining traction in the surgical industry. The marker-based navigation system is the most prevalent sort of navigation system nowadays [2].

In 1895, W. C. Rontgen invented the X-ray and started experimenting with diagnostic imaging. This marks the beginning of the therapeutic use of diagnostic imaging. Computed tomography (CT), Magnetic Resonance Imaging (MRI), Ultrasound (USG), as well as other imaging methodsenable professionals to discover and treat health issues using 2D and 3D photos. Functional MRI (fMRI) and Single Photon Emission Computed Tomography (SPECT/CT) integrate morphological and physiological (or physiologic) scanning. These strategies helped doctors comprehend an area's anatomy as well as functioning.

The most recent innovation in diagnostic imaging emphasizes the collection of information in real-time and the presentation of data. The increased availability of real-time data has become more crucial since the use of these data often results in management and therapy that is completed more quickly and with greater accuracy. This is particularly true in the field of surgery, where having access in real-time to reconstruct pictures in either two or three dimensions while an operation is in progress might prove to be quite useful.The emergence of AR, which would be a combination of displaying computer-generated (CG) visuals as well as real surroundings, has made this connectivity much more accessible.

As augmented reality (AR) may modify our perception of reality in numerous ways, the ability to collaborate with a computer expands surgical possibilities. New procedures based on AR are being developed because of the vast variety of options it presents to doctors. Virtual reality (VR) and augmented reality (AR) may soon be able to completely replace many of the tools needed to successfully execute a successful surgical procedure today. However, few people are aware of the advantages of using AR since,unfortunately, it is not yet at a point where it can substitute for the great majority of standard surgical procedures. The primary goal of this research is to look at the most recent advancements in the rapidly growing relationship between augmented reality and surgeries.

2. LITERATURE REVIEW

Nakamoto et al. stated in their study that surgery is advancing quickly, but there are still limits, such as the inability to adequately view the target organ throughout laparoscopic surgery. They found that laparoscope tracking and organ registration provide geometrically perfect overlaid pictures. Actual laparoscopic pictures, as well as a 3D organ model, were merged to help the surgeon understand anatomy.The author concluded that AR imaging is a major development that increases accuracy in laparoscopy and endoscopic procedures. To better monitor dynamic organ movements and deformation, researchers are exploring new methods [3].

Jeffrey H. Shuhaiber discussed and examined computer-augmented reality in surgery and also its possible aims in education, surgeon learning, or patient therapy. Only publications with a well-defined purpose, methodology, and result were considered. Augmented reality is an effective tool for low-performance surgical dexterous operations; it is mostly decided by stereotactic identification or ergonomics. It's a proven training tool for resident training. Weak evidence suggested an impact on surgical morbidity and death. Cost-effectiveness wasn't established.Augmented reality improves surgical procedures. Further study is required to determine its long-term clinical impact on the patients, surgeons, as well as hospital managers. Its usage and transfer are restricted until registration and ergonomics are better understood.

Christian Hansen et al. discussed in their study that AR is gaining popularity in operating rooms. A meaningful 3D representation of planning data that allows reliable comparisons of distance or

geographical linkages is still a need. Intraoperative viewing of 3D modeling approaches using illustrative rendering and AR. Their study contributed to a distance-encoding silhouette methodfor 3D modeling and random graphics (distance-encoding surfaces). Also, they illustrated resection surfaces. The created algorithms were incorporated into an operating room model. Under controlled situations, researchers tested the effectiveness of the visualization approaches. The research found that the suggested illustrated method is superior to traditional rendering approaches for distance measurement.In surgical augmented reality, distance evaluation is made easier with the help of the offered illustrative approaches. Their study focused on developing methods to improve monitoring or registration accuracy to make the suggested technique a safer option for treatments [4].

Sheik-Ali, S et al. stated in their study that VR or AR gadgets feature high-resolution displays, mGPUs, and position-sensing technology.A quality assessment in Newcastle and Ottawa yielded a median score of 7. According to the study, there is a strong link connecting VR/AR in surgical training programs and boosting the surgeon's ability to accomplish duties, complete a surgery precisely, and hand-eye coordination, as well as bi-manual operations. The documentation praised VR/AR for robotic surgery.VR/AR seems to improve surgical skill learning due to the limited research.Speculative conclusions were all that could be reached because of the many ways in which research was conducted and the relatively novel applications of virtual and augmented reality in surgical education. Additional study is needed, preferably with large samples, rigorous treatment outcomes, or extended follow-up durations [5].

Felix Brent et al. conducted a study that Visual augmented Reality (VisAR) was used to implant 124 thoracolumbar pedicle screws into seven bodies. Four donors had open spine surgery with 65 screws. 3 donors underwent MISS to place 59 screws.Pedicle screw guiding in open and minimally invasive spine surgery was performed with the help of VisAR (MISS). This study's URL was accessible through QR code, printed out, or used with VisAR in the operating room. Digital Imaging and Communications in Medicine (DICOM) data is converted into holographic images and recorded on the donor's back once the code has been wirelessly downloaded and the study has started. The surgeon brought up each pedicle's documented routes through voice control or aligned screws with a virtual guiding holographic. According to the author, VisAR technology inserted 124 pedicle screws with 96 percent accuracy and the total error was 2.4° or 1.9 mm.

3. DISCUSSION

Surgery trainees may hold the better concepts taught in the classroom by using 3D augmented or virtual reality to practice actual surgery. Learning from errors is assisted through debriefing and criticism. Because it doesn't need the presence of training instructors, this kind of simulation is very adaptable. Virtual reality (VR) aids in the visualization of the human body's unreachable internal regions. Anatomical details may be studied in great depth thanks to 3D modeling. When teaching surgical students, it's important to use scenarios that are as similar to real life as possible. Students may utilize a model created from real-time surgical recordings captured from various viewpoints to mimic surgery in virtual reality. Surgery abilities may be improved greatly by virtual reality training sessions. In addition, it reduces the total expenditures, recuperation period, or postoperative problems of the procedure. Businesses may use AR visualization technology to make interactive experiences for their goods that enable customers to connect with

them in a new way. These systems allow users to submit 3D material and alter the image's size, color, and more elements to provide the greatest possible user experience.

3.1.Understanding Augmented Reality's Basic Concepts:

With the use of specialized equipment and software, an augmented reality system may provideduring surgery, the surgeon receives real-time computer-processed image data. The use of monitors, projections, lenses, trackers, and other specialized equipment is required to successfully project augmented reality. Figure 1 illustrates the fundamental operating concept of a fundamental AR system [6].The easiest solution is to superimpose a computer-generated picture over real-world video recorded by a camera and then display the resultant composite on a video projector computer or tablet computer.Portable video projection equipment has been developed in case it is not feasible to permanently place a video projector in the surgery room. In contrast to traditional visualization methods, using AR does not need the surgeon to take their eyes off the surgical site, which is the primary benefit of this technology [7].

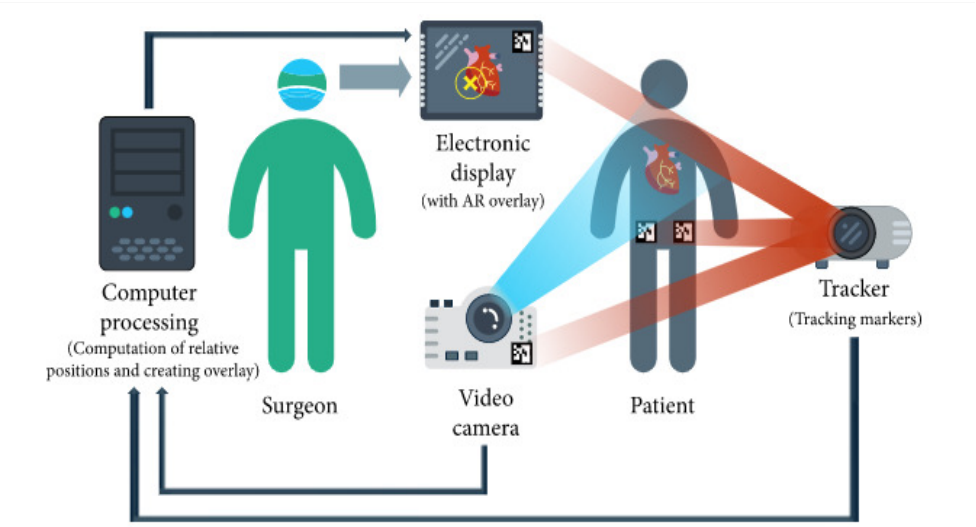

Figure 1: Illustrating the Fundamental Concepts of Augmented Reality.

The need that preoperative healthcare photographs to be reconstructed in three dimensions is now the primary factor that restricts the use of augmented reality.Using Digital Imaging and Communications in Medicine (DICOM) software, either pre-made by a third party or one that one has developed oneself, it is feasible to produce these reconstructions. The precision of the reconstructing technique and the precision of the data input both play a role in determining the quality of the obtained image. These reconstructions could be used for purposes like the digital investigation of target regions, the preparation in advance of an efficient surgical technique, and also the improvement of orientation or guidance within the surgical site [8].

The surgical team's personal choices and also the procedure's needs determine the kind and quantity of data that will be shown. Visualizing key features like main veins, nerves, and other essential tissues is made easier by augmented reality (AR). AR improves the quality of care and lowers operation time by reflecting these components onto the patient. In addition, AR allows you to change the transparency of the items [9].In addition, speech instructions may be made through voice recognition, allowing for the hands-free operation of the system. Surgeons may manage the gadget without help without breaking aseptic standards thanks to this feature.

Additionally, the team may interface with hardware employing action recognition, which can be applied to any surface or air, even when it's clean [10].

3.2.Using Augmented Reality to Improve Learning and Collaboration:

Surgery trainees, other medical personnel, and students benefitted greatly from the use of augmented reality in education and competence evaluation. Surgeons may enhance their abilities in a variety of situations by practicing on specialized training simulators,it might be used toassess their technical competence. This may be of particular use to residents as well as students who are still in training since it will allow them to build intuition and good decision-making skills without having to spend as much time in the clinical setting. With the help of a skilled surgeon, it may be used to simulate some of the most implausible circumstances. Unlike Virtual Reality (VR) simulators, which use computer-generated environments to simulate the whole experience, AR simulators use real-world items in combination with pictures to provide satisfying tactile input. In addition, basic surgical training is more enjoyable because of the use of AR [11].

3.3.Techniques for the Alignment of Images:

Aligning actual and Computer – generated (CG) visuals is crucial. Manually aligning photos is the easiest.Because such a method is time-consuming and potentially erroneous, the registration phase (preoperative picture alignment with the already treated patient) must be continuous to account for organ arrangement changes during breathing.Trackers locate the precise location of the camera as well as the patient's body to match the two photos. These trackers monitor feature points placed on static structures (namely, iliac crest, clavicles, etc.) to provide reference points.According to the literature, there are a variety of markers.To recognize them for navigation reasons, some writers used a set of visual indicators and a specialized camera. Trackers and markers are used to measure the distances between the cameras and also the patient. It was discovered that infrared markers had been used in many investigations [12].

There are several advantages to using these markers, but the most important one is their capacity to stay in the patients after surgery. Furthermore, an endoscopic system that can identify the emitted light is required.Laparoscopic camera monitoring or subsequent automated synchronization of collected images with 3D reconstructions are alternative methods of registration. With the use of a few webcams as well as freeware 3D reconstruction applications. Inoue and his colleagues demonstrated that all medical organizations had the option of purchasing a registration system utilizing basic and inexpensive technology [13].In the future, it may be able to follow organ location in real-time without the use of specific markers by applying multiple methods of examining the operating area. These methods rely on computer power to model and visualize organ movements as well as deformity in advance of surgery. Additionally, it is feasible to do registration without markers using an RGB (red-green-blue) and range camera.

3.4.Accuracy:

When it comes to supplying the surgical team with accurate data, the quality and complexity of the 3D reconstructed images are very essential. It is hard to do a direct comparison of the precision of individual studies because the circumstances are always changing and various methods are used to measure accuracy. According to many studies, the accuracy of optical

systems is within 5 mm [14],which would be rated acceptable for use in clinical settings.The level of accuracy that is necessary varies substantially across different operations and has to be evaluated on an individual basis. With an average precision of 1 mm, the Pulse Code Modulation (PCMA) approach of recording relative locations offers the highest level of accuracy compared to the other approaches that were discussed. Yoshino et al. [16] used operational microscopy in combination with a high-resolution MRI image to recreate a phantom model during an experiment.In addition, they used an optical monitoring program with a stated precision of 2.9 mm 1.9 mm. This has been observed that the precision does not rely on the level of experience that the surgeon has. Because it is impossible to give the projected picture a 3D look or to provide an accurate feeling of depth, the maximum feasible accuracy is further reduced. Displaying things with varying degrees of opacity or in colors that get progressively darker is one of the potential alternatives. Taking the use of motion parallax, which may be achieved by monitoring the surgeon's head and changing the projection appropriately, is one way that will further reduce the severity of this problem and bring it down to a more manageable level [15].

3.5.Applications in Medical Practice:

The preoperative planning phase of surgery, as well as the actual operation itself, are both good candidates for practical applications of augmented reality. Images that have been preoperatively recreated in three dimensions may be altered and made ready for presentation in AR systems. Common applications for augmented reality include customizing incisions or cutting planes to an individual's preferences, determining the best location for trocars, or gradually improving surgical safety by showing the locations of critical organ components [6]. Augmented reality (AR) technologies aimed at the consumer market are already at an advanced stage of development for a wide variety of possible application domains. As the number of articles concerning augmented reality (AR) in the medical fields, surgical intervention, as well as rehabilitation continues to rise, it is clear there is a significant need in the field of healthcare for products and services which can enhance the standard of care currently being provided. This special issue's goal is to provide technologists, computer scientists, or end-users with an assessment of the potential of AR technologies in promoting the creation of practical uses in the early future and also to drive academic studies toward addressing the technical and human-factor challenges that are still present among the most popular paradigms for augmenting the visual sense with computer-generated features.

Another advantage of AR is that it may assist surgeons working in challenging environments after neoadjuvant chemotherapy or radiation has been administered. AR could be used to both visualize and maximize the efficiency of the surgical resection volume. The use of AR-assisted surgeries is analogous to other ways of aid in many surgeries; nevertheless, the choice of the surgeons will determine whether or not such gadgets are used [16].Due to the natural restrictions placed on head and brain movements, augmented reality systems are effective when used in neurosurgical operations. It has been revealed that augmented reality played a significant role in 16.7 percent [17], of neurovascular procedures, making it possible for a greater rate of exact identification of tumors and a shorter amount of operating time as compared to a typical 2D method.When removing superficial tumors, performing epilepsy surgery, or performing neurovascular surgery, neurosurgeons gain greatly from the ability to plot the operation's path as well as pinpoint particular temporal lobes, blood arteries, or critical neurological pathways with extreme precision.

For orthopedic operations and reconstructions, augmented reality is effective since it enables surgeons to examine reconstructions immediately over the patient's body, thereby reducing visual distractions. Arthroscopic resections, bone resections, osteotomies, Kirschner wire installation, and joint replacement are some of the treatments that have been successfully performed with the use of AR.Despite this, the use of AR decreases the amount of radiation exposure that would be received during fluoroscopy, as well as the length of time that is needed to complete the work, while simultaneously lowering the danger of needless hemorrhage. During a non-invasive operation, Rodas and Padoy [18], employed augmented reality to generate a user-friendly representation of dispersed radiation. This enabled the researchers to monitor and display the quantity of radiation that was received. When it comes to the process of generating orthopedic implants, augmented reality has the potential to replace patient-specific models that are 3D printed. It is also conceivable to use augmented reality in orthopedic procedures that are performed with the assistance of robots.

3.6.Advanced Augmented Reality Image Fusion:

In addition to displaying computer-generated visuals, augmented reality may also show images that are not ordinarily viewable. Optical pictures and near-infrared fluorescence images have been effectively integrated into a few investigations. By giving a surgeon more information to work with, this method improves surgical accuracy.Surgeons could be able to discover blood veins below organ surfaces as well as other tissue abnormalities using this technology. Another intriguing idea was put out by Koreeda et al. [19], using augmented reality (AR) in laparoscopic surgeries, equipment seems to be completely obliterated from view. To further improve surgery precision while maintaining appropriate latencies of 62.4 milliseconds, this approach also assisted in successfully reducing needle exit point mistakes.

3.7.Augmented reality has its limitations:

The use of augmented reality in surgical research opens up many new possibilities and introduces novel elements. Surgeons may utilize extra information for decision-making and enhancing efficacy and safety.Augmented reality devices may now provide information with higher precision and reduced latency thanks to recent technological advances. Despite ongoing development, some challenges need to be solved. Currently, all reconstructed pictures have to be fully equipped using complicated techniques requiring expensive computers.Moreover, due to a predicted technological advance, real-time capturing of high-resolution medical scanners or image restoration may be possible.Time spent on surgery may be cut in half with the help of AR, but there are still several restrictions to be aware of. Concerning challenges to be addressed when employing 3D overlays include attentional blindness (that occurs when the surgeon fails to notice an unanticipated item that suddenly emerges in his field of vision) [20].

In certain cases, surgeons can reduce visual clutter by increasing the contrast between distinct elements, decreasing the degree of occlusion caused by augmented reality, and maximizing the geographical connection between the different components. If projection is immediately onto organs, it is also crucial to provide an adequate amount of visual contrast. It would still be challenging to form a reliable mental representation of a scene in three dimensions and to perceive its depths. Concerns also arise due to the system's latencies, which might reduce the surgeon's precision and ease of use.

4. CONCLUSION

Digital cameras and other optical sensors; accelerometers; global positioning systems; gyroscopes; solid-state directions; radio-frequency identification (RFID), and so on are all used in modern mobile augmented-reality systems. These technologies provide varying degrees of precision and accuracy, depending on their particular use.By providing access to real-time data and information about their patients in a quicker and so more precise manner than ever before, recent advances in augmented reality technology could assist in making it easier for physicians and surgeons to make a diagnosis, cure, and operate on their patients.The concept of augmented reality (AR) refers to the process of superimposing digital information whether visual, audio or otherwise onto the actual environment to improve one's perception of that world. Retailers and other sorts of enterprises may use augmented reality to sell their products or services, run creative marketing strategies, or collect data tailored to particular consumers. Research in medicine and technology, if continued, will eventually find solutions to the majority of the world's issues. It would seem that augmented reality is a strong instrument that is maybe capable of bringing about a revolution in the area of surgery if it were used logically. It is conceivable that augmented reality will act as an enhanced human-computer interface in the not-too-distant future. This would enable surgeons to obtain even greater outcomes via their collaborative efforts using AR. However, there is a significant need for more development to realize augmented reality's maximum capabilities or maximize its cost-effectiveness.

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CHAPTER 3

A PROSPECTIVE STUDY OF THE ASSOCIATION BETWEEN SUCCESSFUL IN VITRO FERTILIZATION (IVF) AND ASSOCIATED FACTORS

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ABSTRACT:

In-vitro fertilization, more often referred to as IVF, is a process that involves many different steps that are taken to improve a woman's chances of becoming pregnant, and reduce the risk of having a child with a genetic disorder, or both. The purpose of this research is to inform people who are considering undergoing Fertility treatments about the entire process, the present position of the procedure in India (including the success rate and the rate of live births), including all the applicable adverse effects of treatment on the mother. These adverse effects include the fertility drugs that areutilized in the therapy or their effects on the human body, and also ectopic pregnancies, ovarian hyperstimulation disease, cervical cancer, and also an increased risk of cardiovascular; these are all conditions that have been linked to chemotherapy. IVF therapy may have a detrimental influence on the kid, causing issues such as heart abnormalities, neurological disorders, birth defects, and so on. The author also concluded that as IVF has become more popular, it will change the way a large percentage of the human population reproduces. In several regions of the globe, as many as 10 percent of all children would be born via IVF soon.

KEYWORDS:

Hyperstimulation,Intracytoplasmic Sperm Injection (ICSI), In-Vitro Fertilization (IVF), Infertility.

1. INTRODUCTION

The World Health Organization (WHO) considers infertility to be a disease when a couple expresses a want to have children notwithstanding their inability to conceive [1].In-vitro fertilization (IVF) used to be known as "test-tube babies" due to the stigma associated with the term. Louise Brown, the first "test-tube baby," was born in England on July 25, 1978. She was created outside of her mother's womb using the easier procedure of artificial insemination, wherein sperm is inserted in the uterus. In-vitro fertilization (IVF) is the method that paved the way for this understanding; IVF involves fertilizing an egg or sperm outside of the body in a laboratory setting. When an embryo is fully grown, it is placed in the uterus alongside another embryo. In-vitro fertilization is also known as Assisted Reproductive Technology (ART), in the treatment of IVF may be difficult and costly, which is why only approximately 5 percent of infertile couples try to conceive with it. Despite this, in-vitro fertilization, often known as IVF, has been responsible for the birth of more than 200,000 children in the United States since it was first introduced there in 1981 [2].

Nearly twenty percent of couples throughout the globe and twenty-five percent of couples in developing countries are unable to have children due to infertility, which is after one year of having unprotected sex, infertility is characterized by an inability to conceive a child [3]. Infertility impacts not only the mental but also the physical, sexual, and social aspects of the life of infertile couples [4], highlighting the need of receiving the appropriate treatment.In vitro fertilization (IVF) is a procedure used to help women who are unable to conceive naturally because of problems with their fallopian tubes or difficulties with fertilization.In vitro fertilization (IVF) is the process by which an egg is fertilized by sperm outside of the body.

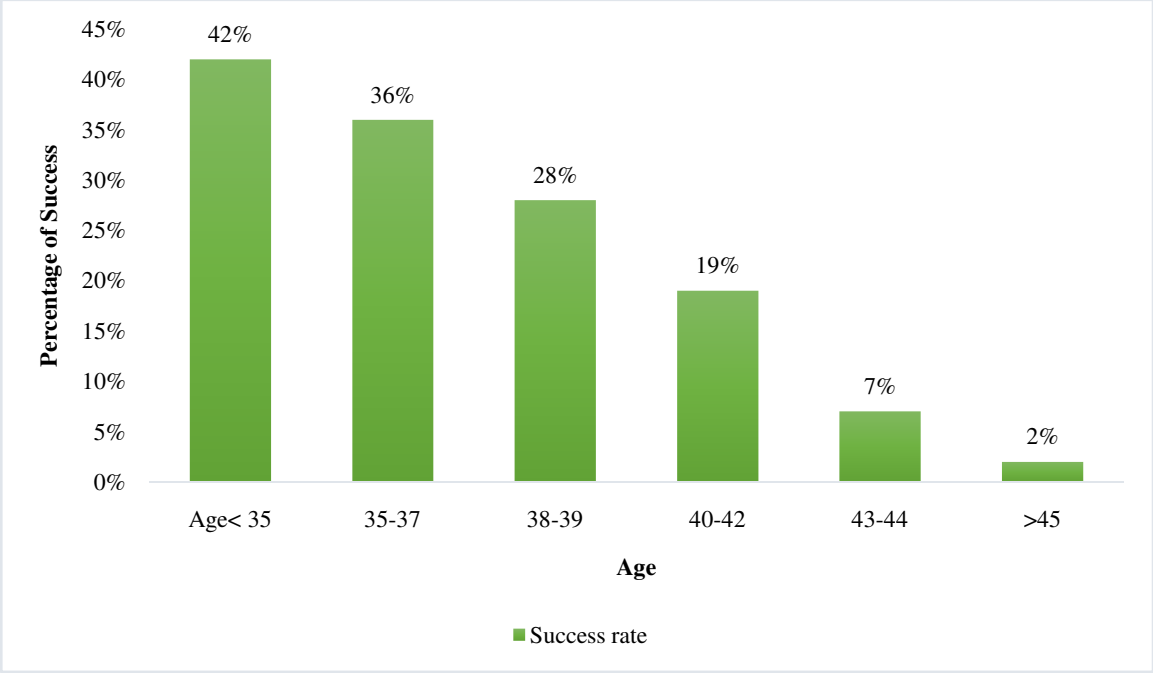

Figure 1:This Chart Shows the Age-Related Average In vitro fertilization success rates Procedures in India.

The rate of success of in vitro fertilization (IVF) in India varies from 30 to 35 percent based on the parameters that were given previously, as shown in Figure 1. The success rate of in vitro fertilization (IVF) procedures performed on young women is around forty percent on average in India. Younger women, namely those under the age of 35, have a greater likelihood of achieving their goals than older women. The number of live births that occur after an embryo transfer is often used to calculate the success rate of assisted reproductive technologies (ART), of which this is the most common kind. The number of live births that occur for every embryo transfer is the live birth rate.

2. LITERATURE REVIEW

MacKenna et al.stated in a study that did not identify any association betweencorrelation between BMI and IVF success, which is consistent with the findings of research carried out in Latin America. The body mass index did not affect the results of the artificial reproductive technology research, which was carried out on a group of women with a high incidence of obesity [5].

Von Wolff et al. discussed in a study that this analysis is supported by the literature on IVF treatment with deformities as well as functional abnormalities. Infertility concerns are highlighted. The danger of congenital abnormalities is about one-third higher in IVF-conceived children than in other children; the Odds Ratio (OR) for cardiac malformations is 1.29 (95 percent confidence interval, [1.03; 1.60]), and also the Relative Risk (RR) for musculoskeletal as well as genitourinary malformations is 1.35 and 1.58, respectively. IVF treatment should only be used in situations of infertility that could be addressed by any other methods since the origins of IVF dangers to children's health are unknown [6].

David F Albertini et al. stated in a study that two-thirds of couples might have a live birth, according to prognostic estimations. Reality is less thrilling. Age or ovarian responsiveness continues to influence success rates throughout cycles. Overtreatment and prescriptions of ineffective, if not dangerous, costly supplementary therapies ('add-ons') are developing concerns. We need a reasonable, evidence-based strategy. Several scientific mysteries need more study. The function of sperm screening, the adult ramifications of in vitro embryo development, and maintenance are of special interest.

3. DISCUSSION

In vitro fertilization (IVF) may be a treatment target in cases where a woman's fallopian tubes are absent or blocked, she has severe endometriosis, the man has low sperm counts, synthetic, as well as intrauterine insemination, kept failing, she has had fertility problems for years, and perhaps the pair needs to avoid any inherited disorders before conceiving. Unfortunately, the effort to undo a tubal ligation operation was not successful.Collection of ova, collection of sperm, observation or stimulating of healthy ovum/ova in the ovaries, fusing of fostered ova and desired sperm in the laboratory by establishing an adequate environment for ovulation and implantation, as well as finally transplantation of embryos into the uterus, that are sequentially given in Figure 2.

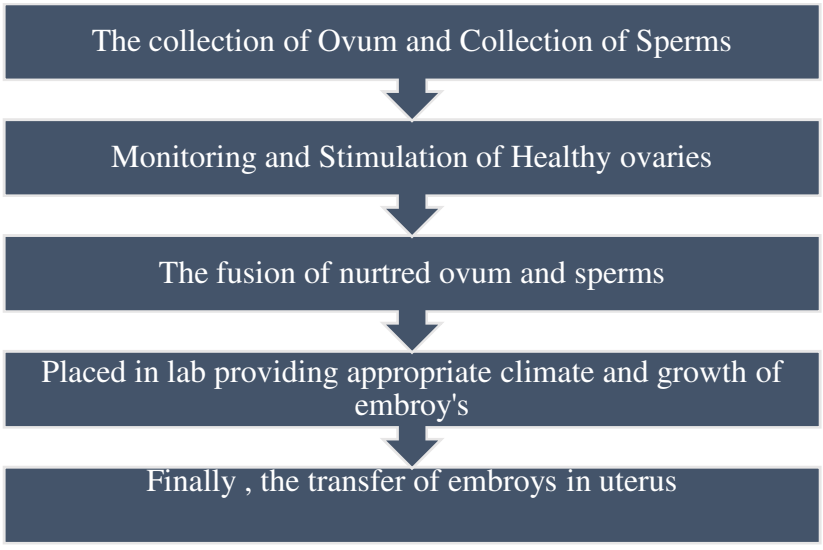

Figure2: Displays the Five Fundamental Phasesduring the In Vitro Fertilization and Embryo Transfer.

- *Step 1:*

Fertility medicines are recommended to manage the maturity ofthe eggs, and also to increase the likelihood of harvesting several ovaries during menstruation, a process known as ovulation induction. Both of these processes are intended to raise the likelihood of a couple becoming pregnant. It is desirable to have several ova since some of the ova will not grow or fertilize after they have been retrieved, and the development of the ovum may be followed by the use of ultrasonography, in addition to the examination of urination and complete blood samples to assess hormone levels [7].

- *Step 2:*

Follicular aspiration was performed by removing ova from the ovaries using a minimally invasive surgical process called ultrasound guidance of a cylindricalthe eggs are withdrawn from the ovaries by inserting a needle into the pelvic cavity. This operation is known as follicular aspiration [8].

- *Step 3:*

The preparation of sperm, which is often acquired by ejaculation, is necessary to achieve fusing with the eggs.

- *Step 4:*

In vitro fertilization occurs in specialized facilities where sperm and ova are incubatedIntracytoplasmic sperm injection (ICSI) might be used if it was judged that fertilization was less likely to occur.Fertilization and cell proliferation of the ova are monitored, and the resulting structures are referred to as embryos if fertilization has been accomplished [9].

- *Step 5:*

As a general rule, embryo transfer happens within 2 to 3 days after egg retrieval, however, in certain situations, it might take up to six days. A two- to four-cell embryo has formed at this time, it's a fertilized egg. During the transfer procedure, the cervix is uncovered by inserting a speculum into the uterus. A catheter is inserted into the womb and a preset quantity of embryos are gently floated in the fluid. A series of blood tests and perhaps ultrasounds are performed to evaluate whether or not implantation has been successful and whether or not a pregnancy may be expected at the end of this process under close supervision [10].

3.1.In-vitro fertilization (IVF) and Gonadotropin-Releasing Hormone(GnRH):

Several factors may be used to determine the success of ovarian hyperstimulation, such as aging, Anti Mullerian hormone production, and Antral Follicle Count. The procedure and dose for ovarian hyperstimulation are determined by this prognosis of poor or excessive reaction to ovary hyperstimulation. Suppressing naturally occurring ovulation with the use of the gonadotropin-releasing hormone antagonist regimen is another kind of ovarian hyperstimulation that may be used to validate personal preference based on when menstruation first began spontaneously in the previous cycle [11].

3.1.1. IVF cycles driven by gonadotropin-releasing hormone (GnRH) are both natural and moderate (IVF):

There are two approaches to IVF that include Gonadotropin-Releasing Hormone (GnRH) as a mediator in normal and mild cycles. A naturalistic IVF cycle is one in which no drugs are used to stimulate the ovaries. However, the Gonadotropin-Releasing Hormone (GnRH) antagonist approach might be utilized in tandem with anti-ovulation drugs. In this way, they know that the cycle would be initiated using natural phenomena.A mild IVF cycle involves the use of a low dosage of ovarian stimulating medicines for a brief time during the woman's monthly cycle. The goal of this cycle is to create between two and seven eggs that can then develop into viable embryos. This extremely developed technology looks to preventsevere side effects for women, although it might save money compared to traditional IVF. Additionally, there is a dramatically reduced chance of multiple implantations or ovarian hyperstimulation syndrome with this procedure[12].

3.1.2. In-Vitro Fertilization (IVF) Preparation Of Ova And Sperm:

The detected eggs are cleaned of any remnants of the external environment and made ready for fertilization in the laboratories. Before fertilization, an oocyte selection could be done to choose the eggs that have the best possible probability of developing into a healthy baby. In the meanwhile, a procedure referred to as "sperm washing" is carried out, the results in the sperm for fertilization by removing inactive cells from the seminal fluid. This is done to prepare the sperm for use. If sperm is being provided by a sperm donor, the semen would normally have been processed before being frozen, separated, and then warmed to make it acceptable for use. If the sperm is being supplied by a sperm donor, the semen also will have been donated. This is done to ensure that it is in a usable condition.

3.1.3. Process OfSperm and eggs:

To ensure fertilization, sperm and egg are placed together in a culture medium in about a 75,000:1 ratio. Two pronuclei are seen in most instances when eggs are fertilized during co-incubation. Intracytoplasmic sperm injection (ICSI) could be used in specific conditions, including when the sperm count or motility is poor (ICSI). Ovum is put into a particular growing medium and left undisturbed for 48 hours till 6 to 8 cells are formed [2].

3.1.4. Growth and Screening of Embryos for IVF:

After the ovum has been removed, the embryos are cultivated when they are between 6 and 8 cells in size. It was discussed in many IVF programs and meetings in Canada, the United States, andThe Australian government has mandated that embryos be placed in an extended culture system and transmitted at the blastocyst stage, around 5 days following harvest. This is particularly important if a large number of high-quality embryos still are accessible on day three [13]. Based on cell count, growth evenness, and fragmentation, embryos are often rejected by embryologists. Several factors, including the woman's age and the results of several medical tests, decide how many embryos may be transplanted. Using a thin plastic catheter put into her vagina and cervix, the "best" embryos are transferred to her uterus. Next, they're surgically placed within the patient. Multiple embryos may be put into the uterus to improve the chances of implantation and subsequent pregnancy [14].

3.1.5. In Vitro Fertilization (IVF) Medicine As An Aid:

To boost the chances of success by improving and enhancing the corpus luteum's activity, which is essential for implantation and early embryogenesis, progesterone, progestin, or gonadotropin-

releasing hormone (GnRH) agonists are administered. IVF cycles even without Intracytoplasmic sperm injections (ICSI) are considerably more likely to result in live births when progesterone is used for luteal support.With a confidence interval of 95 percent, combination effects with GnRH (GnRH), antagonists significantly enhance the live birthrate by 16 percent. On either side, growth hormone and aspirin as supplementary treatment in IVF did not show reasonably concrete proof of their overall effectiveness[15].

3.2.The Following Factor Is Something That Has To Be Taken Into Account:

- The patient must be knowledgeable about both the medications being used and the IVF process itself. Prescriptions for fertility medicine or hormones increase follicle formation, therefore patients undergoing IVF treatment should be informed about these medications or hormones, as well as the negative effects that may be linked with their use.
- The patient should be educated on the process of fertilization either spontaneously or via the use of ICSI, with the resulting consequences.
- An IVF patient must be informed of the risks, like ovarian hyperstimulation, nausea, and sometimes bleeding as an adverse reaction to anesthesia.
- IVF success rates must be communicated to patients.

3.3.The Effects of the Drugs Used in In Vitro Fertilization:

Drugs are often used in IVF procedures to help the body get ready for treatment and to increase the likelihood that more healthy eggs will be produced from the ovaries. However, these medications may have major adverse impacts on women as well as the child.

- A Rising Prevalence of Births to Numerous Infants.
- An alarmingly high rate of spontaneous abortions.
- Symptoms such as severe headaches, nausea, and burning flashes may also be present.
- Overstimulation of the ovaries may cause symptoms such as depression and irritability as well as ovarian cysts or pelvic pain.
- Overstimulation of the ovaries may cause symptoms such as depression and irritability as well as ovarian cysts or pelvic pain.

3.4.Multiple pregnancies as a potential side effect of IVF treatment:

When compared to singleton births, multiple pregnancies provide a higher risk of complications arising from in vitro fertilization (IVF), and these complications affect the mother as well as the unborn child. Due to several pregnancies. Medical issues, including pre-eclampsia (increasing high blood pressure) as well as antepartum hemorrhage, are more likely to occur as a result of the pregnancy (bleeding before the onset of labor). There is also an increased chance of early delivery or developmental delay in newborns, particularly those who are expecting twins [16].

3.4.1. Multiple pregnancies provide several maternal concerns:

Having a miscarriage, having a hemorrhage, pregnancy-related hypertension, diabetes, anemia, polyhydramnios (excessive amniotic fluid around the fetus), and anemia all seem to be possible

health issues for both mother and child. A longer stay in the hospital results in greater overall costs for medical treatment.

3.4.2. Ectopic Pregnancy (often referred to as an iatrogenic pregnancy):

Both Intrauterine Insemination (IUI) and in vitro fertilization (IVF)have a greater chance of developing ectopic pregnancy. An ectopic pregnancy is one in which the significant embryo attaches itself someplace other than in the uterus of the mother. Both the mother's and the unborn child's lives can be in danger if the embryo develops outside of the uterus of the pregnant woman. Even though an ectopic pregnancy could take place whether it's the ovary, the cervix (the womb's neck), or the abdominal cavity, 95 percent of ectopic pregnancies consequence from the embryo remaining in the fallopian tube, according to data from the National Health Service. This is the case and although ectopic pregnancies could occur in any of these locations. Because of this, the damaged organ will burst, which will result in significant bleeding. Because it is very unlikely that an embryo would survive an ectopic pregnancy, the extraction of the embryo is an essential part of any therapy for the condition [17].

The practice of transplanting many embryos at once is the root cause of the most significant danger associated with in vitro fertilization (IVF), which would be the likelihood of having more than one baby at a time. There may be a correlation between having more than one child and having your pregnancy end in a miscarriage, labor and delivery problems, premature delivery, as well as neonatal morbidity, all of which can trigger harm in the long run [18]-[19].Human chorionic gonadotropin (HCG) has been demonstrated to induce enlarged, painful ovaries in 30% of women; this condition is called ovarian hyperstimulation syndrome (OHSS). Some birth problems are likely more common in children created via in vitro fertilization, according to a review of data from the National Birth Defects Study in the United States in 2008. (IVF). Septal heart defects, cleft lips without cleft palate, esophageal atresia, and anorectal atresia were all included in this group of abnormalities. The method of causality behind these congenital malformations still seems to be unknown without any conclusive evidence [20].

3.5.The Impact Of Biomarkers On In fertilization Done In Vitro:

IVF pregnancy rates are much lower and progesterone levels are higher in women whose FMR1 genotypes are ovary-specific. Biomarkers that impact pregnancy possibility in IVF include a higher level of Antral Follicle Number, Anti-Mullerian hormone levels, trying to measure good sperm quality of sperm donor through using DNA fragmentation methods such as the semen quality Comet assay, and advanced maternal age, that also boost the successful and effective pregnancy rates[21].

3.6.IVF's Drawbacks:

IVF therapy is subject to the same restrictions as natural fertility. Infertility treatments like IVF can help older women have a greater chance of achieving pregnancy,however, this is not enough to counteract the reproductive drop that generally hits women in their 30s and 40s.Donor eggs may be necessary if a woman has exhausted her supply of eggs if they are of low quality. A woman's response to the medications is also a factor in IVF success. One egg would be produced normally when she does not react, which significantly reduces the chances of success. If a woman has had a history of miscarriages, IVF is unlikely to be beneficial.

3.7.Option to In-vitro Fertilization:

- *Gamete Intrafallopian Transfer (GIFT):*

Gamete Intrafallopian Transfer (GIFT) is an embryo transfer method that is similar to in vitro fertilization except in which the egg is fertilized within the fallopian tubes. Because of the more "natural" conception process it provides, many infertile couples choose it rather than traditional IVF.

- *Zygote Intrafallopian Transfer (ZIFT):*

In this method of assisted reproduction, the fertilized eggs are not deposited in the uterus but rather in the fallopian tubes. For women to have success with this option of IVF, their fallopian tubes need to be in good condition.

- *Tubal Embryo Transfer (TET):*

According to the ZIFT method, this alternative to IVF involves the transfer of an early-stage embryo back to the mother.

- *Surrogacy*:

Traditional in vitro fertilization (IVF) therapy has an indirect medical option known as surrogacy. There are two different types of surrogacies. In the first kind of surrogate, known asIn conventional surrogacy, a woman agrees to be inseminated with donor sperm and then carries the pregnancy to term on behalf of a person. The relationship between the infant and the surrogate mother is one of biological motherhood. The patient or their partner's sperm and egg are implanted into the surrogate during the gestational surrogacy process. The surrogate then carries the pregnancy to term on behalf of the intended parents. The kid is the patient's and their partner's biological offspring, hence the surrogate mother has no biological connection to the child. These procedures may be explored with competent members of the medical staff.

4. CONCLUSION

In vitro fertilization, often known as IVF, refers to a process that involves several different steps that are carried out to improve a woman's fertility, help avoid genetic disorders, and improve the chances of conceiving a child.Even though in vitro fertilization (IVF) therapy is often carried out by highly trained specialists, there is still the possibility of encountering problems.Although in vitro fertilization (IVF) treatment is not without its risks, it does have a certain level of success, and it is sometimes the only option for infertile couples. The methods of Intra cytoplasmic sperm injection (ICSI) and in vitro fertilization (IVF) have reached a degree of generalization where they are generally recognized and done. Despite this, the status of genetic hazards is still not completely understood. Therefore, the industry needs to make it a priority to need validation of the safety and effectiveness of new technologies before enabling IVF patients to have regular access to these treatments. Because reproductive medicine, and in particular IVF, is quickly revolutionizing human reproduction, it will almost certainly continue to be of essential relevance to both the scientific community and society at large.Advancing development has expanded the procedure's success rate or reduced multiple births. Doctors intentionally restrict the number of embryos they implanted in infertile women, reducing triplets, quads, and quants. Before allowing IVF patients regular access to technologies, the industry must validate their safety and efficacy. IVF is transforming human reproduction, therefore reproductive medicine will continue to be important to the scientific communities and environment.

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CHAPTER 4

AN ANALYSIS ON RELATIVE SUSCEPTIBILITY OF BLACK ROT DISEASE CAUSED BY *XANTHOMONAS CAMPESTRIS*

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ABSTRACT:

Significant production losses are brought on by the bacterial black rot disease, which damages Brassica oleracea L. crops worldwide. In temperate locations, plants with chlorotic and necrotic lesions are often destroyed since they may not be marketable, which results in the majority of yield losses. The most prevalent disease of vegetable brassica crops worldwide is black rot, which is caused by the gram-negative bacterium Xanthomonas campestris pv. campestris (Pammel) Dowson. The genome of Xcc has undergone substantial research, and the whole genome sequence is currently available at NCBI. This disease, mostly affects the above-ground parts of the host plant at any stage of growth, primarily affects plants in tropical and subtropical regions. As they are susceptible towards this pathogen. This research includes several methods to identify the diseases causing pathogen. The disease may survive in infected plant waste and cruciferous weed hosts as well as transmit from plant to plant through its seeds. The disease may be controlled using traditional procedures. Hosts having resistant towards infection, seed treatments with hot water and antibiotics, fungicides, and biological and chemical management strategies are used to overcome the chances for infection.

KEYWORDS:

Black rot, Brassica oleracea, Bacterial Infections, Image Segmentation, Xanthomonas campestris (Xcc).

1. INTRODUCTION

Plant disease diagnosis is both, an art and a science. A doctor's ability to diagnose (i.e. identify signs and symptoms) relies on both intuition and scientific methodology. Over two-thirds of India's population lives off the land, making it a cultivated nation.The quality and quantity of agricultural products are significantly reduced when plants are infected with the disease. Plant disease research focuses on patterns that may be seen visually in plants. Disease surveillance on plants is critical to the farm's ability to successfully cultivate its crops. In the beginning, monitoring and analysis of plant diseases were performed manually by a specialist in that sector. This requires a great deal of effort and takes a long time to complete. Plant disease detection may benefit from image processing methods [1].

To diagnose plant leaf disease, it is necessary to look for many spots on the leaf. Each patch on the plant's leaves differs in form and color, with a fuzzy feel. In addition, plant leaf disease has an impact on the whole plant as a whole [2].In particular, a wide variety of strategies image processing techniques, and computational intelligence have been presented as potential methods

for detecting plant leaf disease. The potentially fatal leaf disease that may be discovered on pine trees needs the illness to be identified at an early stage, which would in turn requires the disease's leaf to be continuously monitored. Because manually identifying plant leaf diseases is a hard procedure that takes a large amount of time, researchers favored using automated disease detection because it is the first step in the process. The common disorders manifest themselves as patches that might be either yellow or brown. The remaining illnesses are caused by viruses, fungal infections, and bacterial infections [3].

1.1.Symptoms Of Leaf Diseases:

The most common pathogens that attack leaves are bacterial, fungal, and viral. An observable consequence of plant disease is the plant is referred to as a symptom of that disease. The plant's reaction to the pathogen might develop as observable changes in its color, shape, and function. These changes may be referred to as symptoms. In this section, the author would go through the signs of this illness that should be remembered if there is a noticeable slowdown in plant growth.

1.1.1. Symptoms of Plant Viral Disease:

The detection of plant leaf illnesses caused by viruses is the most challenging of all the diseases that might affect plant leaves. Viruses don't leave any obvious indicators that are easily observable, thus their symptoms are often confounded with those of nutritional deficits or pesticide harm.Examine the leaves of the plant for any splotches of color, either yellow or green, as seen in Figure 1.Common insect vectors include aphids, leafhoppers, whiteflies, and cucumber beetles, which are caused by the Mosaic virus. The leaves may get wrinkled and curled, and the plant's development might be hindered.

Figure 1: Displays the Mosaic Virus Symptoms of Plant Disease [4]**.**

1.1.2. Symptoms of Bacterial Plant Disease:

Figure 2: Displays the Pepper plants that have been infected with bacterial leaf spots have leaves that have a tattered look [5]**.**

The presence of pathogenic bacteria is the root cause of many major illnesses that affect plants. They must first enter the plant via a wound or one of its naturally occurring apertures since they are unable to enter plant tissue directly. Through activities like trimming or plucking, users incur the risk of sustaining wounds as a consequence of being harmed by insects, various infections, and equipment. The condition is distinguished by the appearance of teeny, hardly visible patches that have a light green color and quickly become waterlogged. As seen in Figure 2, the lesions continue to grow and eventually take the form of a dry, dead patch. These patches may dry out over time, especially in less humid weather, which then causes the diseased tissues to fall off, giving the afflicted leaves a torn look as a consequence of the tissue injury [6].

1.1.3. Symptoms of Fungal Plant Disease:

Figure 3 below describes some of the fungal diseases that may affect plants. The fungus that causes the late blight is responsible for this. In the beginning, it looks like gray-green specks that have been drenched in water. These patches deepen as the fungal condition progresses, and white fungal growth appears on the undersides.

Figure 3: Displays the Fungal symptoms in Potatoes that have been infected with late blight and will have dark brown or black spots on their leaves and stems [7]**.**

Black rot is caused by the fungus "*Diplodia seriata*" (*Botryosphaeria obtusa*). Trunks, branches, leaves, and fruits are all fair game for the fungus. Minnesota's cold winters are no match for the black rot fungus, which may hibernate in branch cankers or mummified fruit. Research on apple postharvest decay and the relative vulnerability of several apple cultivars to decay due to "*Botryosphaeria obtusa*" were carried out, and this study covers both of those discussions. The detection of plant diseases is also a topic that is covered in this study as to how farmers can overcome apple black rot disease.

The bacterium *Xanthamonas campestris PV campestris* is responsible for the disease known as black rot. Although this bacterium is capable of infecting any member of the Brassica family, it most often causes problems in brassica vegetables including broccoli, cabbage, cauliflower, and kale. Infected seeds are a common vector for the spread of black rot in agricultural settings. After it has established itself, black rot may live on in residues. Bacteria may penetrate plants through

tiny holes at the leaf edges known as hydathodes, which are also the locations where morning dew condenses. It is also possible for the bacteria to infiltrate the plant through contaminated seeds, wounds caused by hail damage or other forms of mechanical harm, or insect feeding. According to the findings of certain investigations, the bacteria might be spread from plant to plant by flea beetles as well as other insects. The spread of black rot is facilitated by water. When temperatures are over 77 degrees Fahrenheit and the humidity level is high, black rot is most capable of spreading.

To control black rot, inoculum resources must first be removed, and then fungicide treatments must be used. The relative vulnerability of various cultivars is unclear, and there has not been enough research done onapple rot disease management that makes use of host resistance to pathogens or resistance applications. Field assessments may be challenging to do to identify the relative sensitivity of apple fruit to rot infections since the indications induced by a variety of diseases tend to be quite identical to one another.Lesions on the leaves are the first and most easily identifiable sign of an infection caused by black rot. The lesions have the appearance of being spherical and brown, with a darker rim around them. There are little black spheres, known as pycnidia, contained inside these lesions. These pycnidia serve as carriers for spores that may continue to infect the crop for the current year.

2. LITERATURE REVIEW

Seung-Yeol Lee *et al.* stated in their study that round, brown patches were distinct from other apple illnesses. A morphologically identicalfungal pathogen was grown in a petri dish using potato dextrose agar. Both injured and unwounded Hongro and Fuji apple cultivars had brown patches in a pathogenicity test. *F. decemcellulare* KNU-GC01 had a high degree of similarity (98.3% and 97.6%, respectively) with F. decemcellulare NRRL 13412 in terms of RPB1 and RPB2 sequences, and the two strains clustered together in the phylogenetic tree, indicating that they are closely related at the species level. Brown spots on apples in Korea are caused by *F. decemcellulare*[8].

Khirade *et al.* conducted a study that addressed segmented or extraction and classification algorithms for plant disease diagnosis employing leaf images. Image capture, Preprocessing, Segmentation, Feature extraction, or Final disease classification are the five phases in plant leaf disease detection. Image acquisition using Red, Green, and Blue (RGB) leaf transformation. Preprocessing removes noise and boosts picture contrast. This divided picture is utilized for the extraction of features and then the final classification. Authors study will help to identify plant illnesses [9].

S.S. Miller *et al.* stated in their study that Morning, midday, and all-day shade affect orchard output. From 2002 to 2005, 'Ginger Gold' apple trees were shaded in a field near Kearneys Ville, WV. Morning shadow (MS) consistently inhibited trunk and branch development, whereas full shade (FS) or Afternoon Shade (AS) showed intermediary impacts.Full shade reduced to yield the most, followed by MS and AS. AS leaves had higher soluble carbohydrate concentrations, especially sorbitol.This suggests that MS may have impeded photosynthesis throughout a time of day when net assimilation is typically high. Orchard output may be improved by planting or managing apple trees to decrease morning shadow [10].

Margarita Beer *et al.* discuss in their study that aborted apples may be seen on the trees of certain famous apple types. Organically managed Northern German apple orchards provided inoculated

for laboratory tests that led to black rot caused by *Diplodia seriata Diplodia seriata* or smokey blotch produced by *Peltaster ceophilus* on mature apples. Overwintering fruit mummies released inoculum. Both diseases were far less common once fruit mummies were removed by hand over the winter and by the end of June each year in a field study done over 4 years in that other organic orchard. *Neofabraea Alba* and N. Perennan's storage rot growth were unaffected by the removal of fruit mummies. Organic apple production provides a backdrop for discussing the possibilities, limits, as well as costs of this phytosanitary measure [11].

L. Brockamp and R.W.S. Weber stated in their study that removing fruit mummies manually and applying crop protection chemicals (Myco-Sin and lime sulfur) reduced the risk of black rot (*Diplodia seriata*) on an organic fruit field in the Lower Elbe area (Northern Germany) during three years (2011–2013). Pre-harvest fruit infections caused by *D. seriata* may be considerably reduced if fruit mummies are removed, either alone or in conjunction with MycoSin and lime sulfur treatments. During the surveillance of primary infections, a connection was found between spore potential as well as rainfall volume and intensity as well as high temperatures. Spraying MycoSin was shown to be the most effective method of controlling the storage rots that were produced by *Neofabraea spp*[12].

3. DISCUSSION

The disease known as black rot of cole crops is Campestris (Pammel) Dowson is an extremely damaging infection caused by the pathogen *Xanthomonas campestris PV* that, in the presence of suitable climatic conditions, significantly reduces crop output globally by more than fifty percent. The disease is known as black rot by the presence of pathogenic bacteria that infect the xylem and populate the mesophyll of a plant. *X. campestris PV*. Campestris may live for a considerable amount of time in seedlings, soil, or most importantly in thePlant detritus and cruciferous weeds found across the field. It is possible that different sources of inoculum are to blame for the yearly reappearance of the illness in both the nursery and the main field. Utilizing seeds that have not been exposed to any pathogens is one of the most effective methods for avoiding black rot.

3.1.Techniques of Detection of Plant Disease:

In its most basic form, the process of plant disease detection may be broken down into the four stages seen in Figure 4.The first step was to collect images, which can be accomplished using a digital camera, a mobile phone, or by obtaining them off the internet. In the second stage, the image is separated into some clusters, each of which could be treated using a different way. The subsequent part will include techniques for the extraction of features, and the last step will focus on the categorization of illnesses [13].

- *Image Acquisition:*

The process of acquiring images is the first step in the operation of any vision system. Acquiring a picture requires taking the necessary measures to collect a plant leaf and then using a camera to acquire high-quality photographs of the plant. Images are collected from many locations, including the internet and the agricultural field. The quality of the database pictures will determine how well the notion is implemented. This picture has been formatted using RGB, which stands for red, green, and blue.

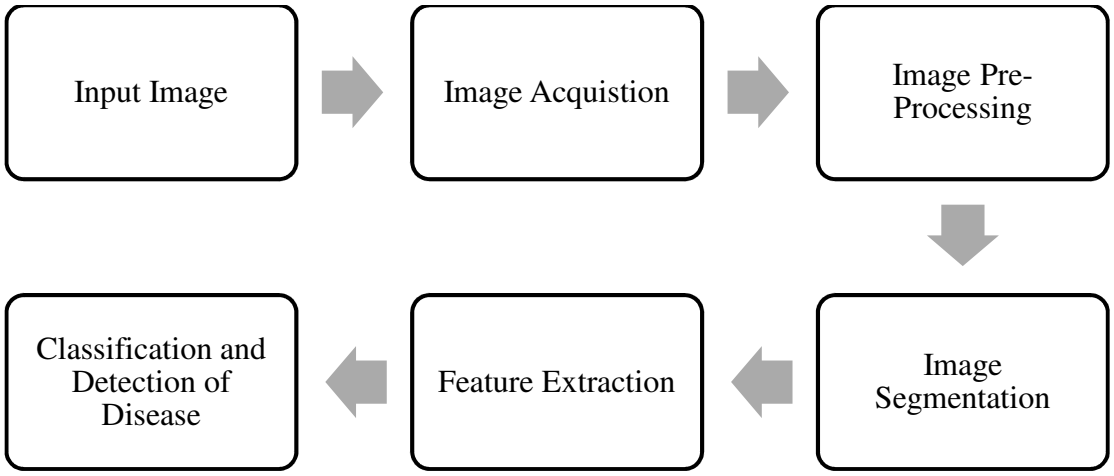

Figure 4: Displays the phased approach to the diagnosis of plant diseases.

- *Image Pre-Processing:*

Image enhancement, converting from RGB to Lab, filtering, and other operations are all part of the pre-processing procedures for an image. In this instance, picture enhancement is performed to boost the contrast. The filtering procedures are used to smooth up the image. When it comes to image processing, you may choose from a wide variety of filtering methods, such as the median filter, the average filter, the Gaussian filter, and many more.

- *Image Segmentation:*

The term "*Image Segmentation*" refers to the process of separating a picture into many different pieces that share similar characteristics or aspects.Several strategies, including otsu's method and k-means clustering, are used to convert RGB pictures into HIS models, and many more, may be used in the segmentation process. The K-means clustering method is put to use when classifying an item into K or more classes based on a collection of characteristics that the object has. The process of object categorization involves reducing, as much as possible, the totalproduct of the item's distance squared from the cluster's center.

- *Feature Extraction:*

The process of identifying an item relies heavily on the extraction of its many features. Following the completion of the image segmentation process, the component of the image about the illness is then extracted. The extraction of features is used in a wide variety of image-processing applications. The characteristics of a plant's color, texture, form, edges, and morphology may all be used in the process of disease diagnosis in plants. Color information is extracted using a variety of methods, including color histograms, color moments, and color structure characteristics. One method for extracting features from textures is the Grey Level Co-occurrence Matrix (GLCM) technique [14].

- *Classification and Identification of Diseases:*

Finally, classifiers are used when putting the datasets through their paces in training and testing. Support vector machines (SVMs), k-nearest neighbor analyses, neural networks, fuzzy logic, and other methods may all be used to create these classifiers. This methodology is used in the

classification and diagnosis of leaf diseases. In general, it is considered that seeds contaminated with bacteria that producethe disease-causing agent that causes black rot are a major factor in the global distribution of the disease. The first signs of a black rot epidemic may be as few as three seeds out of 10,000 total seeds (a rate of 0.03 percent infected seeds). According to testing and certification, there should be no more than one infected seed in every 30,000 seeds in a seed batch.Black rot symptoms may vary widely not just about the host but also cultivar, plant age, and environmental factors. The bacteria can invade plants via natural apertures as well as wounds that were induced by mechanical harm to the leaves and roots.Using the aforementioned cotyledon edge perforations, germs that have traveled on the seed may infect the growing seedlings, and they subsequently systemically spread throughout the seedling. Seedlings that have been infected but have been raised in a greenhouse at temperatures below 15–18 degrees Celsius rarely display any indications of the illness [15].

3.2.The life cycle of Black rot Disease:

Xcc-contaminated seed isthe primary carrier of the disease. The seedling gets infected via the epicotyl while it is germinating, and the cotyledons may acquire blackened borders, wilt, and fall off.The bacteria enter the plant's vascular system and migrate to the young stems and leaves where they cause the disease to manifest as V-shaped chlorotic to necrotic lesions that spread from the leaf margins. When environmental circumstances are humid, bacteria that are present in guttation droplets have the potential to be transported to other plants by the breeze, rainfall, water splashes, or mechanical components (Figure 5) [16].

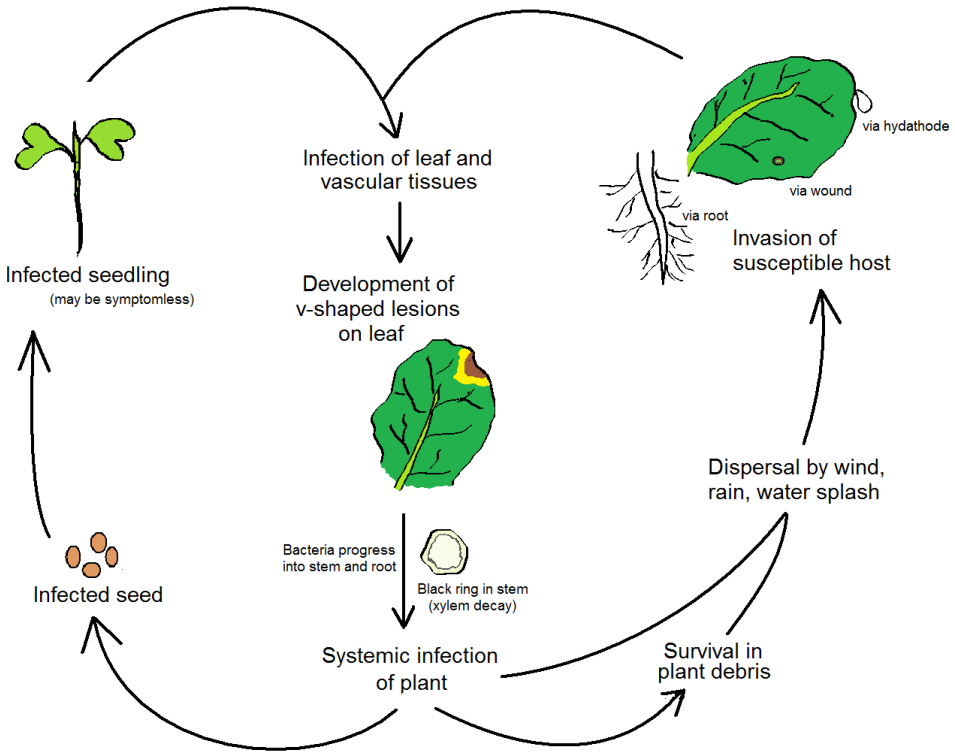

Figure 5: Displays the Black Rot Pathogen's Life Cycle Anthracnose of the Campestris Variety (Xcc) [17]**.**

Hydathodes are the primary access point for Xcc, however, leaf holes produced by insects or plant roots are possible entry points as well. Occasionally, stomata may get infected. It is via the use of hydathodes that the pathogen may get direct access to the plant's vascular system and spread throughout the plant. A sutures vein invasion causes the formation of Xcc contaminated seed. It can survive in the debris of the soil for a maximum of two years, but it cannot do so for more than 6 weeks in soil that has not been affected. Plant waste contains bacteria that may be used as a supplementary inoculum source [18].Some cruciferous weeds may be able to persist year-round and furnish crops with potential inoculum.

The epiphytic survivability of the bacterium on the phylloclade is species-dependent, as shown by Arias et al., who showed that the bacteria could survive for up to 48 days on cabbage, mustard, or lettuce but only for up to 9 days on rice. It has been demonstrated that diseased crops may, in certain instances, serve as a source of inoculum for weeds [19].Wind, insects, aerosols, irrigation water, rain, agricultural machinery, and personnel may all spread the bacterium over short distances. Transplants are used to grow commercial brassica vegetable crops. For plants grown in modules, the use of an overhead watering system enhances the transmission of germs, which may lead to illness in the field. Changing the irrigation technique consequently reduces disease transmission [20].

In most cases, Hydathodes are a possible entry point for the bacterium located on the edges of the leaves.This happens when bacteria-infested droplets from the preventative measures are reabsorbed by the leaf. Different environmental, ecological, and mechanical elements all play a role in determining the entry point. However, the bacteria's ability to exploit the stomata to enter the plant and cause localized darkening is not a certainty, stomata do not seem to play a significant role in Xcc infection. This is because the illness does not typically extend into neighboring tissues. This shows that the migration of germs through the circulatory system is a crucial component in the development of illness. Similarly, machinery, insects, animals, rain, irrigation, or wind may all cause wounds in plants that the bacteria can use to enter the plant.

3.3.Disease Management of Black rot:

The first step in managing black rot is to locate any probable sources of the illness.Furthermore, put an Integrated Pest Management (IPM) strategy into action. This strategy should involve host resistance, the planting of disease-free seeds, the prevention of the disease's further spread, and practicing adequate sanitation. Sanitation is the primary strategy that may be used to lessen, exclude, or get rid of the original causes of illness. Crop rotation, disinfecting seeds, removing unhealthy plants, eradicating garbage mounds, and removing alternate hosts are all examples of general sanitation activities.

- *Treatment of Seeds:*

Inoculum that is carried on seeds is a substantial contributor to the dissemination of bacteria that cause black rot. Only tested and certified seeds, with less than one sick seed in 30,000, or 0.003 percent contamination, should be planted by growers. When it is not possible to determine the contamination level of the seed and when there is a lack of disease-free seed accessible, the seed must be sterilized to kill the bacteria. Farmers who buy transplants must insist on seeing evidence that the seedlings came from disease-free or treated parent stock before making their purchase. Diseased seedlings must not be placed in the fields during the transplantation process [21].

Even following treatments, some bacteria may persist on or inside the seed, potentially hindering its capacity to germinate and flourish. Some research suggests that soaking seeds in hot water at 50 °C for 25-30 minutes is the most effective way to prevent seed-borne black rot. Cauliflower, kohlrabi, kale, rutabaga, and summer turnip seed, among others, may be damaged by being soaked in boiling water for too long. Only soaking for 15 minutes at 50°C in these cases.There has not been any research done to determine what impact the effects hot water seed treatments for crucifer crops have on the wide range of available kinds. Before treating the whole seed lot, growers should first test out the treatment on a small amount of the seed stock by planting it in containers and seeing how it affects the germination rate and the overall vigor of the plant.

- *Avoid the Spread of Disease:*

Black rot bacteria may live on the surface of materials including clothing, tools, and even drinking water. Reducing seeding rates and densities to promote good air circulation, enabling plants to dry quickly, timing watering when plants will dry quickly, and postponing field activities until later in the day when fields are dry may all help to limit the transmission of infections. Working in unhealthy areas last will also prevent the spread of disease to healthy crops. Before going from one field to another, clean or sanitize all types of equipment.

- *Selection of Field and Rotation of Crop:*

Pathogen spread distance affects field selection. Choose fields distant from last year's crucifer fields. Choose well-drained fields which won't get overflow from crucifer-grown regions.Crucifers do best when grown in well-drained, light soils, which are easier to work on earlier in the growing season. Since black rot bacteria can't develop in the winter, early planting helps reduce sickness.Crop rotation is another management approach. Infected crop tissue may live in the soil until it rots. Temperature, soil moisture, and soil type affect how quickly crucifer crop detritus rots. In Georgia and Washington, which have long, warm summers, free-living bacteria may live in contaminated soil for 60 days and in host trash for 615 days. Cool, moist seasons are better for bacteria than hot, dry ones. Ontario recommends 3-year rotations [22].

- *Nutrition for Crops and Weed Control:*

The impact of plant nutrition management on host crop vulnerability tothe infection known as black rot is not well understood. A well-balanced nutrition regimen may lower plant vulnerability to disease infestation. Excess nitrogen encourages luxuriant vegetative development and may make plants more vulnerable. Micronutrients may potentially play a role in crucifer crop disease defense systems. Infected and persistent black rot bacteria may be found on various birdseed rape, black mustard, globe-podded hoary cress, pepper grass, wild radish, and other crucifer weeds (*Brassica campestris, B. juncea, B. nigra, and Capsella bursa-pastoris*).Disease symptoms in weeds might vary from no symptoms at all to tiny yellow V-shaped lesions on leaf margins. Infected plants (including weed hosts) may transmit the disease up to 30 meters to healthy plants. This could live and reproduce on weed seeds and leaves, but it cannot infect or cause illness in cruciferous crops it infects and transmits from weeds to them. Weed removal on farms is crucial, but attention must also be paid to ditches or fencerows for the management of the disease.

4. CONCLUSION

Black rot is caused by the bacteria *X. campestris PV. Campestris (Pam.)*, Dowson is the most severe disease that causes huge losses to crucifers. This illness affects all types of crucifers, including weeds, and it is widespread over the whole planet. When early crop growth takes place in settings that are warm and humid after periods of wet weather, the disease has the potential to cause large production losses. Late infections could provide an opening through which other types of rot organisms could penetrate the food and inflict considerable harm while it is being stored. Although this bacterium is capable of infecting any member of the Brassica family, it most often causes problems in brassica vegetables including broccoli, cabbage, cauliflower, and kale. Infected seeds are a common vector for the spread of black rot in agricultural settings. Eliminate any weeds that may be growing in or near the fields, since they may be a source of the black rot disease. To hasten the process of decomposition, crop leftovers should be buried to a great depth. Sprinkler irrigation should be avoided wherever it is. When possible, it is best to plant varieties that are either less vulnerable to black rot or more resistant to it.

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CHAPTER 5

CONTROL AND MANAGEMENT OF RED ROT DISEASE IN SUGARCANE CROP

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ABSTRACT:

Sugarcane is a key crop for the worldwide agro-industrial sector. Since India is both the world's greatest consumer or second-largest producer of sugar, it must cultivate a sizable amount of sugarcane. One of the most significant crops in the world is sugarcane, however since India is the greatest producer and consumer of sugar, sugarcane output has to be increased. However, sugarcane disease, also known as Red rot disease and sugarcane yield cancer, is a serious worry and the cause of decreased sugarcane productivity. The productivity and yield quality of the sugarcane crop was considerably decreased by this disease. The fungus Colletotrichum falcatum, which is highly diverse and regularly eradicates resistant forms, causes the disease considering how dangerous the condition is. The author discusses the significance of sugarcane disease, how red rot disease affects sugarcane output, and how to prevent red rot disease in sugarcane yields in this essay. There have been several studies on this issue, but more need to be done in the future.

KEYWORDS:

Colletotrichum falcatum, Disease, Red Rot, Sugarcane.

1. INTRODUCTION

Sugarcane ("Saccharum officinarum L.") is the most widely grown commercial crop worldwide. Mobilizing rural resources, contribute significantly to enhancing the standard of living for farmers as well as the national economy, which eventually results in more income and job prospects. It is a tall, resilient, natural grass belonging to the Poaceae family, although it is mostly used for the production of white sugar, which is made using its juice [1]. Jaggery (Gur), ethanol (for fuel), molasses, bagasse, chipboard manufacture, or press mud utilization as a rich produced from organic matter as well as minerals for crop productivity are just a few of the other goods and byproducts created from sugarcane [2], [3]. Sugarcane diseases are now either seed or soil-borne, making it very hard to manage those using agrochemicals after they have spread to the field. By implementing one or even more standard precautions, the occurrence of the illness can be reduced. For the management of sugarcane diseases, no one technique works. The best strategy for eradicating all illnesses is the integrated management of sugarcane infections.

One of the most dangerous sugarcane diseases that are currently known as red rot. In 1893, a characterization from Java was used for the first time. It is common in the nations that produce sugarcane across the world, even though it may be far more harmful in some locations than others. The illness was widespread and very infectious between 1939 and 1942 in eastern United

Provinces and northern Bihar. Due to how damaging it was, the sugarcane crops in certain areas were virtually completely devastated [4], [5].

1.1. Sugarcane Red Rot Symptoms:

The main external symptoms of the disease may be drying, curling or eventual yellowing of the upper leaves. When the entire crown dries out, the entire plant eventually begins to show signs of disease and dies. When the problem isn't severe, the dead matter usually dissolves and turns black, and it moves out of the centers. Since the infection in the stem occurs internally, the existence of the disease is not apparent from the outside. Early in disease progression, plucking a sick cane reveals reddish fibrous-vascular bundles close to the root [6]. The presence of the fungus causes a strong reaction inside the host tissue, with host cells responding or changing before the hyphal invasion. When the tone of the cellular contents changes, a sticky, dull red substance that fills the intercellular spaces oozes from the cells. The cell wall still contains the solvent dye that was found in this sludge, giving the asymmetry a noticeable red drop [7].

In addition, the appearance of red in the fibro-vascular bundle is not always a sign of the disease as the color can be caused by many other conditions as well. As the disease progresses, irregular, discolored spots may be yellowish, reddish as well as white with red edges, as the red color of the disease spreads to surrounding structures, and spread to multiple internodes [8].

1.2. Organisms Causing Sugarcane Red Rot:

Sugarcane red rot is brought about by Colletotrichum factum Vent, as well as Glomerella tucumensis (Speg.) arcs or Müller being the ideal stage of this pathogen. There has been substantial debate over the kind of fungus that causes this illness. Some claimed that because this fungus is just parasitic but not saprophytic, healthy canes cannot be harmed. Others asserted that while it can injure mature canes through wounds, it cannot affect tiny plants. However, young canes are frequently protected by the leaf sheath. It has been asserted in certain regions that the illness is unknown and that the fungus only develops on dead canes [9].

The parenchymal cells of the host tissue are where the mycelium of the organism is produced, both intracellularly and intercellularly. The hyphae are freely stretched, flattened, dull and brittle. Acervuli should only be seen above or below the hubs and have edges or pits. They form clusters and have smooth, black bodies. The acervuli are cauda and arranged incorrectly. Falcate conidia with a single cell up to 20 cm long and 8 cm wide can be found on aseptate conidiophores. Conidia are 16 to 48 nm in length and 4 to 8 nm wide. To the center, they miss a large oil globule. Both intercalary and terminal chlamydospores are possible. Ideal conditions in India were observed on sugarcane leaves in 1952, under cultural conditions, and in 1953 under natural conditions. It is composed of globose, surface perithecia, the lower part of which is submerged in the host tissue. Several asci-carrying ascospores that are aseptate, hyaline, or elliptical are present [10].

1.3. Red Rot Disease Cycle in Sugarcane:

The main pathogen for red rot of sugarcane comes from a faulty stock that is unintentionally carried throughout cultivation, as well as old, crumpled stalks, leaves, or other waste material on which the fungus develops saprophytically. Ratoon crops are also a source of early inoculum. It is debatable if the fungus is purely parasitic and motile. The secondary inoculum is composed of conidia that form in acervuli that form along the central veins of infected leaves during primary

infection, which is shown in Figure 1. They are spread by wind or splashed by rain, insects, or irrigation water. Conidia rapidly germinate by the germ tube, forming an appressorium from which infective hyphae arise upon contact with any hard surface, such as soil particles, as well as plant components [11].

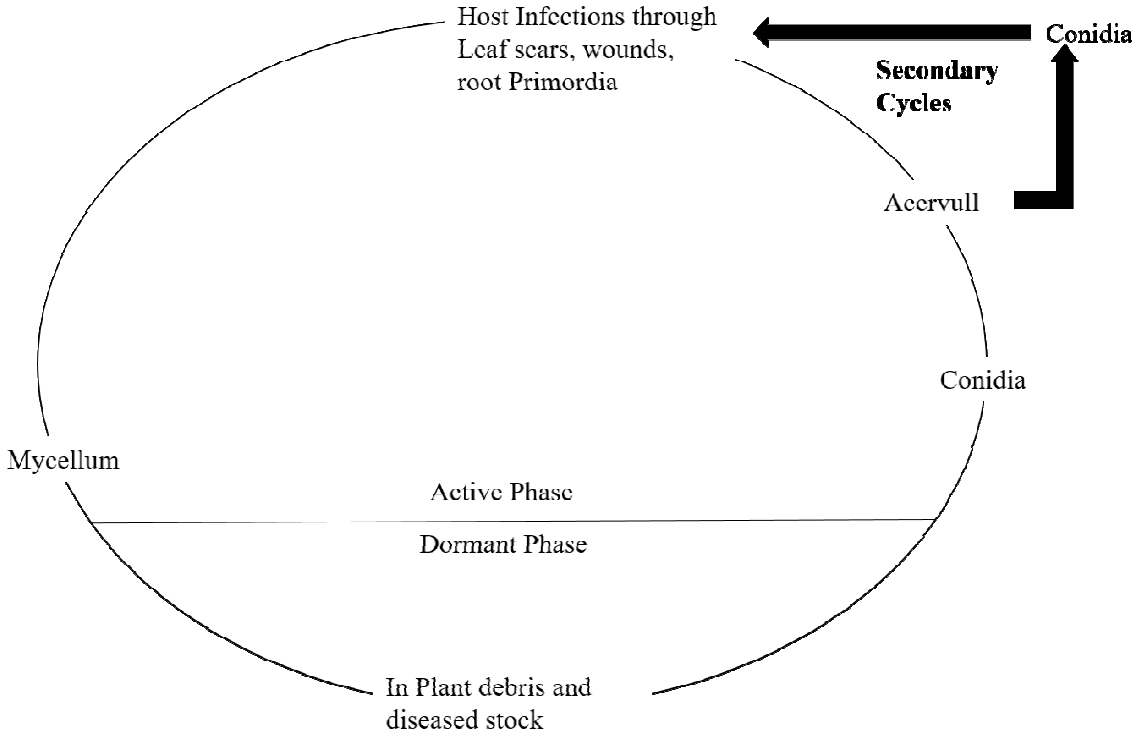

Figure 1: **Illustrate the Disease Cycle of Red Rod of Sugarcane.**

1.4. Control of Sugarcane Red Rot:

Red rot of sugarcane is being controlled because fungicides cannot penetrate the infected tissues of diseased seed sets and because the tails from which the seeds are extracted have suffered significant damage since planting. Therefore, it is advised to carefully select red rot-free seed sets before planting. Buy seeds from nurseries that are periodically inspected by sugarcane conservation organizations to ensure that they are free from contamination [12], [13].

Each seedling should be thoroughly inspected before planting, but those that appear red should be immediately plucked or burnt to the damaged clumps and red rot can be prevented throughout the growing season, by using the green leaves as cow fodder. Can be stopped. Sugarcane tubers should never be placed in areas infested with red rot. Sanitation should always be a priority, and diseased cane stubble should be removed and burned in the field along with other waste. Options for seed treatment can be utilized to lessen the red rot of seed treated in water for two hours at 50°C. For treating seeds, fungicides like Arasan (0.25%) are usually helpful [14].

In all sugarcane regions, red rot control has become a significant challenge. Early-stage identification might not be very simple, but the cane eventually breaks down. Split the canes apart lengthwise to reveal dull red tissue with white spots from across the length of the stalk. These blotches are typical of sugarcane red rot. The monsoon season facilitates quicker disease

transmission or crop drying. Growing only tolerant sugarcane cultivars that have been approved for growth in various sugarcane growing areas is the best way to prevent this fungal disease. All relevant organizations, especially sugar mills, must impose strict domestic isolation measures to prohibit the migration of cane setts from endemic regions to new locations since phytosanitation is the key to managing this disease [15].

1. Observing ethical cultural norms, including cleaning up surplus rubbish from fields or ensuring effective drainage.
2. To prevent rotting from causing poor plant stand, only healthy setts should be planted.
3. To stop water from spreading to nearby farms, bunding should be used to segregate the affected fields.
4. Infected fields shouldn't be rationed under any circumstances.
5. Rotating the crops in the impacted areas may help to minimize the disease inoculum.
6. It's also advised to soak setts in hot water for 30 minutes at 52 degrees C before planting.
7. As a general rule, it is recommended to plant fungicide-treated seed setts to stop the disease from getting into sugarcane fields.
8. Chemical control: Thiophanate methyl, a systematic fungicide, at 0.25 percent, will prevent setts for 3 months against the main red rot infection.
9. Growing resistant or tolerant types can help reduce red rot in addition to the many hygienic measures outlined above. Numerous commercially significant cane varieties, some of which are highly resistant or at least tolerable to red rot, have been produced via inter-generic or inter-specific crosses with Saccharum spp. in the latter instance employing S. spontaneous or S. robustum with S. officinarum.

2. LITERATURE REVIEW

Saurabh Dubey et al. studied about impact or economics of treatment on red rot disease in sugarcane. Red rot has primarily been effectively controlled by resistant types, while numerous fungicides or bio-agents have been proven to be beneficial in vitro but failed to successfully prevent infection in the field. Therefore, at RPCAU, Pusa Farm, SRI, and Pusa, several treatments were evaluated in the field to examine how they would affect various sugarcane characteristics economically. It was shown that the treatment improves both the quantitative and qualitative cane indicators in addition to reducing the incidence of red rot disease. The newly released resistant variant of this fungus frequently turns susceptible after a few periods of development due to the introduction of new pathotypes [16].

K Bhanuprasad et al. studied the management of red rot disease which is a cancer of sugarcane crops, In this essay, the author discusses sugarcane rod rot treatment strategies. Various fungicides or essential oils have been tried in-vitro against Colletotrichum falcatum. In the studies using essential oils, peppermint oil had the highest mycelial growth inhibition (48.00%) or the lowest mycelia growth inhibition (10) at 10 l concentration. It was discovered that chlorothalonil completely inhibited the test-mycelia fungus's growth when combined with five other fungicides (80.0 percent), however, ballet on was less inhibitive (75.71 percent). The outcomes of the virulence comparing tests undoubtedly show that Colletotrichum falcatum separates differ in their virulence as determined by the rate at which they scattered in a resistant as well as a sensitive host, and they partially confirm that these distinctions in all the variances tested thus far are strongly linked with different morphological variations in this regard by the authors of previous preliminary evidence [17].

Muhammad Nasir Subhani et al. studied the effectiveness of several fungicides against "red rot disease" in sugarcane crops. The author of this study investigated various tests to prevent sugarcane red rot disease, 12 fungicides were examined (Colletotrichum falcatum Went.). In this test, fungal growth was entirely (100%) suppressed by Benomyl 50 WP, Foliar, and Radomil 75 WP at concentrations of 5, 10, 20, and 50 g mL-1. The findings revealed that all tested fungicides significantly increased the suppression of mycelial development with an improvement in fungicidal concentrations of 5 to 50 g mL-1 [18].

3. DISCUSSION

Sugarcane, or Saccharum spp., is a significant cash crop farmed in tropical and subtropical areas of the world. It is coveted for its capacity to store significant quantities of sugar and sucrose in the stem as well as for its capacity to make ethanol, a useful renewable biofuel. Sugarcane is an important source of commercial sugar globally, producing over two thirds of the world's sugar supply. Brazil, Mexico, and India together produce 40% of the world's sugarcane. Taking second and third place, respectively, were Thailand, China, the Philippines, Australia, the United States, Pakistan, and Argentina.

With a total area of 4.2 million hectares, sugarcane is grown in the majority of the Indian states (M ha). It ranks as India's second-largest agro-industrial crop after cotton. The sugarcane-producing Indian states and their proportionate share of output are shown in Figure 2. With rising population demands, sugarcane output has to be improved. Diseases are the main cause of worry, even though other biotic and abiotic variables contribute to its low yield. A hundred distinct sugarcane diseases have been documented throughout the world. In various regions of the world, parasites of sugarcane include more than 100 fungi, ten bacteria, ten viruses, and roughly fifty kinds of nematodes [19].

Epidemiology Origins of the infection Red rot can affect mature sugarcane stalks, and the midribs of leaves, including planting materials, which causes significant losses in crop productivity or sugar quality. Red rot spreads via infected setts/canes, diseased stubble/debris, and latent propagules in the earth. The main reason red rot in sugarcane recurs every year is infected seed cane and stubble, which the disease distributes to future crops. Although the fungus is not a true soil-borne organism and only lasts in the soil for around 5 to 6 months, its influence on the disease's recurrence may be modest. The pathogen starts attacking the cane plant during germination, which fails to germinate or even the death of seedlings. Young developing shoots are typically infected after germination by latent mycelia found in bud scales [20].

Red rot can manifest in many ways depending on the type of infection, the season, as well as the local climate. During or after the monsoon, diagnostic signs are seen. Pre-monsoon rains cause the disease's symptoms to emerge, and the monsoon, which arrives at the ideal time for the weather, causes the condition to fully manifest. Infection of the mid-rib, leaf sheath, lamina, or stalk arises from the fungus' secondary transmission even during monsoon through irrigation, rain splash, or rainwater, whereas air currents help the disease spread throughout the winter. Conidia secreted across the rind promote infection via nodes after washing down with water. Because the spore mass is mucilaginous, wind dispersal of the inoculum seems to be more challenging. However, it appears that the inoculum was spread aerially because the disease was found in the upper portion of the canes. Wintertime environmental variables make it difficult for the fungus to infect crops which may not seriously endanger the cane crop, but they do cause the

appearance of early infections in the nodal area, which includes the roots, bud scales, buds, and primordia.

3.1. Acute Infection Mode:

The virus mostly affects canes through nodes, with leaf scars, growth rings, root primordia, or buds serving as the major entrance points. The infection could also enter the stalk through the cut ends of the setts, growing fissures, and rootlets. Mycelium may move across cells when the fungus infects the stalk's tissues, leading to the development of the gum in genotypes with a low level of resistance. The vascular bundles can allow the spread to happen more quickly. Internode tissues that have been infected produce a rot with a distinctive red color that frequently contains white patches that are areas of normal color.

Favorable circumstances for the development of illness

- A mean temperature between 29.4 and 31°C is ideal for the disease's development.
- pH 5-6
- Dry environments throughout the first stage of development.
- High levels of ozone (90%).
- Waterlogged soil conditions.
- A lack of cultural norms that encourage weed growth.
- Ongoing field cultivation of the same cultivar.
- The environment contains sensitive species.

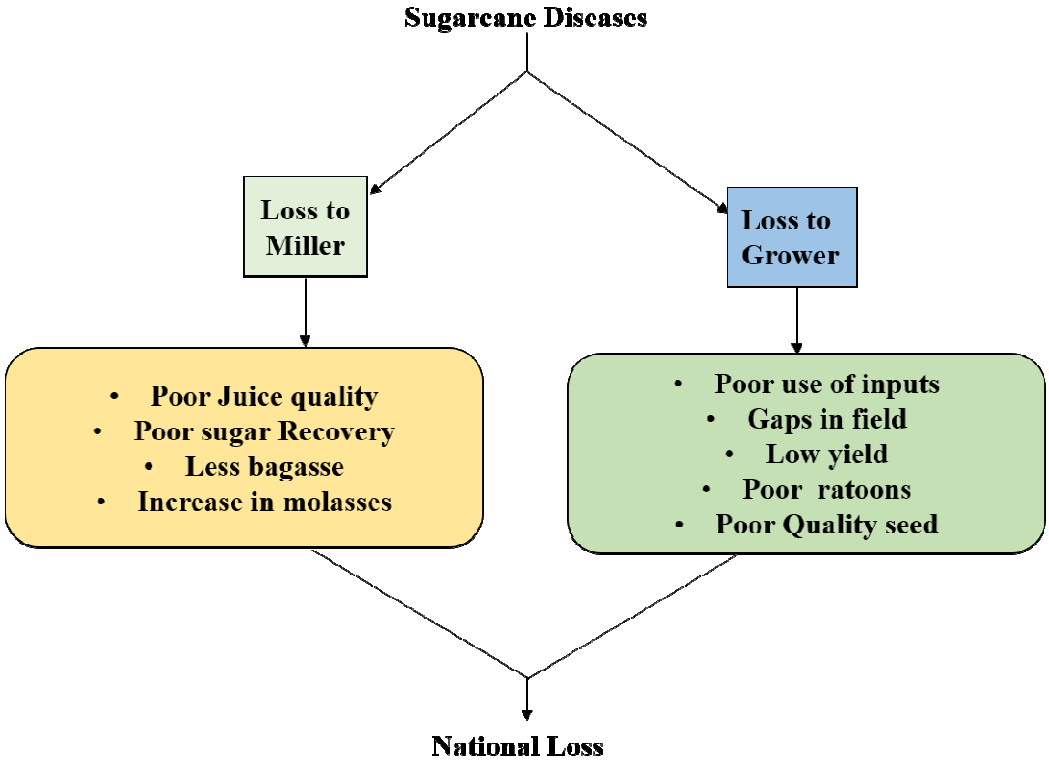

Figure 2: Illustrate the Cycle of Red Rod Disease of Sugarcane.

Sugarcane, or Saccharum spp., is a massive cash crop grown all over the world in tropical and subtropical regions. It is prized for its ability to retain significant amounts of sucrose and sugar in the stem as well as more recently for its capability to deliver ethanol, a useful sustainable biofuel source. Sugarcane, a vital source of commercial sugar globally, produces over 66% of the world's sugar production. The major Indian states with a high level of production are shown in Figure 3.

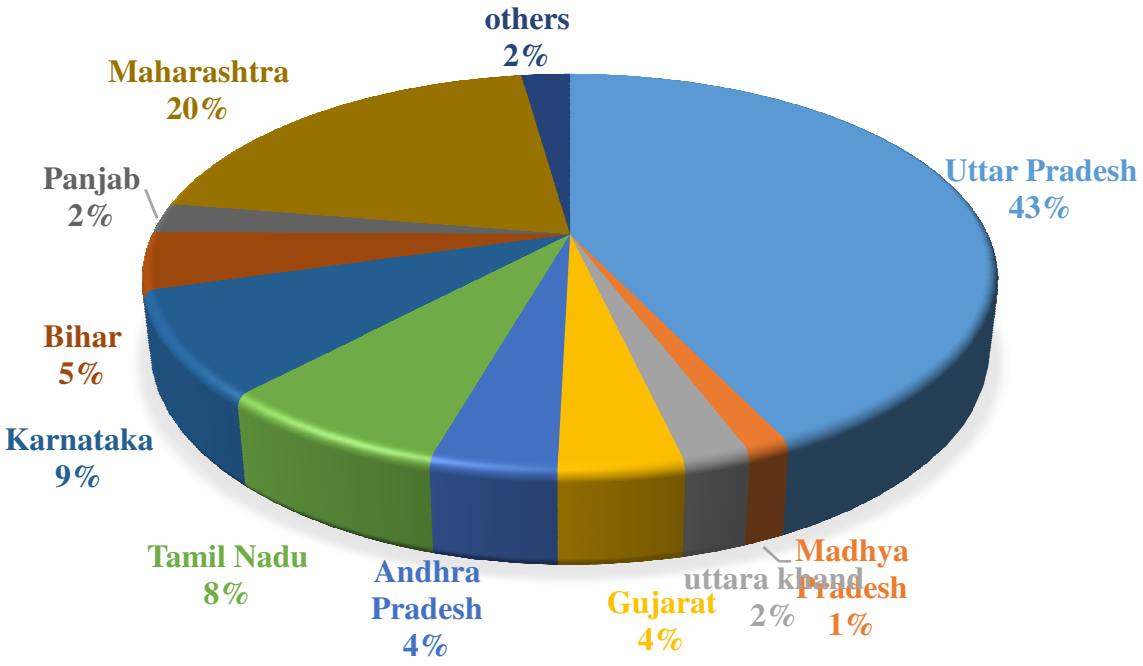

Figure 3: Illustrate the Some Major States of Sugarcane Production in India.

3.2. Management of Red Rot Disease in the Sugarcane:

The contagious fungus can be dispersed by rain, wind, or insects. From one year to the next, the illness may linger in the soil, decaying leaves, or unraked crop residue. The illness would spread to the field if a farmer grows canes that have already been infected. In India, the Gramineae (Poaceae) family member "Saccharum officinarum", is a widely grown crop. India is well-known for being the world's second-largest producer of sugarcane, with Uttar Pradesh taking the lead. Additionally, it makes significant contributions to the government treasury and employs more than a million people directly or indirectly.

Whole standing crops can be destroyed by the red rot disease, popularly called the cancer of sugarcane plantations. The illness can cause the sugarcane to lose 29% of its weight and produce cane of low grade. Infected cane has up to a 25-75% lower sugar content than healthy cane. The most harmful condition that may affect sugarcane is red rot. Every component of the plant just above the earth is impacted by the illness. As the condition worsens, the leaves progressively wither downward. Usually, the fourth or fifth leaf from the top is injured, and gradually the entire crown deteriorates and wilts.

Traditional methods for managing the red rot disease include cultural practices, the selection of resistant types, planting resources free of the disease, biological, physical, or chemical control, and others. These techniques aim to reduce the likelihood of red rot following replanting to boost

sugarcane plants' output. Furthermore, the management techniques used to reduce the occurrence of red rot had not produced satisfactory results. No one strategy has been successful in reducing illness incidence to this point. One of the best methods for illness control is integrated vector management (IDM). In comparison to non-IDM techniques, integrated disease management strategies raise growth metrics, reduce the prevalence of red rot, or improve sugarcane performance qualities. All disease control strategies are included in integrated disease management. This topic will be covered in the debate that follows.

3.2.1. Cultural and Agricultural Practices:

The highest importance should be given to choosing excellent agricultural practices and using cultural or biological management approaches as a preventative strategy. Red rot disease may be considerably reduced by using nutritious planting materials, field sanitation, certified seeds, crop rotation, or appropriate drainage facilities. It has been hypothesized that these cultural methods can lessen crop losses as well as inoculum from the field. The inoculum level is increased by monoculture of the same crop under the same cultivar, which leads to the development of the disease. The crop in the heavily diseased field should be changed every three to four years or cycles, and rationing should be avoided. Unmanaged nursery methods ought to be used since red rot is a disease that is spread by seeds or seedlings. Programs for child care must be governed by law. Guaranteed seedlings, pairings with other types, and seedlings themselves must be pest- and disease-free. The use of disease-free sets represents the most effective strategy for knowing how to prevent.

To control the red rot disease, sugarcane growers are now removing infected elements that compromise cultural practices. The most practicable red rot disease control strategy is sanitation, at least until C. falcatum-resistant cultivars are fully developed. A comprehensive genomic study on C. falcatum is also badly required. Nevertheless, due to the success of field disease control, the incidence of disease destruction has indeed been greatly decreased. In-depth breeding efforts to create red rot disease-resistant sugarcane varieties or create biological control methods are crucial for the sugarcane industry's survival.

1. Red rot, which is often referred to as sugarcane cancer, is currently unmanageable. Moreover, the diseases may be managed by employing integrated red rot management measures.
2. Infected settlements permitted the disease to spread. Select only healthy, disease-free seed canes as a consequence. Setts with reddening in the nodal region or at the cut end need to be thrown away.
3. Either grow a seed crop on seed canes that have been soaked in Thiophanate Methyl (ROKO)+Pseudomonas overnight or heat and aerate seed canes for just an hour at 54°C.
4. Rotate paddy or green manure crops in your fields. Avoid ratooning.
5. During the rainy season, the disease spreads quickly. The injured field could be bundled to stop the infection from spreading by rain or floodwater.
6. Roguing diseased plants with spindly leaves in the first few weeks of May and June and spraying the nearby soil with a 0.2% carbendazim solution.

4. CONCLUSION

Red rot disease is difficult to cure chemically because of the impermeability of the rind as well as the tolerance of the fibrous nodes to absorb fungicides. Due to non-specific or general damage by chemicals to soil micro-flora, it is now important to seek innovative ecologically friendly treatments for the problem. To examine the potential for producing systemic resistance to C. falcatum, novel ways should be taken into consideration in addition to the present management techniques. The author of this paper discusses the significance of sugarcane disease, how red rot affects sugarcane productivity, and how to prevent red rot in sugarcane yields. When adequate defense genes have been found, further research will concentrate on developing transgenic sugarcane with built-in resistance to red rot.

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CHAPTER 6

EPIDEMO-SURVEILLANCE STUDY OF THE INSECT-BORNE XYLEM-LIMITED BACTERIUM *XYLELLA FASTIDIOSA*

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ABSTRACT:

The estimation of novel contagious disease occurrence, whether linked with new microbes or not, continues to remain a challenging and debatable subject. Prominent aspects directing widespread diseases are usually simply acknowledged some ages after epidemics, largely disclosing that a restricted amount of causes are related to the development of a particular group of bacteria. However, the current overview portrays the outline of the insect-borne xylem-limited bacterium Xylella fastidiosa, a pathogen linked by numerous fresh plant infections in diverse regions of the world. Evolving plant infections often have substantial commercial, ecological, social, and cultural effects. Xylella is a plant disease caused by the bacterium in plants having broadleaved structures such as oak and palm trees, affecting other plant species also such as grapes, coffee, citrus, and almond. The bacteria target the xylem section of the plants causing chlorosis, discoloration, and leaf necrosis.

KEYWORDS:

Citrus Plants, Epidemic, Leaf Necrosis, Pathogen, Xylem-Limited Bacterium, Xylella fastidiosa.

1. INTRODUCTION

X. fastidiosa is a gram-negative aerobic bacteria of the genus *Xylella.* It is found in the larger environment and is a weak growing bacteria with a rod-shaped structure and non-flagellated. In comparison with other bacterial species, it is capable of multiplying within the majority of the plant species.Investigations from the past years focusing on some severe infection occurrences have directed to significant modifications in an understanding of *X. fastidiosa* ecology, biology, and progression. This additional evidence has not only introduced novel understandings into facets of the bacteriology of this microorganism and its relationship with the plant and insect hosts but has also correspondingly prepared an accessible phylogenetic structure that has permitted for improved extrapolations concerning causes leading to the development of infections. This study identifies and discusses the main paths that lead to epidemics triggered by *X. fastidiosa*. The host plants usually don't show any signs of symptoms when encountered with the bacterium. But later on, shows few symptoms such as leaf chlorosis [1].

X. fastidiosa is categorized into three subspecies based on their mechanism to select a host: *X. fastidiosa* subsp. *fastidiosa (*causes of the deadly grapevine disease known as *Pierce's disease.),* *X. fastidiosa* subsp. pauca(much acknowledged for citrus chromatic chlorosis and olive quick

decline syndrome) *X. fastidiosa* subsp. *multiplex* (mostly linked with *Prunus* spp.). Another disease caused by *Xylella fastidiosa* which is prevalent across Brazil is citrus variegated chlorosis (CVC). The disease typically affects the different varieties of "sweet oranges". Possible symptoms of the disease include noticeable 'variegations 'on the leaves that are old along with necrotic patches on the upper side and lesions formation in brown color. The fruits affected by the disease are usually hard and small also these fruits serve with no commercial significance. The only preventive measure for treating the CVC infection includes the removal of shoots of the infected plants by the use of a technique known as pruning. The use of insecticide or using healthy plants can also be done in order to broaden the area of prevention against the disease. Infection-causing bacteria have a set mechanism for spreading the disease in the host plant starting with affecting one part of the plant body and eventually propagating to the other section of the plants which if left unnoticed leads to complete damage to the plant and sometimes results in death.

The detection method for *Xylella fastidiosa* needs to be quick and secure to detect the presence of the pathogen in the early developing stages of the infection inside a host body. The prevention of the disease caused by the bacteria will only be set into action once the presence of the causing pathogen is detected and restricting the organism from further causing the infection. Conventional molecular biology techniques comprise of enzyme-linked immunosorbent assay 'ELISA', Polymerase chain reaction 'PCR', short sequence repeats (SSRs), and so on can be used for the detection of the pathogen. ELISA as a molecular biology tool is used for the evaluation of either the existence of an antigen or to determine the existence of an antibody in a testing sample. Mainly used in the evaluation of a 'serum antibody concentration'. Since the technique is widely used in different approaches it has applications in the detection of the presence of probable food allergens that triggers allergy via food, usually eggs, peanuts, milk, or even gluten [2].

Toxicology is also a branch of biology that uses ELISA for the detection of different drugs. ELISA assay comprises of series of various steps. Some of the stages in the assay consist of restricting and washing to avoid the chances of binding of non–specific molecules. The binding of non-specific molecules takes place when the adsorption of the protein occurs with each other or against the surface of the plastic of the ELISA plate through the interaction involving 'off target'. Such binding is regulated by the hydrophobicity in order to be excluded by some percentage through the application of washing and blocking. The steps in the mechanism of blocking consist of the protein introduction into the sites of the plate plastic in order to be adsorbed by the well. Followed by the next consecutive step which comprises of inclusion of detergents to minimize the binding of hydrophobic off-target interfaces among the proteins and to exclude the proteins that are unattached by the system. The process of PCR is based on a principle of replication of enzymes present inside the Deoxyribonucleic acid (DNA). In a concise form, the technique involves the amplification of a short stretch of DNA with the help of a primer governed by the enzyme. The enzyme "DNA Polymerase" constructs a fresh strand of DNA that are corresponding to the "DNA template". The enzyme "DNA polymerase" adds a nucleotide to the 3' – OH group, the already existing group. *Xylella fastidiosa* causes many diseases in plants such as almond leaf scorch, 'Pierce's disease' in grapevine, oleander leaf scorch, and alfalfa dwarf following are the vectors of *X. fastidiosa* for the spread of the pathogen [3]–[5] (Figure 1). The diseases cause visible symptoms in leaves, shown in Figure 2.

Various experiments on the mechanism of transference and targeting the host cells have shown that isolated *X. fastidiosa* from grapes causes alfalfa dwarf. Whereas vector leafhopper of *X. fastidiosa* for a grapevine is found in almond orchards. The bacteria transmit from almond or grape plant source to healthy plants of almond and grapes in new combinations. It invades the host plant by targeting the xylem section of the plant, blocking the natural transfer of water and nutrients across the plant system which leads to the death of the host plant [6]–[8]. Coming to the pathogenesis part *X. fastidiosa* blocks or disrupts the xylem vessels by making a xylem-limited biofilm leading to disruption in water and nutrients transportation causing water stress.

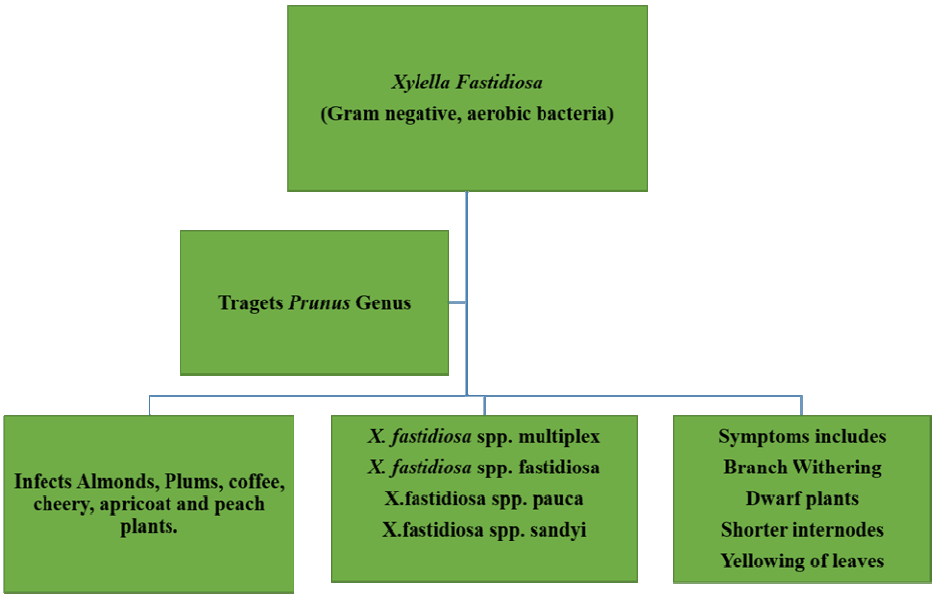

Figure 1: The Target Species of *X. fastidosa* and its Subspecies Along with the Common Symptoms Associated with its Invasion.

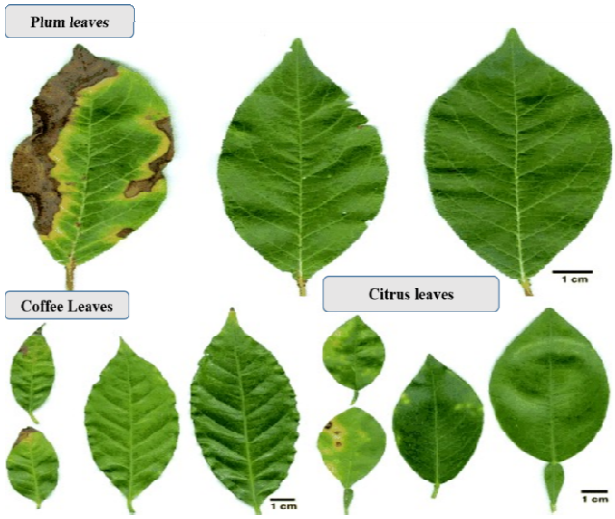

Figure 2: Pictorial Representation of Visible Symptoms of *Xylella fastidiosa* in Plum, Coffee, and Citrus Leaves

Some of the cases reported in the past show the secretion of toxins produced by the bacterium causing a disturbance in the photosynthesis mechanism. The bacterium technically invades the host plant in two ways firstly by attacking the network of network tissues of the infected plant or by targeting the insect's foregut which feeds on xylem sap [6]. *X. fastidiosa* movement is dependent on adjacent vessels that transpire over intact or broken pit tissues, which is an essential procedure for effective intraplant movement. The lifecycle of *Xylella fastidiosa* begins when the insect feeds on the infected plant and later spreads the bacteria to every new plant it feeds on. The bacteria expands and restricts the movement of water from transferring through the plant eventually contributing to the death of the host plant [9], [10] (Figure 3).

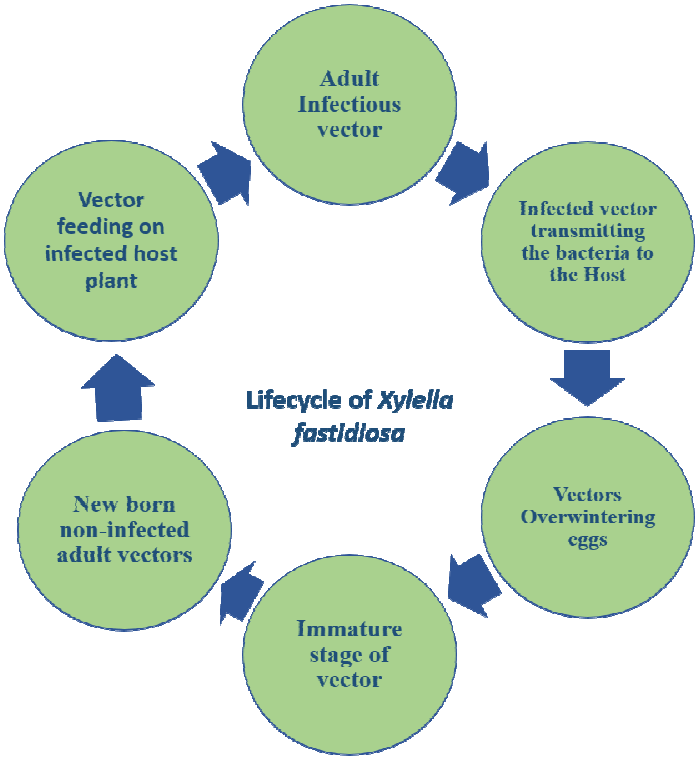

Figure 3: Representing the Stages of Evolution in the Lifecycle of *Xylella fastidiosa.*

Management strategies to control the widespread bacterium *Xylella fastidiosa* which spreads Pierce's disease include the use of biological control in a large environment by reducing the amount of *H. coagulata* in vineyards and citrus. Employing the use of kaolin and neonicotinoids help protect vineyards against the bacterium. Another strategy that includes to affects the *H. coagulate – X. fastidiosa* association is the elimination of infected grapevines. Presently, around there are no procedures presented to intrude the grapevine and *X. fastidiosa* interface. However, these strategies are not productive but could help in starting the management of the bacterium spread [11]. For agricultural land, different formulations of zinc and copper as a spray is in use at current to combat the problem of bacteria colonization [12], [13]. A combination of zinc and citric acid (4%, 2%) are currently been employed to tackle the cell concentration of '*X. fastidiosa* subspp. *Pauca*' among olive trees. *X. fastidiosa* depends upon insect vectors for the transmission of the infection. One of the common diseases prevalent among the *Prunus* genus trees in Mexico and Central America is '*Pierce's disease'*. A larger category of crops such as weeds and vines are greatly affected. Transmission happens via insects that act as a vector and transmit the

bacteria to the grapevine and other plants. The choice of host selection and specificity sometimes does not result in disease. Spittlebugs and Sharpshooter leafhoppers are the main vectors. The insect vector should be a xylem feeding agent, by this means it will be able to disrupt the passage of nutrient transportation. The transfer of *Xylella fastidiosa* is because of the three main reasons firstly, procurement of the target plant. Secondly attaching and withholding the bacterium against the foregut of the vector and thirdly, removal and introduction in a fresh target host. The transmission process also comprises of expansion of the disease in various stages of the proliferation of bacteria post-inoculation. However, the causes responsible for procurement are greatly understood although the process of inoculation and consistency in the host plant is still yet to be discovered. A high number of bacterial cells in a host plant also reflects the penetration and attachment efficiency of the *Xylella fastidiosa.* Interaction of insect vectors with other target elements is majorly responsible for the survival of the bacterium[5], [8], [14]–[18].

2. LITERATURE REVIEW

Rodrigo P.P. Almeida *et al.* demonstrated the transference of *Xylella fastidiosa* by vector and strategies for the management of diseases caused by the bacteria. Bacterial ecology plays a vital role in determining the transmission of disease. The authors have elucidated the characteristics and the causes affecting vector transmission of the bacterium and strategies for managing the diseases using elementary investigation [11].

M.J. Davis *et al.* discussed the pathological development of Pierce's disease caused by *Xylella fastidiosa* in almond leaf scorch also known as golden leaf scorch. The authors have conducted a research study that comprises isolation of bacteria causing similar infections like pierce's disease. The bacterium was isolated from 17- 20 *Prunus amygdalus* infected with symptoms of almond leaf scorch. The collection was restricted to 16 non-symptomatic trees in two orchards in California. Isolation of the infection-causing bacteria was done from infected trees in both orchards through one season of summer with attempts recorded in 22 of 23 (95.7%) and 34 of 60 (56.7%). The study concludes that Almond leaf scorch symptoms were greatly widespread and it was easy to re-isolate the bacteria from trees that were inoculated with bacteria via injection rather than leafhopper transference [9].

Alexander H. Purcell and R.P.P. Almeida illustrated the biological attributes of *X. fastidiosa* obtained from almonds and Grape strains. The researchers reported the hypothesis testing of Almond leaf scorch is caused by Pierce's disease in grapevine. Strains of Almond leaf scorch in the field were found that both groups of *X. fastidiosa* promote the spread of almond leaf scorch infection while hibernating inside the almonds post mechanical transmission. The authors also stated that in conditions of a greenhouse atmosphere, every isolate triggered almond leaf scorch, and isolates from grapes generated pierce's disease [19].

Rodrigo P.P. Almeida and Leonard Nunney explicated a review study on the disease emergence by *X. fastidiosa.* The authors have stated the association of different plant diseases by the bacterium and how the bacteria invades the host plant causing the proliferation of disease. The study also includes a discussion about the factors responsible for emerging diseases by *X. fastidiosa* [6].

Davide Greco *et al.* demystifies an overview study on the expansion of *X. fastidiosa* and the association of diseases in the GenusPrunus. The authors have discussed various methodologies for handling outbreaks caused by the bacterium. According to the research conducted and

evidence collected almond is the common host for the bacterium. Furthermore, there are different pathogenic infections linked with stone fruit plants which depict relevant severity concerning the cultivar [3].

Stefano Pavan *et al.* expounded their views on the determination of genotypically resistant species of olive against the bacteria *Xylella fastidiosa.* The study accounts for the 'Olive Quick Decline Syndrome' epidemic triggered by the *xyllela fastidiosa* subsp. pauca causing alteration in the ecosphere of the peninsula of Salento. Researchers have also accounted for the use of a simple sequence repeat marker for the evaluation of putatively resistant plants (PRPs) [20].

A.H. Purcell and D.L. Hopkins outline the reasons for Pierce's disease in Grapevine: *Xylella fastidiosa.* The research article elaborates on the general characteristics of *Xylella fastidiosa* and its association with Pierce's disease. The authors have successfully described the symptoms of the disease along with the different strains responsible for causing a particular symptom in the host plant in varied geographical locations [21].

Chira Roberta Girelli *et al.* conducted a research study on treating infected olive trees 'Olive quick decline syndrome' by '*Xylella fastidiosa* subsp. *pauca'* with the use of an H-NMR-based metabolomics study. The authors have nicely explained the procedure of metabolic fingerprinting, a potent technique for monitoring the progression of the disease, and the influence of treatment given to the host plant with the help of detection with a biomarker. With the H-NMR technique, the researchers were successful in generating data for the management of 'Olive Quick Decline Syndrome.

In this present paper, a detailed review study is presented on the diseases originated by *Xylella fastidiosa* and its characteristics along with the mention of subspecies responsible for particular symptoms in Prunus Genus across different geographical locations. The study also reports a few management practices for controlling the progression of the infection by the bacterium.

3. **DISCUSSION**

The increasing rise of '*Xylella fastidosa'* in the *Prunus* genus has created havoc due to its rapid widespread and lesser options of management. However past research and data have provided an insight into the ecology and biology of the bacterium and its subspecies. The strains of the subspecies have different target mechanisms for varied host plants ranging in diverse geographical areas. Association of plant diseases is linked with the particular strain invasion in the host plant. The main objective is to educate critical queries and concerns for researchers and supervisory agencies identical, meanwhile, the data produced through the past period has equally elevated new queries but moreover elucidated the past ones. The current review article emphasizes a comparative discussion of various past-reported research articles and research papers on *X. fastidosa*. Figure 4 demonstrates the image of the bacteria *Xylella fastidosa* under electron microscopy.

A review study by Davide Greco *et al.* on 'Diseases trigged by *X. fastidosa* in Genus *Prunus'* discusses the importance of plants coming under the genus as these plants are both commercially and ecologically essential. The association of *Xylella fastidosa* with the plants of this genus helps in understanding the interaction of bacteria with its host plant. Almond, plum, and citrus plants are at constant risk of this bacterium. The paper also reports the election and innovation of resistant and tolerant cultivars along with the research study of the mechanism of resistance used

as a defense mechanism. In addition to this recording observations in case of plants highly infected with the infection can be an important approach for an assortment of resistant and tolerant materials amidst the local germplasm [3].

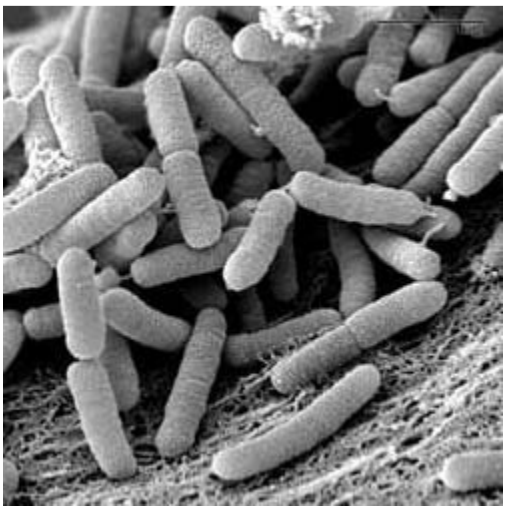

Figure 4. *Xylella fastidiosa* visualized under the Electron Microscopy.

Another review study by Leonard Nunney and R. P.P. Almeida on 'Emergence of plant diseases by *Xylella fastidiosa'* elucidates briefly the characteristics of the bacterium and its interaction with host plant and host insect. The data mentioned in the paper helps in understanding the targeted diverse geographical regions across the globe. The invasive nature of *Homalodisca vitripennis* vector of *X. fastidosa* in expansion to different areas leads to the origin and increase in Pierce's disease and oleander leaf scorch disease along with the repercussions [22].

Alexander H Purcell and Jao spotti lopes explained the 'Transmission of *Xylella fastidiosa* by the vector and use of existing data for the management of diseases. The authors explicate *X. fastidosa* as a xylem obstructing bacterium that targets the xylem vessels of the host plant leading to restricting its water movement. The study further discusses the role of Sharpshooter Leafhoppers as a prime vector for infecting plants like citrus, almond, coffee, and ornamental trees and another major vector called *H. coagulate* that is primarily responsible for the cause of Pierce's disease in the U.S region. Features of the vectors such as the frequency and the potency to cause invasion in target plants were also reported. Management strategies for Pierce's disease proliferated by the *H. coagulate* include complete interruption of the host plant with the infecting agent and employing the use of 'biocontrol means' such as pathogens [11].

Enrico M. Bucci in a review delineates that about the virulence of Xylella fastidiosa andhow it is a threat to agricultural farming around the globe with suggestive solutions for disease management. The authors have focused on describing the 'Olive Quick Decline Syndrome' (OQDS) triggered by different strains of the *X. fastidosa*.The sap of the olive tree serves as the target for the bacteria as it resides there and divides in numbers restricting nutrients and water transportation. Insects feeding on the sap of the olive tree further spread the bacteria in the distinct parts of the plant [10].

5. CONCLUSION

Bacteria *X. fastidosa* is an aerobic bacterium that targets Genus *Prunus*. It is an aerobic gram-negative bacterium that obstructs the xylem vessels causing restriction in nutrient and water transport across the host plant that later on results in the death of the infected plant. *Prunus* harvested species are been cultivated across the globe. The *Prunus* genus plants are also a major financial source of income, especially for farmers. Some of the varieties of crops such as peaches, almonds, and citrus have a significant link to the culture and traditions of a particular section of society. The review study elaborates on the characteristics of the *X. fastidosa* its mode of transmission with vectors and also enlists its subspecies along with the symptoms during the invasion. For disease management, strategies have been reported for tackling the infection widespread. At present, there is no effective process or tool to prevent the infection but certain measures can be taken to manage the infestation such as completely terminating the infected tree to further stop the contamination chances to other trees /plants or by the use of biocontrol the diseased plant could be managed.

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CHAPTER 7

A COHERENT STUDY OF DESIGNER FOODS PRODUCED BY USING RECOMBINANT DNA TECHNOLOGY

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ABSTRACT:

The genetic modification of living things like animals, plants, or microbes is a specializeduse of gene technology to alter the genetic makeup of an individual. Recombinant DNA technology (RDT) refers to the practice of combining genes from multiple species, and the organism that results is referred to as "Genetically modified (GM)," "Genetically engineered," or "Transgenic".This study includes, compilation of worldwide regulatory landscape as well as the advantages of easily accessible designer foods such as probiotics, designer eggs, designer milk, designer grains, and designer meals with designer proteins or micronutrients and macronutrients. Furthermore, this study explores the process of group actions with numerous biotechnological tools that are utilized to carry out genetic modification as well as their advantages. India has given its approval for the growing of certain genetically modified crops; nevertheless, this approval has led to results that have been limited due to a lack of sufficient understanding as well as religious reasons,which have overlooked environmental purity and impoverished sections' hunger.As a result, more research is still needed to ensure that it is safe for human consumption.

KEYWORDS:

Designer Foods, Genetically Modified Foods, Genetically Modified Organism, Recombinant DNA Technology (RDT), Transgenic.

1. INTRODUCTION

Biotechnology, more especially genetic engineering, is already being used as a helpful resource in a variety of fields, including health, industry, and agriculture. It has begun to enjoy the practical fruits of genetic engineering, like novel medicinal cures or higher agricultural production, and this is expected to continue doing so [1].Genetically modified (GM) foods are created from creatures whose DNA has been altered in ways that do not occur normally, for as by the insertion of a gene from another organism. This area of research is also known as "Gene Technology," "Genetic Engineering", and recombinant DNA Technology (RDT)". However, most commercially accessible genetically modified (GM) foods are produced from plants, although in the future, meals generated from microbes or GM animals would likely be introduced. To boost productivity, genetically modified crops are often engineered to be more resistant to pests and fungi or to tolerate higher concentrations of herbicides. Improved yields and consistency are two ways in which genetically modified crops might help bring down the cost of food.

In 1946, researchers found evidence that DNA could be transmitted from one creature to another [2].Genetically Engineered Foods (GE Foods), Bioengineered Foods, and Genetically Modified Foods (GM Foods) are all terms for foods that have been modifications inserted into their DNA using genetic engineering technology. When compared to conventional breeding approaches like selective breeding or mutant breeding, genetic engineering provides a more efficient as well as precise means of introducing and manipulating novel features.The cultivation of genetically modified (GM) crops has been the source of much debate. There have been significant ideological and scientific worries raised as a result of the implementation of biotechnology in agricultural settings, and these worries are still being reiterated in scholarly and popular publications [3].

The production of Genetically Modified Organisms, sometimes known as GMOs, makes use of a variety of scientific techniques, such as recombinant DNA technology or reproductive cloning.Reproductive cloning involves extracting a human nucleus from an individual cell and inserting it into the empty cytoplasm of a fertilized egg. This results in the formation of a new egg (egg cells that have had their nuclei removed are called "enucleated eggs"). Following the procedure's completion, an offspring will be generated that is genetically indistinguishable from a donor. Dolly, a sheep, became the first animal to be cloned to use a nucleus from an adult donor cell when she was born in 1996. (as opposed to a donor embryo).

Since then, several new species have been created using reproductive cloning. Recombinant DNA technology, in contrast, hand, includes incorporating one or even more genes from one species into the deoxyribonucleic acid (DNA) of another species. Although this approach has been documented, it is now only employed for basic scientific study. As seen in Figure 1, this includes introducing one bacterial genome into the "cell body" or cytoplasm of another microbe [4].

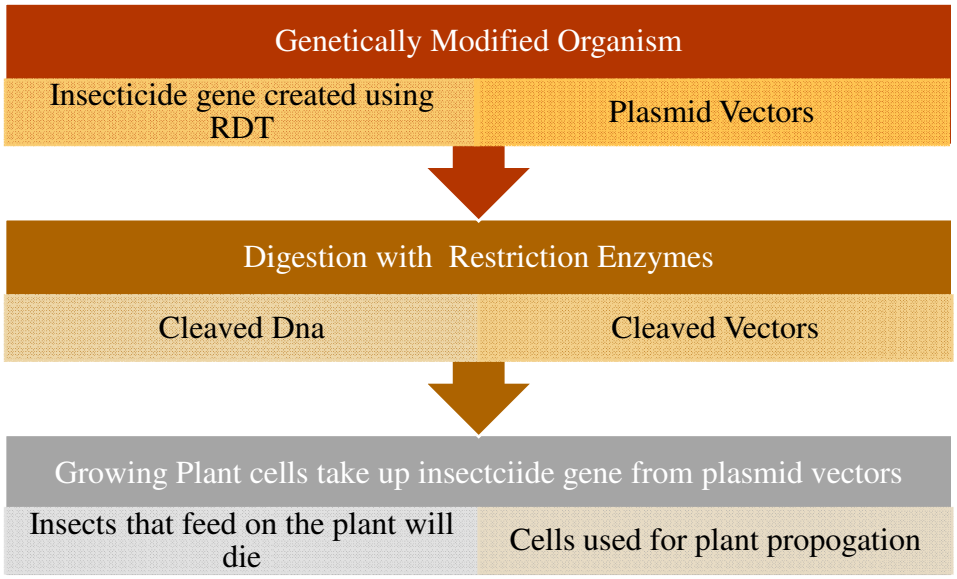

Figure 1: Using Scientific Procedures Including Recombinant DNA Technology (RDT), Genetically Engineered Creatures Are Generated.

Agriculture, health, research, and even environmental management all use genetically modified organisms (GMOs) created by modern biotechnology. The manufacture of genetically modified

organisms (GMOs) remains a very contentious issue in many areas of the globe, even though they have provided numerous benefits to human civilization.Designer food is food that has been created to provide additional health advantages beyond those provided by the dish's original ingredients.The same concept is under several names: fortified food, designer food, and functional food, they are simply terms for foods that have had nutrients added to them or that have had additional nutrients added to them to supplement what is already there. Developed in Japan in the 1980s, the phrase "functional food" describes manufactured foods that include nutrients that have health advantages beyond their basic nutritional composition [5].

This study addresses some of the most persistent issues about the health risks, environmental and ecological dangers, and safety problems associated with genetically modified foods and recombinant technologies and also examined the current worldwide regulatory landscape as well as the advantages of various designer meals. This review study focuses mostly on the significance of genetic engineering, the potential dangers involved, and the current condition of the public discussion around genetically modified organisms in particular.

2. LITERATURE REVIEW

According to a study by Kaya *et al.* titled in turkey urban consumers' opinions toward Genetically Modified Organisms (GMO) and food unfavorable attitude is due to the factors connected to the health of consumers, the environment, and also the biological variety and natural resources.Consumers in Turkey's major cities feel that consuming genetically modified foods is harmful, which are known to be harmful, carcinogenic, allergic, biological pollution, intoxication, infertility, or organ failure may lead to antibiotic resistance when they are manufactured or ingested [6].

The majority of female respondents in Algan Ozkok's research (2015) on consumer perceptions of genetically modified foods were found to have an unfavorable GMO-related attitude 73.2 percent of interviewees (n=957) think that GM foods contain hormones. 72.1 percent of participants(n=969) consider that individuals who eat GM foods may develop cancer, whereas 71.5 percent (n=962) consider that GM foods might induce allergens in humans, and 66.1 percent of participants (n=967) feel that GM goods are dangerous to humans [7].

Magdalena *et al.* stated in a study that an animal model was used to investigate the cholesterol-lowering and inflammation-reducing effects of CLA-enriched eggs. Atherosclerotic plaque size, as well as thestiffness of smooth muscle cells and the density of atherogenic macrophages, were all shown to be considerably reduced in CLA-enriched eggs compared to CLA-supplemented eggs, according to the scientists. The author concluded that Adding chromium to hen's feed at a dosage of 200–800 ppb reduces cholesterol in the egg yolk by 13.9–33.7 percent [8].

Marta Kramkowska *et al.* proposed in a study that unbiased information on the possible advantages and hazards of eating transgenic food. The potential for genetic manipulation of animals and plants to enhance the world's food crisis, enhance agricultural yields, boost food nutrition, or create medicinal formulations with proven therapeutic importance are all compelling arguments in favor of the practice. The author of recent research came to this conclusion: We need more comprehensive studies that reliably assess the impacts of eating food engineered using genetic engineering methods [9].

Accurate flavonoids such as quercetin, naringenin, and catechin inhibited the production of Hakimuddin *et al.* discovered that normal "Human Mammary Epithelial Cells" (HMEC) surpassed MCF-7 cancer cell growth. This study also found that red wine polyphenols have an anticancer effect against breast cancer cell lines (MCF-7). The research discovered that cytotoxicity was rather low in healthy people [10].Cankaya and Iscen stated in a study that investigated the levels of knowledge possessed by potential science and technology instructors as well as their perspectives on GMOs. According to the results of the survey, almost all of the individuals who are considering careers in teaching believe that genetically modified organisms are hazardous and that they should avoid ingesting GM foods. [11].

Genetically modified foods are foods that have had gene sequences either added or removed to produce them. The term "nutrition" refers to the incorporation of necessary nutrients into one's diet.The goal of this discussion is to conduct an impartial investigation of the scientific basis for the majority of the benefits of GM foods for evaluation.Cereals lack the important amino acids Lysine, Tryptophan, and Threonine, while pulses lack the sulfur-containing amino acids Cysteine, Threonine, and Methionine. Diseases including kwashiorkor, infertility, blindness, anemia, and abnormal childbirth are all linked to poor nutrition. Numerous researchers have tried to enhance the nutritional content of food crops using genetic engineering to meet this requirement.Two scientists, Watson and Crick, identified the double helix structure of DNA, a finding that has sparked a revolution in biology. Transgenic alteration of plants or animals' food was made feasible by the work of other scientists [12].

2.1.Genetically Modified Food:

Crossing one plant with some other plant of the same kind of a related species was previously required to combine the desired genes in a single plant. This included mating one plant with some other plant of the same kind or of a species that has been related to it. Growing crops that have a better yield or enhanced quality, resistance to pests or diseases, and sensitivity to heat, cold, or drought is favorable from both an economical and an agronomic point of view. The presence of desirable genes in plants may endow them with the ability to withstand harsh environments.Transgenic technology has made it feasible to mix beneficial genes drawn from a broad range of living sources in a very simple manner.

Recombinant DNA technology (RDT) holds the promise of facilitating the development of a humanly idealized and purposefully crafted living being. Any food that contains or is produced from a genetically altered organism is considered GMO [13].Trees and grain crops have both benefited from this kind of genetic alteration. It is common practice to utilize DNA-coated particles as carriers in the physical approach of biolistic transformation, through which the desired genes are injected into plant cells [14]. Electroporation is a technology that is similarly helpful in facilitating the incorporation of foreign genes into thehost organism's genome. This technique is appropriate for use with plant tissues that do not include cell walls. DNA is introduced into plant cells via tiny holes that are momentarily created by electromagnetic pulses [15]. Crystals on a small scale are another potential source of these holes. Microinjection, which involves the direct incorporation of DNA into the genome, is an additional approach that was developed more recently [16]. Antisense technology is also an effective approach for inactivating certain genes, like those that are accountable for the ripening process as well as the defense of plants against viral diseases [17].Foods that Are Typically modified genetically are:

2.1.1. Golden Rice:

Ingo Potrykus was the one who came up with the idea.Genetically modified to produce a Precursor of beta-carotene (pro-vitamin A) in edible rice parts, "golden rice" has several health benefits (endosperm). Vitamin A is a nutrient that is lacking in the diets of more than 120 million children throughout the globe. Golden Rice can contribute to the reduction of the one million to two million fatalities that occur annually due to a lack of this nutrient.The endosperm, which would be the portion of rice that humans consume, can synthesize beta-carotene spontaneously.Naturally occurring beta-carotene is a pigment found in rice leaf tissue that plays a role in the photosynthesis process. Furthermore, because photosynthesis does not take place in the endosperm of the plant, it is not natural for the plant to create the color in the endosperm. Two genes, 9 Psy (Phytoene synthase) and 9 Lyc are responsible for the production of phytoene in rice (lycopene cyclase) responsible for beta carotene production in rice were introduced to make golden.

To ensure that Phytoene Synthase (Psy) and Lyc (Lycopene cyclase) were exclusively generated in the endosperm of the rice grain, it was required to integrate the genes into insert them into theplant's nuclear genomes and control them through the use of an endosperm-specific regulator.The rice would become red if the plant collected lycopene, but this would be counter to the intended outcome. Recent research has identified an indigenous enzyme in the plant responsible for the yellow coloration of yellow rice. This enzyme transforms lycopene into beta-carotene in the endosperm. It's because of this that the rice is a certain shade of brown.

2.1.2. Cold tolerant tomato:

The incorporation of an antifreeze gene from a cold-water fish allowed scientists to create a tomato plant that could withstand temperatures as low as 0 degrees Fahrenheit. The chilled water flounder, a fish that could survive in very cold circumstances, is the source of the freezing resistance genes. This gene in the flounder allows it to create a chemical antifreeze. This is created when DNA from the refrigerant is fused with a plasmid. Recombinant DNA is the name given to this kind of hybrid DNA that results from the mixing of DNA from 2 distinct origins. A bacterium is used to house the recombinant DNA, which includes the gene for the antifreeze protein. It is permitted for the bacteria to multiply several times, which results in a large number of copies of the recombinant DNA.There are two naturally occurring genes in tomatoes: Old-Gold-Crimson (OG-c), which acts as an antagonist to the Hp (high pigment) gene that causes the absence of beta-carotene, and the high pigment gene, which expresses beta-carotene. The expression of the hp gene causes the fruit to become red and boosts its beta-carotene contents by 40 percent.When the OG-c gene is activated, the fruit turns a deep red hue and the amount of beta-carotene in it reduces by a ratio of 40.

2.1.3. Amflora Potatoes:

The Granule Bound Starch Synthase (GBSS) gene was silenced by inserting an antisense copy of this important enzyme in the biosynthesis of amylose. The bioengineered potatoes with added protein:

- In contrast to cereal grains, potatoes are low in the essential amino acids lysine, tyrosine, methionine, or cysteine.
- Amaranth seed albumin (AmA1), a tuber-specific protein, has been employed in potato transformation.

- The AmA1 protein has an optimal combination of amino acids. It has a higher concentration of beneficial amino acids than is reported by the World Health Organization.
- The absence of allergens in the isolated protein was a major factor in its selection. Total protein content increased by 35–45% in transgenic tubers after AmA1 gene insertion, with increases of 2.5–4 times in lysine, tyrosine, methionine, or cysteine.

2.1.4. Genetically modified maize (corn):

There are now corn strains that are resistant to glyphosate herbicides. Pioneer Hi-Corn hybrids resistant to imidazole herbicides have been sold by Bred under the brand name "Clearfield," although the trait for herbicide tolerance was developed into these hybridsbarring genetic engineering from usage.The regulatory structure that governs authorization, usage, advertising, as well as consumption of genetically modified crops, on the other hand, does not apply to imidazole-tolerant maize. This is because of the way that the corn was genetically modified. Corn that is genetically modified to be resistant to herbicides is being cultivated in the United States [18].

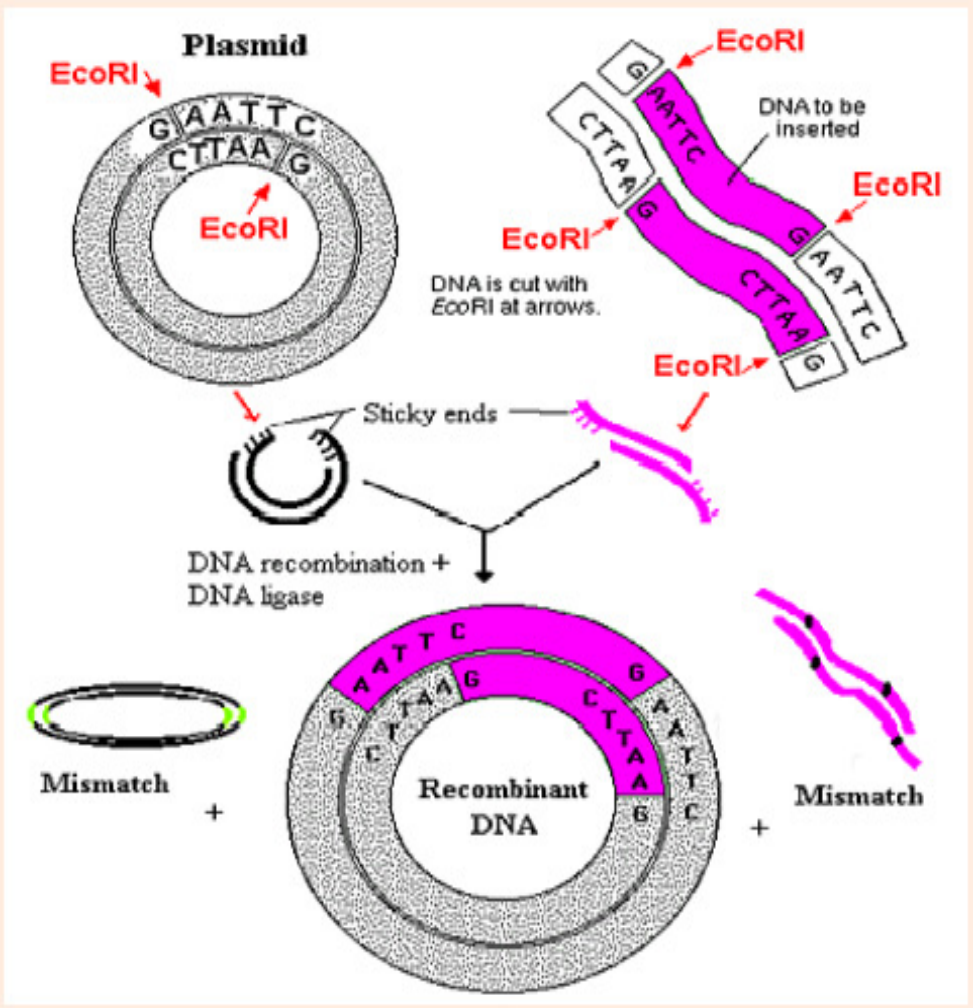

Figure 2: Gene flow: The Transfer of Genetic Material Across Living Organisms [19].

As shown in Figure 2, track out an organism that has the sought-after quality. Determine the specific sequence of genes responsible for the desired characteristic. Sequences of genes may be introduced into a plant's genome. Let the modified cell mature into a plant. The plant must be allowed to multiply. DNA may be transported through a vector. A gene gun, virus, or bacterium may all serve as vectors for gene transfer.The transformation system must integrate DNA into the host genome in a way that does not alter the structure of the host genome, and the whole process may be broken down into three stages:

- The change is caused by the incorporation of a certain number of DNA copies.
- Long-term maintenance of the altered phenotype.
- Modifications to the inserted gene that affect phenotype.

The utilization of RDT technology has enabled selected advances to be made in a variety of fields, including crops, pharmaceutical industries, gene therapy, vaccine design, and bioremediation, to name a few of these fields' applications. The other refers to a method of waste management known as bioremediation, in which genetically modified organisms are purposefully released into an area to neutralize environmental contaminants (converting dangerous materials into less toxic or non-toxic compounds), with the end goal of purifying polluted soil or water in an efficient, quick, and cost-effective manner. While making an informed decision about whether or not to use genetic modification on an industrial scale, it is important to take into account not only the potential advantages but also the potential costs associated with each application.

3. DISCUSSION

Designer food is defined as foods beyond just their normal nutritional content to provide additional health advantages "Designer food," "functional food," and "fortified food" all relate to the identical thing: food that has been artificially enhanced by the addition of vitamins, minerals, and other nutrients. In the 1980s, the phrase "designer food" was used in Japan to describe processed meals containing nutrients that conferred health advantages beyond those provided by the foods' nutritional make-up. In China, designer food is utilized in traditional medicine under the name "health foods" [20].

Many different types of meals and dietary components were grouped under the umbrella term "functional foods" because of their purported health benefits. These benefits ranged from lowering the risk of certain illnesses to mitigating the negative effects of stress and obesity. Regular foods may be made more nutritious via the processes of fortification and nutrification. Functional foods include fermented foods with living cultures and genetically modified foods with increased levels of health-promoting ingredients. Considering that it comprises nutrients for brain and nervous system development, infant formula could be the first designer meal. Designer foods include, but are not limited to supplementing with live bacteria and nucleotides to improve immunity and performance in athletics as well as the inclusion of docosahexaenoic acid (DHA) in healthy beverages to improve brain or visual development.The following Table 1, provides a summary of the numerous designer meals' positive effects on one's health. Food may also be modified via the process of fermentation. Traditional medicine in many nations, such asIndia, China, and Japan, has a long history of consuming fermented foods for their purported health benefits. These fermented foods include fermented red wine, yogurt, tempeh, red yeast rice, as well as other foods.

Table 1: Displays the Overview of the Nutritional Value of Designer Foods.

Macro and Micro Nutrients	Designer Foods	Health benefits
Conjugated linoleic acid (CLA)	Eggs and milk with added CLA	Inhibitor of adipogenesis, carcinogenesis, atherosclerosis, and inflammation
Omega 3 fatty acid	Eggs, oils, and milk fortified with omega-3 fatty acids	Treatment for Heart Disease, High Blood Pressure, Autoimmune Disease, Allergies, Nervous System Disease, Pregnancy, or Rheumatoid Arthritis
Glucoraphanin	Sprouts of broccoli fortified with glucoraphanin	Reduce the cancer risk
Vitamin D and calcium	Milk with added calcium and Vitamin D	Reduces PTH levels, slows bone resorption, and protects postmenopausal women from gaining weight.
Vitamins	Golden Rice	Medications and treatments for vitamin deficiencies
Folic acid	Grains that have been enriched with folic acid	Decreases the possibility of babies being born with neural tube defects

3.1.Function Of Designer Meals In Cancer Prevention:

Many cancer-fighting compounds may be found in diet or plants. To effectively prevent cancer, those components must be used. Incorporating "designer foods" into a healthy diet is a promising strategy for increasing the consumption of cancer-fighting compounds.The use of designer foods as a cancer prevention strategy has been shown effective by several studies. The incidence of tumors, when bovine milk lactoferrin and polyphenols from black tea were combined, the development of hamster buccal pouch carcinomas, the effectiveness of carcinogen-metabolizing enzymes, and cell redox state were all considerably decreased given to the animals through their diets [21]. Inhibitory activity on breast cancer cells has been shown for polyphenolic chemicals found in red grape wine, like anthocyanins and flavonoids. Selective cytotoxicity against MCF-7 cells was produced by the polyphenols' induction of calcium release that occurred due to a disruption in mitochondrial activity caused by membrane damage. The polyphenolic fractions also had a distinct antioxidant impact on cancer cell lines, in addition to their anticancer activities.

Many cultures throughout the globe like drinking tea. Tea's protective effects against diseases including heart disease and cancer have been shown in scientific studies. The *Camellia sinensis* plant, which is used to make tea, is grown all over the world because of the polyphenols it

contains. In animal trials, both black tea and green tea showed promise in lowering the incidence of numerous malignancies including those of the lungs, stomach, esophageal, duodenal, pancreatic, brain, chest, or colon.

3.2.The use of biotechnology in the manufacturing of designer foods:

The development of gene technology made it possible to manipulate genes in crops and to employ plants as "pharma" factories for the production of various medications. By modifying a significant milk component in high-producing dairy cowsit is feasible to improve the functional properties of dairy milk by using transgenic technology. The most rapid advancements in dairy biotechnology, in particular those that modify the milk's chemical make-up, have cleared the way for the production of designer milk Sabikhi [22]. Biotechnology has advanced to the point that it is now possible to genetically modify mustard (Brassica juncea) to produce large quantities of beta-carotene, which is the precursor of vitamin A.Because of the prevalence of vitamin A deficiency in India, this action was taken to address the problem. Brophy *et al.* study introduces infusing multiple copies of the bovine casein genes (CSN2 and CSN3) into bovine oocytes to produce bovine kappa-casein or beta-casein [23]. This increased the amount of casein that was present in the milk. Another research was done by Hyvonen *et al.*[24], found that the amount of human lactoferrin present in cow's milk had risen.

3.3.Consumption of genetically modified foods is linked to some health risks:

Studies have shown that animals fed GM crops suffer health problems and even die. The young sperm of rats fed transgenic potatoes and soya were aberrant, and many cows, goats, buffalo, pigs, as well as other animals that grazed on specific biotech corn had birth defects, BT-maize, GM cottonseed, miscarried, and died as a result. Furthermore, this is a controversial topic, since the biotech crop manufacturer's research found no ill effects from consuming GM crops in rodents.Although the Agri-biotech industry does not identify a clear link between GMF use and a public health problem, opponents provide various examples. Foodborne disorders, like soya allergies, have become more common in the United States and the United Kingdom during the past 10 years, and there has been an epidemic of Morgellons syndrome in the United States. Hundreds of Indian farmers and cotton workers have reportedly experienced skin allergies, according to media reports. Bacillus thuringiensis corn produces an allergenic protein that modifies the body's immune responses, according to the latest studies.

It would indicate that the testing procedures that are currently being employed in biotechnology firms are insufficient. For example, only the results of chemical analyses of certain nutrients are published, and in general, genetically modified crops are considered to be on par with traditional crops when there are no notable discrepancies found in the compound compositions of either product. It is suggested that this strategy will ensure that the genetically modified crop is healthy enough to be trademarked or grown commercially.There are further challenges in determining whether or not GM crops cause allergic reactions. Identifying whether or not a GMF is allergic is facilitated by in vitro studies where the gene responsible for allergenicity is recognized,cod proteins, and the alpha-amylase anti-nutrient factor gene. Of course, animals and human experiments are necessary, and data bank investigations are beneficial, for determining the strength of GMF compounds in the digestive tracts. It is challenging to determine if a novel GM crop with a gene transplanted from and before it enters the food chain, an unknown allergenicity source is allergenic because the incorporation of a non-allergic gene could produce excessive production from an already existing small allergy.

4. CONCLUSION

Genetically modified (GM) crops are living, which means they may travel and expand to distant locations. These demands sending strong signals to the biotech industry to move cautiously and avoid doing any damage to people or the environment by accident. Consumers should be able to demand that GM food items be labeled as such, that their security or environmental implications be independently tested, then those responsible for any harm caused by GM crops be held financially liable.The designer food method has many benefits, including the ability to consistently provide the necessary nutrients without requiring a shift in the general population's eating habits. The current food production and delivery system can be seamlessly integrated with it. Designer foods were crucial in the Western world in enhancing the dietary quality and eradicating nutritional gaps.By improving production or decreasing dependency upon synthetic pesticides and herbicides, GM crops can facilitate environmental protection while also addressing global hunger and inequalities. Safety testing, legislation, regulations, and food labeling are all sectors that still need improvement. Many think that genetic engineering will play a major role in the future and that must embrace it because of the great advantages it promises.A lot of work has to go into figuring out how individuals feel about this gene technology. Also significant to mention is the public's skepticism of organizations and also the widespread belief that such bodies are not adequately addressing real concerns about GMOs as part of the risk management efforts.

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CHAPTER 8

A COMPREHENSIVE STUDY ON SYSTEMATIC CANCER MANAGEMENT WITH OPPORTUNITY FOR FUTURE RESEARCH

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ABSTRACT:

Cancer is a disease that can damage different body organs and its uncontrolled cell division inside the primary organ affected by the illness occurs. The uncontrolled growth of cells occurs inside the target organ during the early stages of cancer, but as the disease progresses, it also spreads to other organs. In this paper the author discussed the stages of cancer when a patient has early-stage cancer, there are many different treatment options available, including hormone therapy, chemotherapy, bone marrow transplantation, surgery and medication therapy. As a result, the study sheds information on the pharmacokinetics and action of several cancer-treating medications inside the body. The author concludes that the action of anti-cancer medications inside the body in terms of limiting the growth of cancer cells and the method through which the drugs are released from the body. The current study creates opportunities for future research into several additional disorders and potential treatments.

KEYWORDS:

Anti-cancer, Cancer, Drug, Lung-Cancer, Pharmacokinetics.

1. INTRODUCTION

Cancer is a condition in which the organ's cells multiply out of control. There are several forms of cancer in which the body's cancer cells multiply uncontrollably. Worldwide, there are a large number of cancer-related fatalities. Cancer cells can multiply uncontrollably and can invade other organs to do the same to those organs. Cancerous cells divide into a specific organ during the early stages of the disease, but eventually cancerous cells spread throughout the body.

Despite being the illness that results in the second-highest number of fatalities worldwide. In recent decades, there have been fewer fatalities overall. The decrease in fatalities is attributable to technological innovation, the development of novel medications, and novel methods of cancer therapy. Skin cancer, prostate cancer, kidney cancer, breast cancer, bladder cancer, liver cancer, lung cancer, and many more forms of cancer exist.Figure 1 illustrates the Global cancer Incidence in different countries [1]–[3].

1.1. Lung-Cancer:

The sort of cancer that primarily affects a patient's lungs is called lung cancer. In the patient's lungs, cancer cells proliferate. The main contributors to lung cancer are smoking and unchecked pollution. Additionally, passive smokers have a significant chance of developing lung cancer.

With each additional cigarette smoked, a smoker's chance of developing cancer rises. Shortness of breath, coughing up blood, chest discomfort, chronic cough in the lungs, weight loss, and severe headaches are among the symptoms. Lung cancer detection is done using a variety of techniques, including physical examination, lab-based tests, imaging tests, and biopsy.

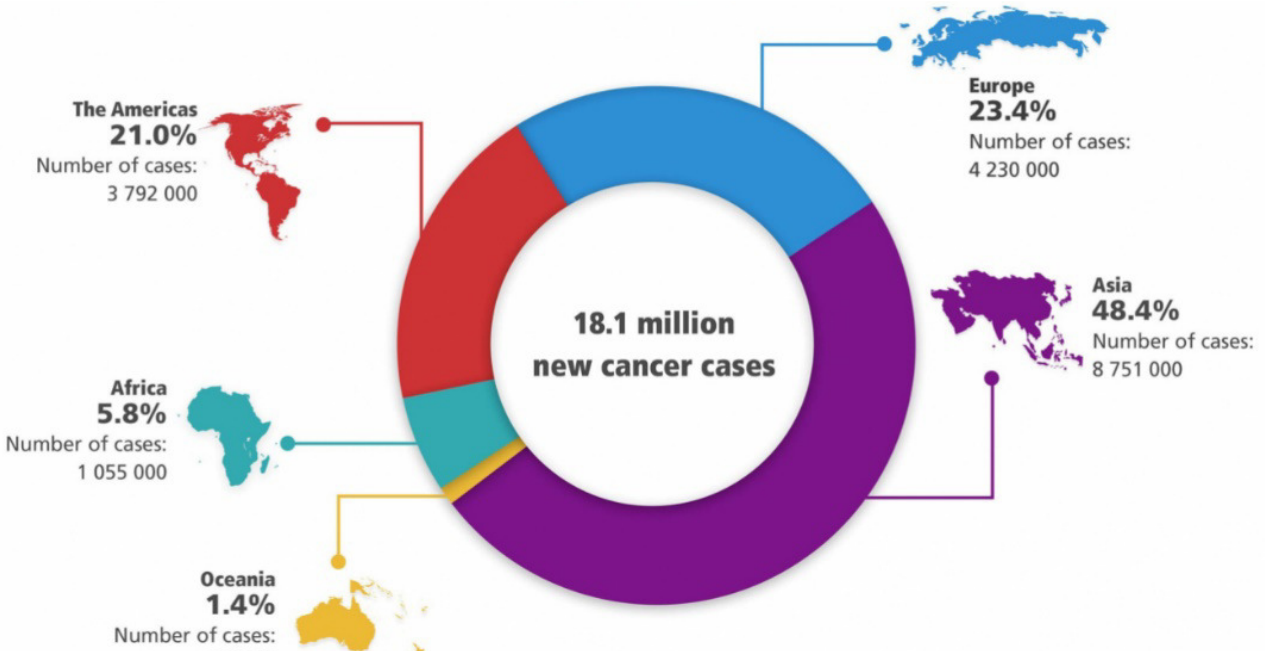


Figure 1: Illustrates the Global cancer Incidence in different countries [4]**.**

1.2. Treatment Of Lung Cancer:

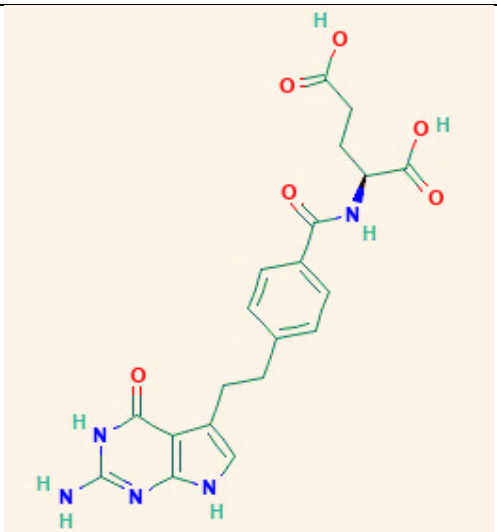
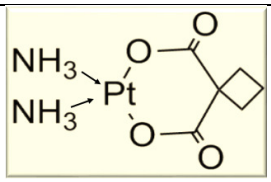
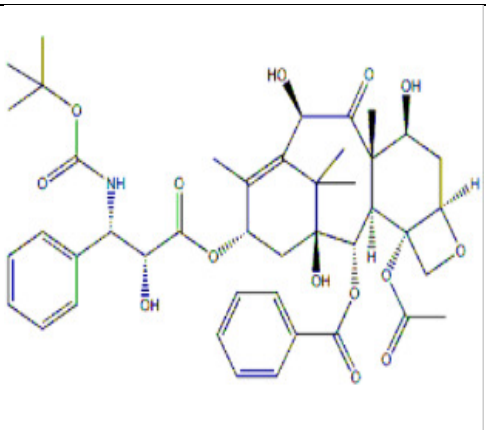
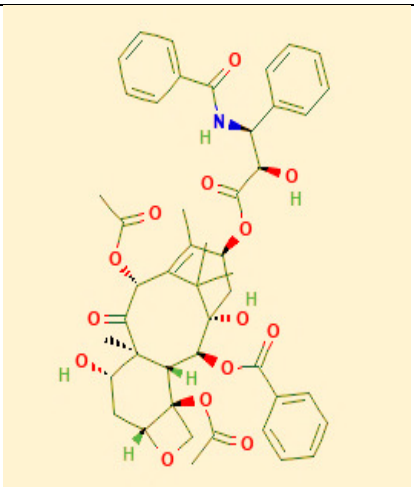
Depending on the stage of the disease, a doctor may recommend a certain method of therapy. Different types of therapy include: Chemotherapy involves administering medications to the patient to kill cancer cells. In the course of surgery, cancerous cells are removed from the organ.

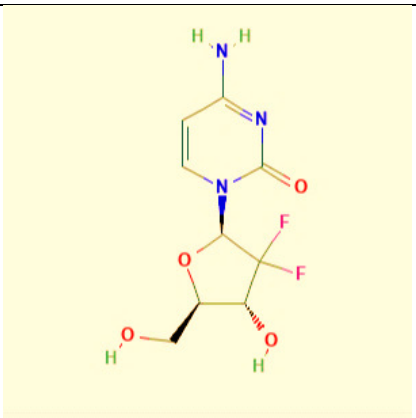
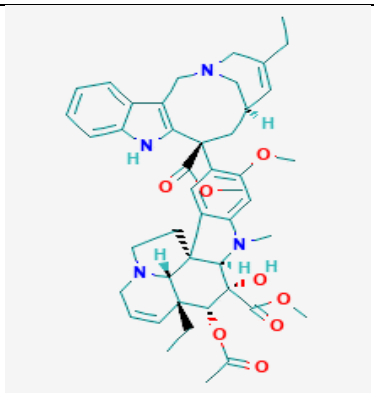
In radiation therapy, High-intensity radiations, such as protons and X-rays, are either retained inside the body, as in brachytherapy, or directed on the organ by an external machine source. Bone marrow transplant: This procedure is sometimes referred to as stem cell transplant. The doctor may administer a high dosage of chemotherapy because the bone marrow inside the bone produces blood cells. Immunotherapy uses the immune system to fight cancer cells.

Many patients' immune systems could not recognize the presence of cancer cells inside the body [5]–[7]. Immunotherapy allows the body's immune system to recognize cancer as foreign and attack it. In hormone therapy, in the case of prostate and breast cancer hormones secreted inside the body act as a fuel for the growth of cancer cells. Thus, restricting the secretion of hormones help in limiting the spread of cancer. Table 1 shows the list of various drugs used for treating cancer.

Table 1: Shows the list of various drugs used for treating cancer [8]**.**

Sl. No.	Drugs used for treating Lungs Cancer	Structure

1.	Pemetrexed	
2.	Cisplatin/Carboplatin	
3.	Docetaxel	
4.	Paclitaxel	

5.	Gemcitabine	
6.	Vinorelbine	

- *Pharmacokinetics of Gemcitabine:*

The drug hinders the elongation of deoxyribonucleic acid (DNA) of cancer cells and thus acts as an anti-cancer drug. The peak concentration of Gemcitabine drug in blood plasma reaches within 15 to 30 minutes of applying an intravenous injection. The peak concentration observed is 10 to 40 mg/L. The drug also gets delaminated inside the body. The deamination reaction takes place liver, kidney, blood, and other tissues, and following the route the drug is excreted out of the body via urine [9]–[11].

- *Pharmacokinetics of Paclitaxel:*

The drug gets attached to plasma protein. Almost 89 to 98% of drugs get attached to plasma protein. The drugs like ranitidine, cimetidine, and diphenhydramine do not affect the protein binding of paclitaxel. Within 120 hours of infusion of dose, 71% of the drug is excreted through feces. Whereas, 14% of the drug is excreted via urine

- *Pharmacokinetics of Docetaxel:*

Docetaxel binds with protein. 94% to 97% of the drug binds with protein, majorly with albumin, a1-acid glycoprotein, and lipoproteins. The drug is metabolized in the liver. Docetaxel is excreted through the fecal route as well as urine. But, excretion of the drug via fecal route is at the rate of 6 to 75%. Pharmacokinetics of Vinorelbine, The absorption of the drug takes place rapidly and reaches peak serum concentration within 2 hours. The protein binding of Vinorelbine drug is 80 to 90%. The drug gets distributed in various organs like the heart, liver, brain, and kidney. The highest quantity of drug is found in the kidney and liver and traces of drug reaches the brain and heart. The drug gets metabolized in the liver and excreted via the hepatic route.

Excretion through urine is 20% in case of intravenous injection, and 30% to 60% of the drug is excreted via the fecal route. The mechanisms of action of many anti-cancer medications are listed in Table 2.

Table 2: Shows the Mechanism of Action of Various anti-cancer drugs.

Sl. No.	Anti-cancer drug	Mechanism of Action
1.	Carboplatin	Carboplatin is a chemical compound used in treating cancer. Carboplatin is an anti-cancer drug attached to platinum. The mechanism of action of carboplatin includes the activation of carboplatin inside the cancer cells and forming a platinum-complex inside cell. The platinum complex form inter and intra DNA strand cross-linkage inside the cell. Thus modification of the structure of DNA takes place and alters the synthesis of DNA. Thus, alters the phase cycle of DNA synthesis of the cancer cell.
2.	Pemetrexed	The drug is an antifolate drug that targets multiple enzymes like dihydrofolate reductase, glucosamine ribonucleotide formyltransferase, and thymidylate reductase. These enzymes are involved in the synthesis of pyrimidine, purine, and the metabolism of folate in cancer cells. The drug has vast antitumor activity and has shown good efficacy in treating various cancers when used along with cisplatin drugs. An enzyme thymidylate synthase, an enzyme dependent on folate acts as a catalyst in the transformation of deoxyuridine-monophosphate to give deoxythymidine-monophosphate. The antifolate drug, Pemetrexed reduces the secretion of thymidylate synthase, thus reducing the secretion of thymidine required for the synthesis of DNA. Pemetrexed also reduces the secretion of GARFT (Glycinamide-Ribonucleotide-formyltransferase) which is an enzyme-dependent on folate and plays a significant role in the synthesis of purine in cancer cells [12]–[14].
3.	Vinorelbine	The drug shows anti-cancer activity by restricting the metaphase of mitosis. Vinorelbine attaches with tubulin and thus hinders mitosis. The drug attaches at the spindle position of mitosis. This alters the segregation of chromosomes during the process of mitosis. Vinorelbine mainly targets the microtubule of cancer cells that are made up of polymer.
4.	Gemcitabine	Gemcitabine is a potential analog of deoxycytidine. Gemcitabine is converted into active compounds gemcitabine-triphosphate and gemcitabine-diphosphate. The active compounds are formed by phosphorylation via deoxycytidine kinase. The active compounds are nucleosides and show anti-cancer properties. Triphosphate and diphosphate form combines

		to inhibit the elongation of DNA of the cancer cell. DNA chain is terminated by masked-DNA chain. As the gemcitabine triphosphate gets incorporated into the DNA chain, fragmentation of DNA, termination of chain, and death of cancer cells take place.
5.	Paclitaxel	Paclitaxel alters the normal functioning that is required for the growth of the microtubule of the cancer cell. Colchicine and similar drugs depolymerize the microtubule. Paclitaxel hyper-stabilizes the structure by binding with tubulin's beta subunit. Tubulin is the building block of the microtubule. The complex of paclitaxel-tubule alters the length of the microtubule i.e. shortens or increases the length. Chromosomes of a cancer cell depend on the ability of the microtubule for mitosis. Thus, paclitaxel causes the death of cancer cells [15]–[17].
6.	Docetaxel	Docetaxel binds with one of the microtubules. Microtubules are utilized by cancer cells during mitosis. Thus alteration in the length of the microtubule results in the death of cancer cells. The drug also initiates programmed death of cell/apoptosis in cancer-causing cells. Apoptosis is caused by binding with protein B-cell-leukemia-2 of the cancer cell.

2. LITERATURE REVIEW

Thandra et al. in their study embellish that research on lung-cancer epidemiology in the United States and China and suggested that although there is a huge cultural and environmental difference between China and The United States, both countries have a high rate of mortality due to lung cancer and cases of people suffering from the disease are also very high. A link between high air pollution and a high rate of smoking in both countries is observed. Thus, it is the responsibility of both countries to analyze the pattern of cases of lung cancer and to take efforts to reduce the cases. Efforts must be made by controlling the air pollution in both countries and the prevalence of smoking in both countries. Thus, the study determines the common pattern of lung cancer cases in both countries and suggests that measures should be taken to prevent the prevalence of lung cancer cases in both countries [18].

López Escalera et al. in their study illustrate that lung cancer is a serious problem in the U.S and across the globe. The advancement in medical sciences is happening to reduce the death rate, but, the death rate due to lung cancer is the highest among all types of cancer. In the United States, the mortality rate has reduced in the last few years. But, in other countries across the globe, the death rate due to lung cancer is very high. The countries that are developing and where the middle-lower income group population is a high show high mortality rates. The cause of the high mortality rate is the increasing use of tobacco. The stopping of the use of tobacco in the U.S has reduced the mortality rate. But, it is equally important that the role of non-tobacco in causing lung cancer must be analyzed including non-cigarette-tobacco-smoking products. Efforts are required to stop people from smoking to reduce air pollution and inhaling carcinogens [19].

Nasim et al. in their study embellish that there are various ways at the legislative level to reduce the occurrence and casualties related to lung cancer. The legislative work in making strategies to reduce the use of tobacco must be done. Legislation must be made to restrict the advertisement and limit the access of tobacco and cigarette to the young population. The access of cigarettes to children and smoking in the workplace must be prohibited. If all the suggested strategies must be made and implemented in the right way a significant reduction in cases of lung cancer and mortality rate will be seen shortly [20].

Several research has been conducted to assess the instances of lung cancer and the death rate brought on by lung cancer, according to the mentioned literature. The study contends that despite significant environmental and cultural differences, lung cancer incidences in China and the United States are primarily caused by the same factors. Both nations have considerable smoking rates and severe levels of air pollution. According to research, there are a lot more incidences of lung cancer in emerging nations and nations with a population in the low- to middle-income bracket. Tobacco availability for children, workplace smoking, and cigarette marketing must all be reduced by legislative action. Therefore, efforts performed in all directions will ultimately result in a decrease in lung cancer incidence in the upcoming years. The recent study shed information on the pharmacokinetics of numerous types of medications used to treat lung cancer.

3. DISCUSSION

Cancer involves the uncontrolled multiplication of cancer cells inside the target organ. In a later stage, cancer cells affect other organs also. Various types of cancer include breast cancer, bladder cancer, skin cancer, prostate cancer, kidney cancer, liver cancer, blood cancer, lung cancer, and many more. Lung cancer targets the lungs of the patient. The main cause of the lung-cancer is smoking, passive smoking, and uncontrolled pollution, smokers are at a high risk of suffering from lung cancer. Cancer cells multiply inside the lungs of the patient the risk of cancer increases in smokers with the increased number of cigarettes the person has smoked. The symptoms include short breath, the release of blood while coughing, pain in the chest, persistent cough in the lungs, weight loss, and severe headache [21]–[23].

3.1.Pharmacokinetics of Gemcitabine:

The drug hinders the elongation of DNA of cancer cells and thus acts as an anti-cancer drug. The peak concentration of Gemcitabine drug in blood plasma reaches within 15 to 30 minutes of an intravenous injection. This anti-cancer drug also gets delaminated inside the body. Pharmacokinetics of Vinorelbine is the drug that gets rapidly absorbed and reaches peak serum concentration within 2 hours. The drug gets distributed in various organs like the heart, liver, brain, and kidney. The protein binding of Vinorelbine drug is 80 to 90%. The pharmacokinetics of Paclitaxel is the drug that gets attached to plasma protein. Almost 89 to 98% of drugs get attached to plasma protein. Thus, this anti-cancer drug does not remain readily available to the target cancer cell. Drugs like ranitidine, cimetidine, and diphenhydramine do not affect the protein binding of paclitaxel. Pemetrexed drug is an antifolate drug that targets multiple enzymes like dihydrofolate reductase, glucosamine ribonucleotide formyltransferase, and thymidylate reductase.

In cancer cells, these enzymes have a role in the creation of pyrimidine, purine, and the metabolism of folate. When combined with cisplatin-containing medications, the medicine has strong anti-cancer action and is effective in treating a variety of malignancies. Based on the

drug's mode of action, different medications destroy cancer cells. To identify the medicine with the best pharmacokinetics and mechanism of action for treating cancer. To compare how anti-cancer treatments work within the body to destroy cancer cells and how pharmaceuticals are eliminated from the body, the present study sheds light on various drugs and their effects inside the body [24]–[26].

A few anti-cancer medications include carboplatin, a platinum-attached anti-cancer medication. The method of action comprises forming a platinum complex inside the cancer cells and activating carboplatin inside the cancer cells. The intricate process of DNA strand crosslinking inside the cell. Therefore, changing the DNA's structure will change how DNA is made. Affects the cancer cell's DNA production phase cycle as a result. By limiting the mitotic metaphase, the medication has anti-cancer efficacy. Vinorelbine binds to tubulin and prevents mitosis by doing so at the mitotic spindle location. Affects the way that chromosomal segregation occurs during mitosis as a result. Vinorelbine primarily targets the polymer-based microtubule seen in cancer cells. The pharmacokinetics of numerous medications demonstrates the plasma peak concentration and route of excretion of various pharmaceuticals. Drugs become unable to reach the target location due to protein binding. Less medicine will be available to quickly influence the target cancer cell if there is a higher percentage of protein binding.

4. CONCLUSION

Lung cancer mostly affects the patient's lungs and is defined by the unchecked growth of cancer cells there. The leading cause of mortality worldwide is lung cancer. Despite being a major cause of death, lung cancer deaths have decreased dramatically as a result of technological advancements and the creation of new treatment options. Several therapeutic modalities include: Chemotherapy is the process of killing cancer cells by administering medications to the patient. Cancer cells are removed from the organ during surgical therapy. Radiation therapy high-intensity radiations, such as protons and X-rays, are either held inside the body, as in brachytherapy, or delivered onto the organ by an external machine source. Giving anti-cancer medications is part of medical treatment. Depending on the pharmacokinetics and mode of activity of the medicine, the best medication to treat cancer. To compare how anti-cancer treatments work inside the body to prevent cancer cells from proliferating and how they leave the body, the current research sheds light on various drugs' actions and effects inside the body. Depending on the patient's underlying medical issues and the severity of their cancer, each patient receives a unique prescription for medication. As a result, the current study creates opportunities for future research into several additional disorders and potential treatments.

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CHAPTER 9

A REVIEW ON COMMON DISEASES IN *SACCHARUM OFFICINARUM* PREVALENT IN INDIA AS A CAUSE OF ECONOMIC LOSS

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ABSTRACT:

Sugar cane (Saccharum officinarum) belongs to the family of Poaceae and is a perennial grass. It is a major crop employed for the production of sugar and alcohol and is used as a sweetening agent in beverages and jams. Apart from these advantages, the crop is also been used as an energy source in a form of biogas. It also assists in fixing the nitrogen in the atmosphere. Brazil contributes around 40% of total sugar cane production in the world whereas India produces 20% of it. The crop is affected by no of diseases caused by bacteria, viruses, and fungi such as red rot, wilt, mosaic disease, leaf scaled disease, and many more. Past research has reported the use of sugarcane in making rum and the isolation of sucrose. Currently, researchers are working on detecting diseases early caused by pathogens to lower crop wastage. The present review study discusses different types of infections prevalent in the crop, their detection techniques, management, and future scope in increasing production.

KEYWORDS:

Diseases, Detection Technique, Ethanol Production, Red Rot, Sugar Cane.

1. INTRODUCTION

Saccharum officinarum is a perennial grass from the family *Poaceae,* with dense steam separated into two sections of internodes and nodes. The height of the plant sometimes ranges from 2 to 4m in length. A major food source that produces alcohol, sucrose, and sweetening syrups. Warm temperature and humid conditions of tropical and sub-tropical countries support the growth of the plant. Countries like India, Thailand, Russia, Brazil, China, Mexico, and the USA produces sugarcane to meet the global food requirements as shown in Figure 2.

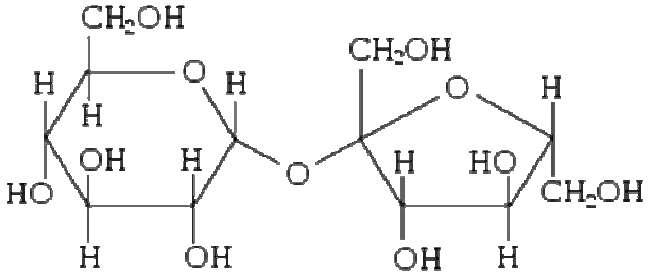

Figure 1: **Chemical Structure of Sugar or Sucrose Obtained from Sugarcane Crop Stored in a Region called Pith.**

Brazil, India, and China are the top 3 producers of the plant. The architecture of the plant is composed of thin and extensive leaves, the wide surface area of the leaves assists the plant in the process of sugar formation through the process of photosynthesis. The stem of the plant consists of lignin and cellulose that are effectively used as an alternative source of energy and chemicals with major employment in industries. Straw and bagasse are the byproducts of the cane sugar that can be employed in preparing ethanol Pith is the storage area for storing sugar, protected by waxy solid bark (Figure1) [1].

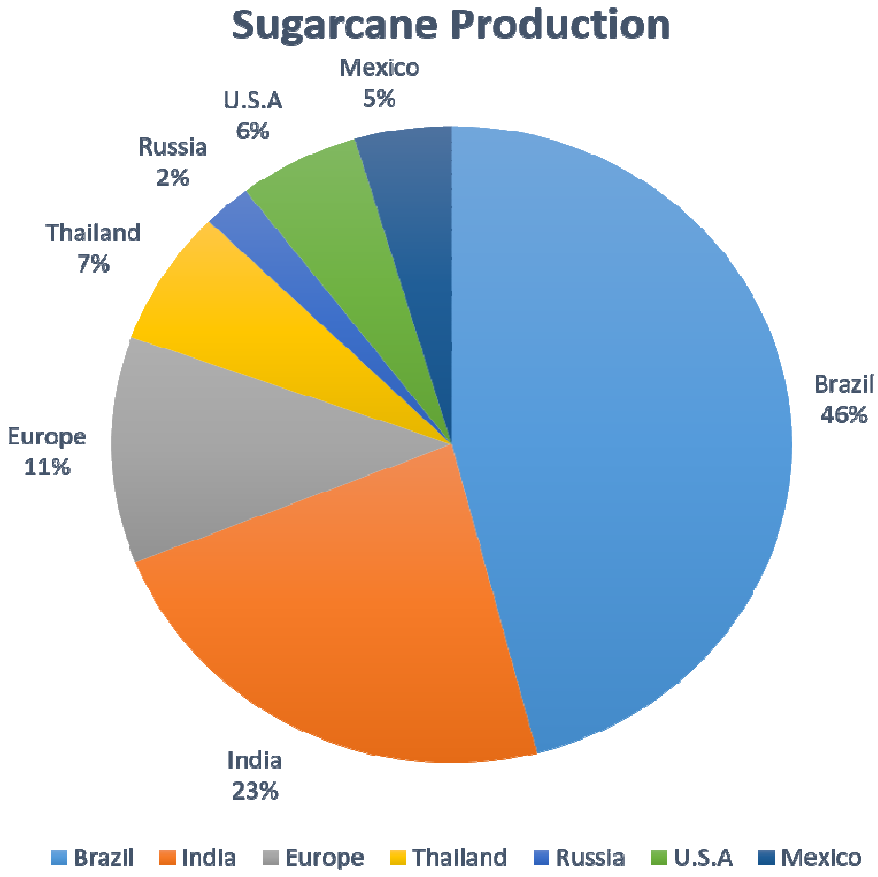

Figure 2: **Distribution of Sugarcane in Different Countries of the World. Brazil Is the Top Producer of the Crop Followed by India.**

1.1.Nutritional value

Sania Arif *et al.* discuss the nutritional benefits of sugarcane and explained the different components in the sugarcane juice as shown in Table 1. Two important energy-producing sources from sugarcane are fructose and glucose, the phenolic and flavonoids contribute to building the antioxidant activity of the sugarcane juice. The majority of the world population relies on the use of sugarcane as a sweetening source in the preparation of varieties of food products. Molasses and brown sugar are acquired as a byproduct after the process of refinement, while molasses is used in ethanol production, brown sugar has been opted for as an alternative to white sugar since it is healthy in comparison. Apart from the nutritional benefits, sugarcane also adds an advantage in medicinal use. The stem and roots of the plant help in managing skin diseases, urine infections, and issues with fluctuating blood pressure [2].

Table 1: Different Components Present in the Sugar Cane Juice Along with the Percentage Concentration.

Components in Sugar Cane Juice	
Sucrose	13-15%
Fiber	10-15%
Water	75%
Non reducing sugars	10-21%
Reducing sugars	0.3-3%
Organic substances	0.5-1%

1.2. Bagasse

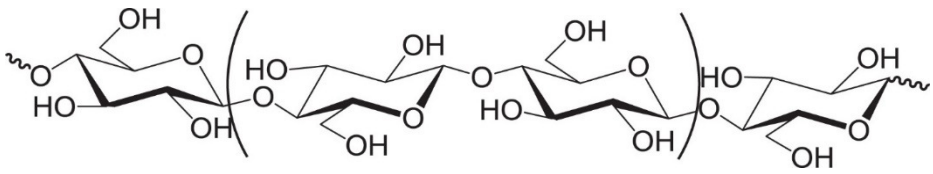

Figure 3: **Chemical Structure of Bagasse, by Product of Sugarcane Obtained After Crushing the Crop.**

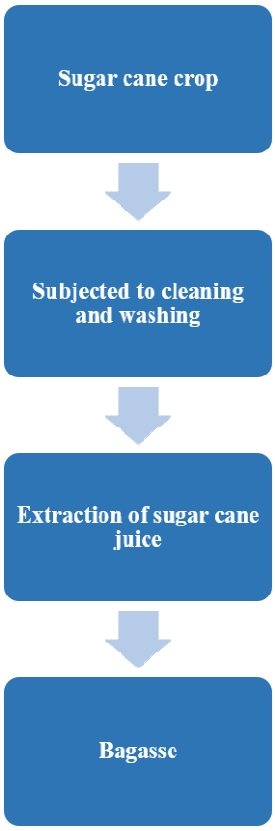

Figure 4: **Illustration of Process Involved in Obtaining Bagasse is the Byproduct of Sugarcane.**

Sugarcane is an essential crop economically as well as agriculturally around the globe. An important source for producing sucrose and alcohol. Post the process of mechanical harvesting, the byproducts from the sugarcane are obtained such as molasses and bagasse.

Figure 3 explains the chemical structure of bagasse, it is a dark brown colored fiber structure material that is employed as a biofuel. Figure 4 is illustrating the process of obtaining bagasse. The production of bagasse is around 540 million metric tons worldwide. The typical composition of bagasse consists of hemicellulose 20-25%, cellulose 45-55%, lignin 18-24%, and ash 1-4%.

1.3.Cultivation techniques

S.M. Nalawade *et al.* discuss various techniques for planting sugarcane. There are certain farming techniques to use, starting with a manual method which includes preparing the agricultural land with a help of a machine (tractor) and then harvesting the "plant canes by machetes and proceeding with de-trashing and application of insecticides to prevent the plantation from pathogenic infection".

Another method of plantation is "Ring or pit planting", this technique includes digging pits circular in shape with a diameter of 90 cm and a depth of 45cm maintaining a distance of 150cm within two pits, and covering them with 5cm loose soil. Thirdly by the use of distributors plantation of sugarcane can be done, it requires a machine with huge loading space that will dispense entire sets [3].

1.4.Common diseases in Sugarcane

Sugar cane is infected by several pathogenic agents which comprise fungus and bacteria. Red rot is caused by a fungus known as "*Colletotrichum falcatum*" the symptoms of the disease include drying of 13th and 14th spindle leaves and the propagation of the infection leads to discoloration and hollowness of stalks of the plant. One of the major identifications of the disease when encountered with the plant also makes the plant's internal tissue turn red with white spotsintermixed transversally.Leaf scale disease is caused by the bacteria *Xanthomonas albilinearns*, the visible symptoms include color fading or bleaching of the leaves of the plant and later resulting in cloudy blue-green or brown.

Another fungal infection prevalent in the sugarcane crop is "wilt disease" triggered by *Fusarium sacchari*, the visible symptoms include dropping of leaves and branches, turning crown leaves into yellow in color plus change of color in ground tissue usually in very dark brown or purple (Figure 5). Other diseases are grassy shoots, red-stripped disease, and mosaic disease [4], [5].

(A) Red rot (B) Leaf scaled disease (C) Wilt disease

Figure 5. Classification of Diseases Prevalent in Sugar Cane Pant Along with Their Symptoms.

2. LITERATURE REVIEW

Abeer A. Elsharif *et al.* described their view on the detection of various diseases in a sugarcane plant. The paper talks about the importance of sugarcane crops around the globe, and the temperature conditions should be subtropicaland tropical. Furthermore, the authors also discuss the economic significance associated with the crop and sucrose production. The authors put forward a method called CLIPS along with the employment of language Delphi for the disease diagnosis for making the planation work easy for the cultivators [6]

Xiping yang *et al.* explicated the research on studying the resistance mechanism of loci against orange rust along with yellow leaf virus in species of Saccharum. The paper discusses the occurrence of "sugarcane orange rust and sugarcane yellow leaf virus" in sugarcane leading to 50-40% harvest loss, to improve the situation use of "DNA sequence variants" are employed to manage the growing infection. The authors proposed a study associated with studying the genome for high-density markers and resistance to disease. Later it was found out that "91 putative DNA markers and 82 candidate genes were responsible for getting related to resistance in one of the two diseases" mentioned above [7]

R Viswanathan and P. Malathi discussed the Use of bioagents to prevent diseases caused by fungi in sugarcane crops. The authors have explained the use of "chaetomium" and "Trichoderma" as a fungicide against fungal diseases such as red rot. Furthermore the "presumed solution of Trichoderma" was found to be efficient for treating wilt disease under field conditions. Antagonistic use of different formulations of fungicide or single-use helps in the management of the disease [8].

Sammy V. Militante *et al.* elaborated on the research study on the diagnosis of the common disease with the help of deep learning. The authors share the disadvantages a farmer survives with when the cops are infected with the disease, to deduce the changes of unawareness the researchers proposed the mechanism of detection of disease by machine learning. The results provide 95% of accuracy and classify and identify the diseases in sugarcane [9].

C.G. Hughes demonstrated a review study on Sugar cane diseases. The author discusses various diseases like leaf-scaled disease, eyespot, red rot, and yellow spot. Further, the paper elaborates transmission mechanism, origin, distribution, and classification of the pathogenic microorganism along with the management techniques. The paper highlights the plight of farmers as the diseases cause a direct impact on the economic conditions of the farmers and result in loss of revenue [10]

H. Park *et al.* reported a research study on "employing the image-based technique for the detection of diagnosis of diseases in the crop by the use of deep learning algorithm". The authors proposed a framework for disease diagnosis and estimation by collecting the data using the module to translate the image into binary data post this step other step includes the use of testing and a learning engine for identifying the diseases from the image data [11].

Prakash Lakshmanan *et al.* outlined a research study on the biotechnology of Saccharum officinarum. The authors explicate the new advancements and consequences related to the crop. However, there have been efforts made constantly in decoding and controlling the genetic makeup of sugarcane by the use of different techniques of molecular biology. Most of these studies have resulted in the invention of new transgenic species which has added an advantage in understanding its storage and transportation [12].

Youssef Abu Ahmad *et al.* delineated the study on "Genotypes of four yellow leaf virus species concerning their location". The authors designed a primer study for evaluating the four species of the yellow leaf virus via Reverse Transcriptase Polymerase Reaction (RTPCR), the researchers took eighteen diverse sugarcane species from all over the world along with 245 sample leaves already infested with the virus [13].

3. DISCUSSION

Sugarcane is a crop with high consumption and demand all over the world. The crop is grown with different agricultural techniques and is infected with several fungal and bacterial diseases. The management and expansion of diseases is a priority to save the farmers from economical losses and also to cope with the growing requirements (Figure 6). Also, Machine learning algorithms can be used for predicting future diseases [14]. With the advancement in biotech techniques, storage of germplasm is now possible. It is one of the efficient methods to save the genetic traits of a particular species from extinction or for future use [15]–[17]. The exchange of germplasm is a significant step for sucrose industries as it offers an alternative clone for economical uses with applications in breeding. One of the major advantages of preserving germplasm is that it offers "disease-resistant" species of sugarcane. Other management techniques differ from disease to disease such as prevention. For example, red rot can be managed by selecting the setts from the area which is not affected by the disease another management strategy is to remove the "stocks", plants attacked by the virus from the roots should also be removed completely. For the management of wilt disease use of a biological agent is necessary, using *Trichoderma* in the soil helps in managing the disease. Management strategies for treating leaf scald, replacement of species susceptible to the disease against the species who are resistant to it and seed cane can also be given the treatment of warm water to destroy the microorganism. Other factors also contribute to the deficient growth of sugar cane (Figure 7).

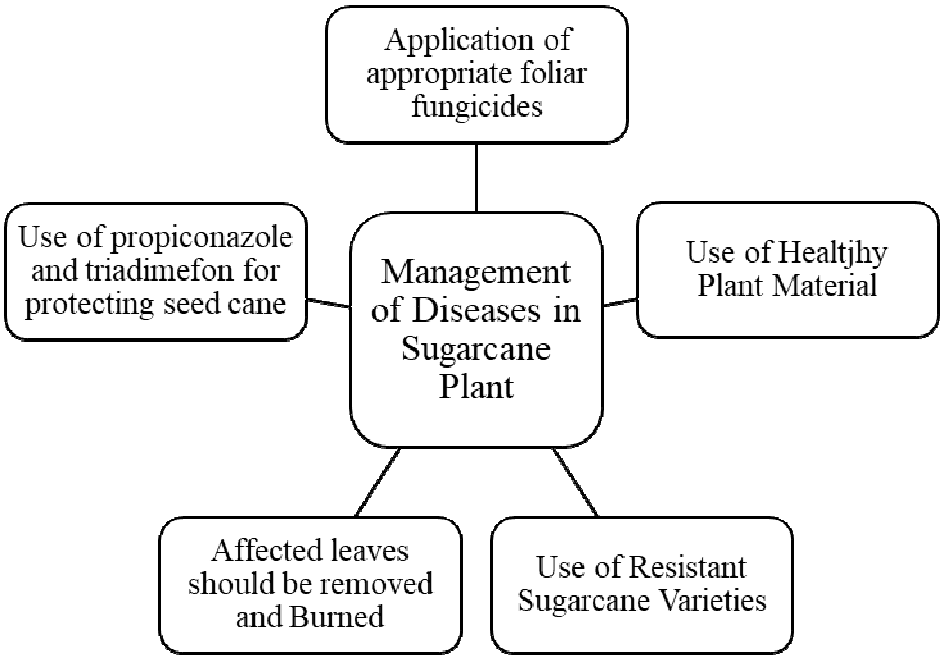

Figure 6: **Different Preventive Measures Employed for Treating Various Diseases Prevalent in Sugarcane Plant.**

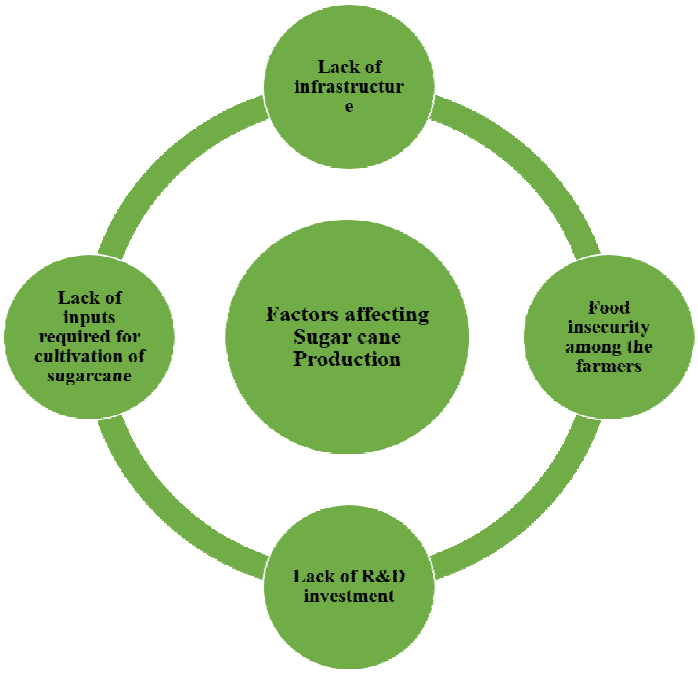

Figure 7:List of Factors Other than Diseases Affecting the Production of Sugar Cane

4. CONCLUSION

Sugar cane is a crop with worldwide demand. The plant requires certain prevention and safety measures to protect it against diseases. The above research study conducted talks about the characteristics of the crop, and its worldwide production along with a mention of countries being the top contributor to the highest production. Sugarcane also known as*Saccharum officinarum* is a plant that is easy to grow and cultivate. The paper describes and discusses various cultivation techniques that can be used for its production. Sucrose or sugar is obtained from sugarcane, since the crop has other benefits, its byproducts are also used as a biofuel. The crop is susceptible to many pathogenic diseases and infections such as red rot, wilt disease, leaf scald disease, etc. the paper elaborates on management strategies which include various steps and preventive steps for managing the economic losses associated with it.

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CHAPTER 10

APPLICATION OF NANOTECHNOLOGY TO INCREASE SHELF LIFE OF PRESERVED FOOD

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ABSTRACT:

Food preservation is the process of retaining the healthy quality and properties of a meal for as long as after preparation. Various techniques of food processing are used to increase the shelf life of the food stuff. Use of Nanotechnology can be a better choice to maintain the quality of the food stuff for a long period. The Nano-technological applications include food processing, packaging, storage, transportation, functioning, and other aspects of food safety. Microbial contamination of food is linked to pathogenic illnesses and nutritional deficiencies. Food has been effectively shielded against degradation using nano antimicrobials with better outcomes. To decrease food loss at every step, from harvest to consumption, new methods of food preservation are required. This study summarizes some of the drawbacks of food preservation techniques like nano encapsulation. This study also explores the possibilities of ozone treatments to keep heat-sensitive foods fresh. This paper explores the issue of food preservation using nanotechnology is brought forward. Nanotechnology has great potential for extending the food shelf life. High-quality food may be produced when the right nanotechnology is used.

KEYWORDS:

Food Preservation, Illness, Nanotechnology, Nanomaterial, Shelf life.

1. INTRODUCTION

Food preservation is the act of managing and preparing food to prevent its spoiling by preventing the assault and development of microorganisms that cause foodborne illness, preventing the oxidation of lipids (rancidity), or preserving the food's nutritional content, texture, or flavor. Food conservation is often referred to as food processing [1].It is generally accepted that exposure to chemicals, microbes from the surrounding environment, or food enzymes could cause food items to degrade. To be eaten, food or food items should be transported from one site to another.

Food may degrade, lose or diminish its physical attractiveness, and lose nutritional content while it is in transportation. The World Health Organization (WHO) estimates that each year, contaminated food sickens and kills over 600 million people, or around one in every 10 human beings on the planet, leading to the loss of 33 million healthy lives. Diarrhea kills an estimated 2.2 million children in underdeveloped nations every year. Poor sanitation, a scarcity of potable water, inadequate food storage facilities, and a lack of education about food safety contribute to

outbreaks of foodborne illness in developing nations. Mortality and morbidity are the most common consequences of smoking. Food-borne illness outbreaks are becoming increasingly common in many parts of the globe as a result of contaminated food. Consequently, food preservation measures must be made to ensure prolonged shelf life, stability in the product's quality, and no change in flavor [2].

To get energy and maintain development, food may be ingested either raw or prepared. In recent years, food waste has become a big problem throughout the globe. The food manufacturing and consumption process wastes a large quantity of food.According to the findings presented in the study that was compiled by "Rethink Food Waste through Economics and Data (ReFED)", the data that can be seen in Figure 1 depict the distribution of food waste across the main categories of food items. Daily, a significant number of people throughout the world go hungry as a direct result of inefficient supply networks, an expanding population, and changing climatic conditions [3].A comprehensive investigation of the amount of food waste generated by various food communities. There was 20 percent manufacturing, 1 percent processing, 19 percent distribution, and 60% consumer-produced waste in the amount of food waste created. Cooking concerns, supply chain issues, and high consumer standards were among the main causes of food loss, as well as changes in weather conditions, soil runoffs, and legislative limits [4].

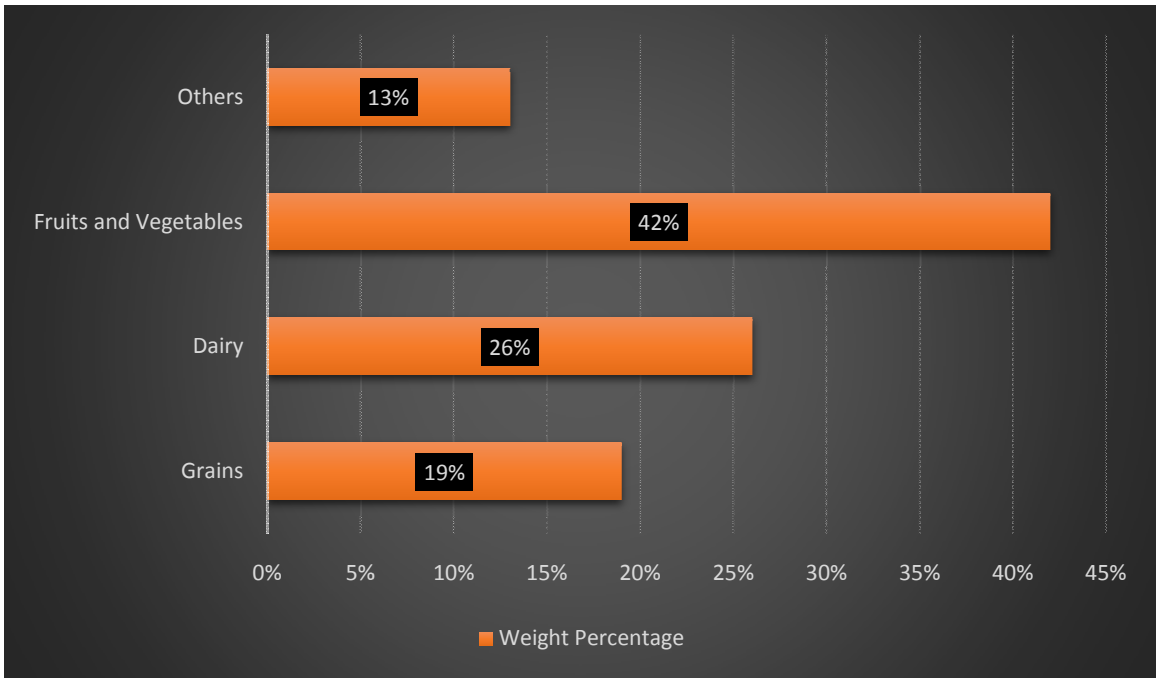

Figure 1: Proportion of food that is wasted across a variety of food ingredients depending on weight.

Furthermore, as a result of the rise in population, customers have an increased desire for food that is not only recent but also nutritious and wholesome. Even if every day there is enough food created to feed the globe, neither the technology nor the food that is produced makes it to hungry people. As a result, food waste has emerged as a primary obstacle for all areas ofthe food manufacturing business. Nanotechnology's advantages include, but are not limited to, better agricultural yields and quality, enhanced cosmetics, targeted drug delivery, and sensing applications.

In the food business, nanotechnology technologies may be used to identify illness in packaging or to improve color and safety by improving barrier properties of flavor and color ingredients and ingredients themselves. Technology based on nanoparticles has considerable potential for improving food items and the environment in which they are found. Microorganisms such as bacteria, yeast, as well as mold, enzyme activity, or other chemical reactions within the food itself all contribute to food deterioration, as do infestations by insects, parasites, or rodents, insufficient temperature for the food, moisture gain, or loss, oxygen reaction, light reaction, physical stress or misuse, and time. Heat, cold, drying, acid, sugar, salt, as well as other substances, are among the most powerful weapons for stopping the development of bacteria, yeasts, and molds. When it comes to protecting anything from the elements like water, air, and light, the most popular solution is to invest in protective packaging [5].

2. LITERATURE REVIEW

Gamazo *et al.* simplified the allergen delivery. They lowered the dosage, and the amount of allergen exposure to mast cells and/or basophils was reduced by using nanoparticles as an allergen delivery mechanism. Bio-inspired Nanostructured materials (NSMs) that are known to be free of hazardous effects are also being studied in recent years and might be used in the food business [6].

Citral essential oil is an antimicrobial that can be transferred into the food supply by the use of a nano-emulsion that was made by researchers using ultrasonic power and encapsulating citral essential oil as mentioned by Lu *et al.* in a study. The antibacterial nano-emulsion that was produced as a consequence had a size of 100 nm and was shown to be extremely effective [7].

Liqing Qiu *et al.* performed research that analyzed the danger and regulations of nanomaterials, the processes by which aquatic items go bad, and the relatively new applications of nanotechnology-related preservation approaches for marine products. Nanotechnology-related conservation strategies have been found to successfully prolong the shelf life of marine items without compromising their quality. Since concerns about nanotechnology's security have persisted, any potential applications must be evaluated with caution.

Prakash J *et al.* discussed in a study that nanotechnology is in the food industry. Antimicrobial bioactive components are contained in nanoparticles (NPs) to promote food preservation. Biocompatible and nontoxic NPs are needed. Some companies use NPS for food packaging due to advancements in this industry. The metal oxide is the most frequent NP in food. As zinc oxide and titanium dioxide NPs have antibacterial action in food, they may be employed to preserve food with increased functional qualities. Nanotechnology's influence on food's nutritional and sensory properties was briefly reviewed, along with safety requirements on nano-based food composition and preservation [8].

Nanotechnology is an interdisciplinary field that incorporates physics, chemistry, biology, and engineering. It is the application of nanomaterials, the dimensions of which nanoscale structures vary from 1 to 100 nanometers [9]. When confined to nanoscale dimensions, materials take on new and interesting features that were not present in the materials in their more familiar forms. The objective of nanoscientists all around the world is to get an understanding of these one-of-a-kind features to generate new and better goods using environmentally friendly techniques [10].

As a "snapshot" of the current condition of nanotechnology methodologies as well as implementation methodsstandards for long-term storage, little risk of contamination, and secure transport of food, this evaluation has been formulated to recognize the underlying mechanistic actions relating to safety or packaging issuesbecause of nanoparticles implications on the food business. It also examines nanotechnology's potential for the present and the hazards it may pose in terms of toxicological consequences, as well as the effectiveness and efficiency of the agencies now in charge of overseeing it. Also included are discussions of contemporary initiatives to solve these concerns and others connected to nanotechnology development, comprehension, and marketing shown in Figure 2 [11].

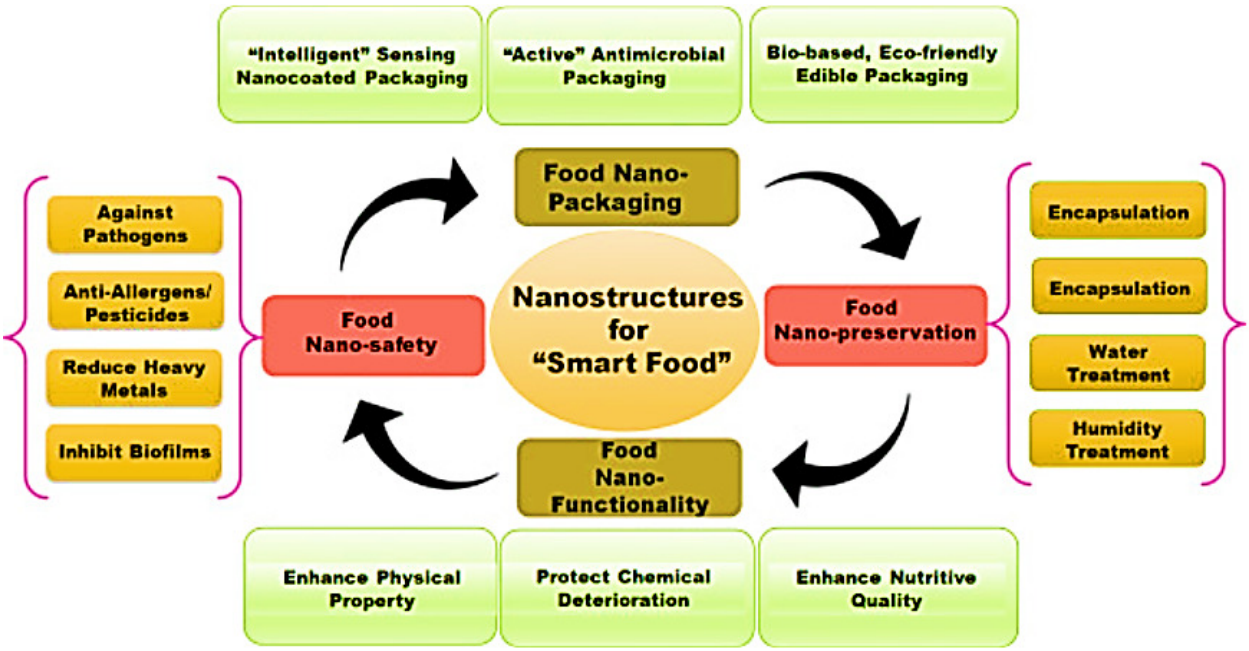

Figure 2: Displays the Systematic depiction of nanoparticle applications in the food sector [12]**.**

2.1.Encapsulation is a Preserving Food Technique that makes use of Nanotechnology:

Edible nano-coatings applied to a wide range of food materials can prevent spoilage through moisture and energy consumption, supply colors, anti-browning ingredients, tastes, antioxidants, and enzymes, and also increase the shelf-life of packaged foods after the package has been removed [13]. By modifying the interfacial layer characteristics, it is possible to slow down chemical breakdown processes by encapsulating functional components inside droplets. The use of nanometer-scale encapsulation using films, layers, coverings, or even micro dispersion is referred to as "Nanoencapsulation".

Using this technology to create functioning meals with increased functionality and durability allows bioactive ingredients like vitamins, carbohydrates antioxidants, and proteins to be preserved. This approach also enables the manufacture of much more stable nutritious meals [14].The use of nanotechnology in the food industry is in the development of packaging materials for food. Other examples of how nanomaterials are being used in packaging include antimicrobial or oxygen-scavenging nanoparticles, "intelligent" packaged food that can monitor and evaluate the status of the food, and biodegradable polymers nanomaterial composites [15].

2.2.Technologies that are often used to preserve food:

- *Thermal treatment:*

Wurlitzer *et al.* Food preservation methods that use heat or thermal treatment are deemed unique. A variety of food industries, including bread, dairy, and produce, have used this method for so many years. The procedure often entails heating the meal to a temperature that is greater than 75–90 degrees Celsius and keeping it at that temperature for 25–30 seconds. An investigation on preserving pasteurization and maize heat treatment was used to improve the taste, digestibility, glycemic index, fragrance, color, and sensory features of apple juice beverages [16]. The germs in food are reduced when it is heated. A substantial body of research, on the other hand, has shown that the food matrix suffers from nutrient and energy losses, taste shifts, and a drop in nutritional content.

- *Freezing:*

Cheng *et al.* studied that cooling, as well as freezing, have been used a lot to keep leafy vegetables, spices, and milk products from going bad and to keep their taste and nutritional value. Freezing may be accomplished in a variety of methods, including through air blast, cryogenics, close communication, and absorption. A few of the most cutting-edge techniques for freezing include electromagnetic disturbance freezing, dehydration freezing, high-pressure freezing, and ultrasound-assisted freezing [17].Cooling time, ice crystal formation speed, storage costs, and the need for specific conditions are all concerns that should be taken into consideration even though cooling or freezing were successful in their own right.To better understand the mechanisms of heat transmission and also fluid flow with different dietary components and to provide a solution to this issue, technological approaches like computational fluid dynamics simulations or 3D- mathematical equations were explored.

- *Ozone Treatment:*

An increasing number of consumers are looking to eat healthfully and sustainably, which has led to a growth in the demand for organic goods. Customers want a functional meal that is devoid of chemicals and preservatives with a reasonable shelf life. Because of this, ozone therapy is becoming more popular.We chose ozone because of its unique properties and quick breakdown. It is an efficient antibacterial and antiviral drug due to its quick disintegration into oxygen molecules and high oxidation potential (2.07 V). Fisher *et al.* compared it to additives and preservatives like chlorine (1.35 V), hydrogen peroxide (1.78 V), and hypochlorous acid (1.79 V) [18], the instantaneous production of ozone by Pandiselvam *et al.* [19], eliminates the need to store dangerous substances. In contrast to heat treatment, which places a greater emphasis on shelf life, minimum energy is also necessary.

- *"Pulsed Electric Field (P.E.F)":*

An innovative pre-drying treatment, food treatment using pulsed electric field technology, with a shorter retention duration is used. The continuous functioning and minimal demand for electric fields (1–5 kV/cm) made this approach well-known. Because it takes just 40 degrees Celsius to work, the method could be employed to replace heat drying and increase food drying[20]. The procedure that is involved in the treatment of liquid meals and pastes employing a pulsed electric field is shown in Figure 3, which depicts a typical schematic of the process. As part of the process, pulses of electricity are delivered as a non-thermal technique of food preservation, a

food item may be frozen. The procedure produces high-quality food with little loss of nutritional value or taste.To use the pulse electric field method, food is placed between two electrodes, and a high voltage pulse (50 kV/cm) is applied for a short period. After this, the food is treated with an electric field. The method utilizes both electroporation and electro-permeabilization as its underlying principles.

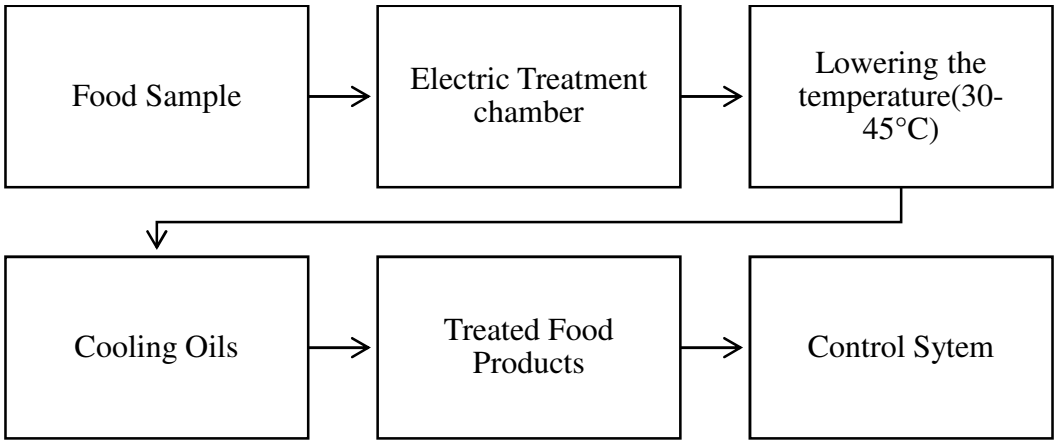

Figure 3: Displays thePulsed electric fields (P.E.F) that are often employed to treat liquids and pastes in the food industry.

2.3. Nano precipitation:

Solvent displacement is another name for the Nanoprecipitation procedure. Emulsion formation occurs when the polymer, drug, or solvent is dissolved in water and then released into the aqueous exterior phase.Nanoprecipitation is a process where polymers are precipitatedwhen the organic solvent disperses into the surrounding aquatic media from an organic solution.The movement of the solvent produces both Nanocapsules and Nanospheres. Recently used biodegradable polymers include “Poly (Lactide-Co-Glicolide) (PLGA)”, “Eudragit”, and “Poly (Alkylcyanoacrylate)” (PACA), “Polycaprolactone (PCL)”, and “Poly (lactide) (PLA)”[21].

2.4.Nanocapsule production method:

2.4.1. Emulsification:

By using an interface agent, the emulsification procedure makes it possible to combine two liquids that would not ordinarily be able to mix (surfactant).With the help of this technique, it is possible to combine a lipid with an aqueous medium or the other way around. This is accomplished through the formation of droplets (during the scattered phase) that are then continued to be spread during the continuous phase.

2.4.2. Solvent Extraction:

The vast majority of the approaches for producing nanocapsules are carried out in a medium consisting of a solvent. It is common knowledge that the use of solvents brings with it a variety of drawbacks, some of the most notable of which are the potential for microbiological contamination, higher costs, and physicochemical instability. In this scenario, it may be essential to get rid of the solvent to switch to a powdered form that can be re-dispersed. Spray drying or lyophilization are the two methods that are used most often for this reason.

3. DISCUSSION

The advance in nanotechnology is a major technological advancement with far-reaching implications for the development of sustainable practices. It encompasses all of the areas of applied science, from biology and physics to food technology and environmental management to healthcare and materials production.In its simplest definition, nanotechnology is the study of substances and particles with dimensions on the order of hundreds of nanometers or less. Nanotechnology has the potential to improve the safety of food in several ways, including the detection of viruses in packaging, the delivery of enhanced flavor and color, and also the fortification of packaged food barriers. There is a lot of hope that nanotechnology will be able to deliver advantages not just inside food goods but also in all surrounding food products [22].

Plastic is increasingly used in the packaging of certain foods. The biggest problem with plastics is that they do not break down and thus linger in the environment for a very long period after being abandoned as garbage. Nanotechnology allows for the production of biological plastic containers that naturally biodegrades. Because they are derived from plants, these polymers are better for the environment. Carbon nanotubes are useful in FP as well. Food spoils because these chemicals emit oxygen and carbon dioxide gases. Because of its unique qualities, such as delayed release, target specificity, precise action on active areas, and large surface area, the technique is favored. Nanotechnology's success may be attributed to its promising outcomes, zero pollution, low energy consumption, and smaller footprint. Additionally, nanotechnology has shown several uses in the fields of agriculture, food, environmental safety, toxicity, and risk assessment, all of which are for future development.

3.1.Safety concerns:

Numerous nanotechnologies depend on tiny components that are contained inside closed systems and, as a result, are unable to come into direct touch with living creatures, including the human body or microorganisms found in the environment. Because of this, their exposure is very low, and as a result, their risk is also quite low. This is in addition to the fact that for certain nano components, it is extremely difficult to conceive of any kind of direct danger at all.It's possible that nanostructures in the food industry won't have a direct impact on people's health; but, the fact that these things are Nanoscale may generate some inevitable side effects.Nanoscale edible coatings have lately emerged as an interesting solution for food quality preservation, extending storage life, and preventing microbial decomposition. This paves the way for direct exposure of people to nanomaterials.

Inhaling, ingesting, or absorbing nanoparticles via the skin are all ways in which engineered nanomaterials and other nanomaterials might enter the body. The toxicity of nanoparticles depends on several factors, including the particles' characteristics, the nanoparticles'mode of entry into the body, the concentration of nanoparticles, the period of exposure, the exposed individual's sensitivity, and also the organism's state. When the oral route of transmission was investigated, only very large dosages of nanosilver or nano -TiO2 exhibited indicators of toxicity [23].Recently, the usage of nano-biocomposites in packaged foods has improved their capacity to operate as a shield against gases. This improvement came about as a result of recent advancements in the field of nanotechnology. Recent developments in the food packaging industry are favoring the utilization of biodegradable polymers that are supplemented with environmentally friendly nanofillers.

4. CONCLUSION

Proper food preservation is critical in light of food-borne illnesses caused by the ingestion of damaged food. Although food preservation techniques have been around for a long time, new and more efficient methods must be explored.Nanotechnology research must also consider the possible harm that nanoparticles might do to the human body. Maintaining a good and accurate balance between engineering and innovation and cost is critical. More natural preservatives with great antioxidant and antibacterial characteristics, which are safe to eat or remove the need for processed food, are also being searched. Several contemporary technologies have been created throughout time to preserve various foods for varying lengths of time. These methods function well even though certain procedures have particular shortcomings and are being superseded by others. Furthermore, in the future, the use of nanotechnology in food preservation would improve the quality, storability, safety, or security of food, which would also help farmers and consumers.Researchers need to learn more about how NSMs move about in food, how harmful nanoparticles are to humans, and what effect they may have on people's health and the environment.

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CHAPTER 11

AN EXPLORATIVE STUDY ON PCR BASED DIAGNOSIS OF FUNGAL PATHOGENS IN *CUCURBITA PEPO.*

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ABSTRACT:

Cucurbita pepo (Pumpkin) belongs to the genus Cucurbita. The subsp. pepo is also known as summer squash. The pumpkin seeds are highly nutritious and full of magnesium, copper, zinc, and protein. Various varieties of fruit are available such as Acorn squash, yellow summer squash, gem squash, etc. Cucurbita pepo has a wide range of applications in medical uses including the treatment of certain diseases such as urinary ailments, antioxidant, anti-diabetic, and antiviral. At the same time, the fruit is susceptible to many fungal infections which results in economic loss worldwide. Past reported studies have shown that the Detection of fungal infection is essential because of no symptomatic signs when encountered with the pathogen. The diagnosis of the infection-causing agent can help scientists to develop techniques in advance for controlling the common prevalence of the disease. In the present study, Polymerase chain reaction (PCR) technology is used as a detecting tool for the early detection of the responsible fungal infections among the Cucurbita pepo seeds.

KEYWORDS:

Cucurbita pepo, Detection, Diagnosis, Fungal Infection, Polymerase Chain Reaction (PCR).

1. INTRODUCTION

The fruit *Cucurbita pepo* L. hails from America, where it has been originally harvested from Texas and the northeast region of Mexico. One of the most widely used kinds of vegetable, with a temperature favorable to “moderate and subtropical” weather conditions full of antioxidants and polyunsaturated fats, and other important nutrients. It is a climber basically “monoecious” which is grown all around the globe for the consumption of its fruits, Figure 1 is representing the structure of *Cucurbita pepo*.

The framework of stems is composed of setose, stout with multifold vines that are thick. The structure of leaves is non-complex, triangular blades of 20-30cm in length with an uneven five lobe “cordate and setose” present at the base, the apex is acute. The fruit has big individual flowers with a “setose pedicel” of 2-20 cm in size, region of the corolla is orange in color with a layered membrane funnel-shaped five lobed that can propagate to 10 cm in length. Calyx, in the male flower, is in an “infundibula” shape around 10cm in length. The male part of the flower consists of 3 stamens with filaments of (1.5cm) long. The gynoecium has a concentrated calyx along with an ovary (oval with a single locus) [1].

Cucurbita pepo is categorized into 3 subsp. namely “*C. pepo ssp.* fraternal and *C.pepo ssp ovifera*” both of which are from the wild taxa. Fruit seeds are white and smooth in appearance

with a flat surface. Flowering plants of small order called Cucurbitales consist of "7 families with 129 genera and 2,295 species". The species consists of 'Begoniaceae' belonging to the family of begonia. "Anisophylleaceae, Coriariaceae, Corynocarpaceae, Datiscaceae, and Tetramelaceae come under the family of cucurbitales"[2]–[5].

Figure 1: Picture Representation of Pumpkin (*Cucurbita pepo*) With Big Broad Leaf Structure

1.1.Nutritional value

The seeds of the pumpkin are high in minerals and vitamins such as vitamin K and magnesium. Both the nutrients help in healing a wound. Minerals like zinc aid in building immunity against microorganisms like viruses and bacteria. Table 1 shows the different nutritional properties of pumpkin fruit.

Table 1:Tabular Presentation of Data Consisting of Nutritional Properties in Pumpkin.

***Cucurbita pepo* Nutritional Properties**	
Protein	1.8g
Fats	0.17g
Fibers	2.7g
Carbs	12g
Calories	49g

1.2.Top producers of Cucurbita pepo

Worldwide Production of *Cucurbita pepo* is distributed thoroughly in the US, crop production requirement changes from region to region. Every other state in the US contributes to the production of pumpkin among which some states harvest the highest cultivation. According to estimation, a total of around 19 million contributed to the worldwide production of pumpkins. China is the largest manufacturer of squash and pumpkins with an annual production of 5.6

million tons followed by India contributing in a range of 3-5 metric tons of pumpkin. Other countries also add their respective values in increasing the word-wide consumption of *Cucurbita pepo.* Figure 2 is describing the ratio of percentages of pumpkin production on a global level [6].

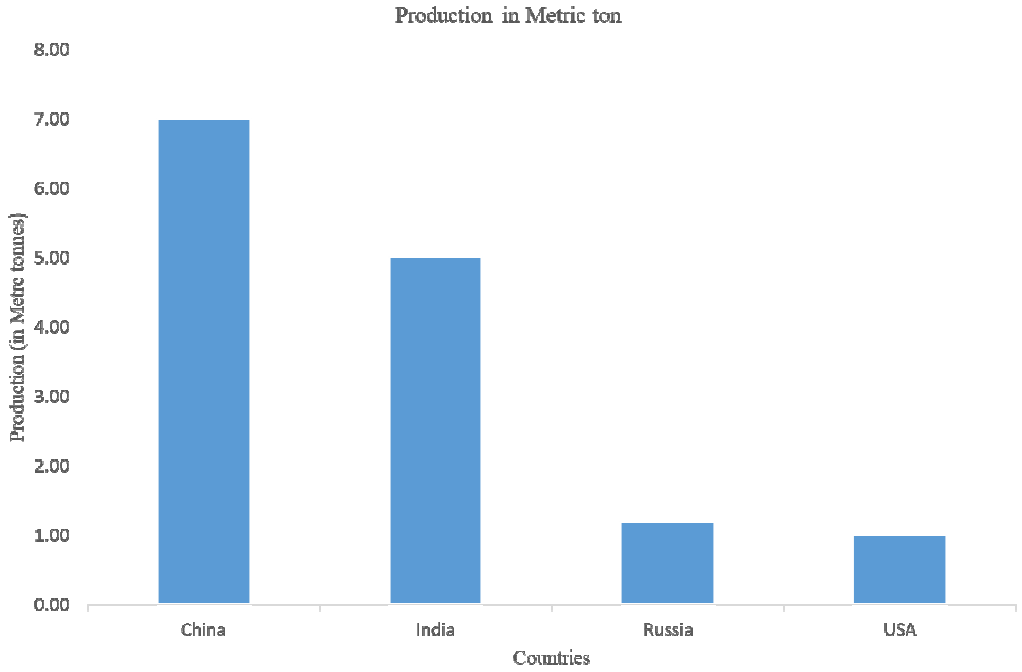

Figure 2: Pictorial Representation of the Numerical Data (Production of Pumpkin in Tones) by Various Countries of the World.

1.3.Common fungal diseases in Cucurbita pepo

Species of *Cucurbita* described in Figure 3,can be infected by various infections such as Fusarium fruit rot, gummy stem blight, and Alternaria leaf spot. Gummy stem blight (GSB) is a disease that is caused by a fungal species "Stagonosporopsis" and 3 subsp: Stagonosporopsis cucurbitacearum, Stagonosporopsis, and Stagonosporopsis citrulli". Among the diseases mentioned above GSB are the major infectious disease prevalent in pumpkins. "Winter squash and pumpkin" are typically resistant to BR where seeds are attacked by a flower or a fruit [7].

2. LITERATURE REVIEW

V. Manici *et al.* documented a review paper on the detection of pathogenic agents using diagnostic procedures in seeds of the vegetable. The authors record the importance of early diagnosis and detection of fungal diseases in vegetable seeds that will help in the prevention of financial losses. Further, the paper describes the difference between the traditional method and the new method and how the technology has evolved over course of time. Employment of " (PCR) and DNA extraction" can prove to be a good method for the identification of diseases in vegetable seeds [8].

Binyam Tsedaley explicated a research study on the detection of diseases in seeds and evaluation of tests in the diagnosis of the health of a seed. The author describes the techniques and their advantages used in modern agriculture, it also explains the measurement of seed health which is

how many seeds are susceptible to a fungal infection. Moreover the use of "(PCR)" can be a great molecular tool and detecting seeds born with infection [9].

Ravindra Kumar *et al.* described a review paper on seed-borne fungal disease identification and their diagnosis. The authors gave a comprehensive summary of the food losses associated with fungal disease and other pathogenic agents. The study further discusses the "conventional and modern method for the detection of seed born fungal diseases". The use of PCR and "Enzyme-linked immune sorbent assay ELISA" could overtake the traditional detection methods [10].

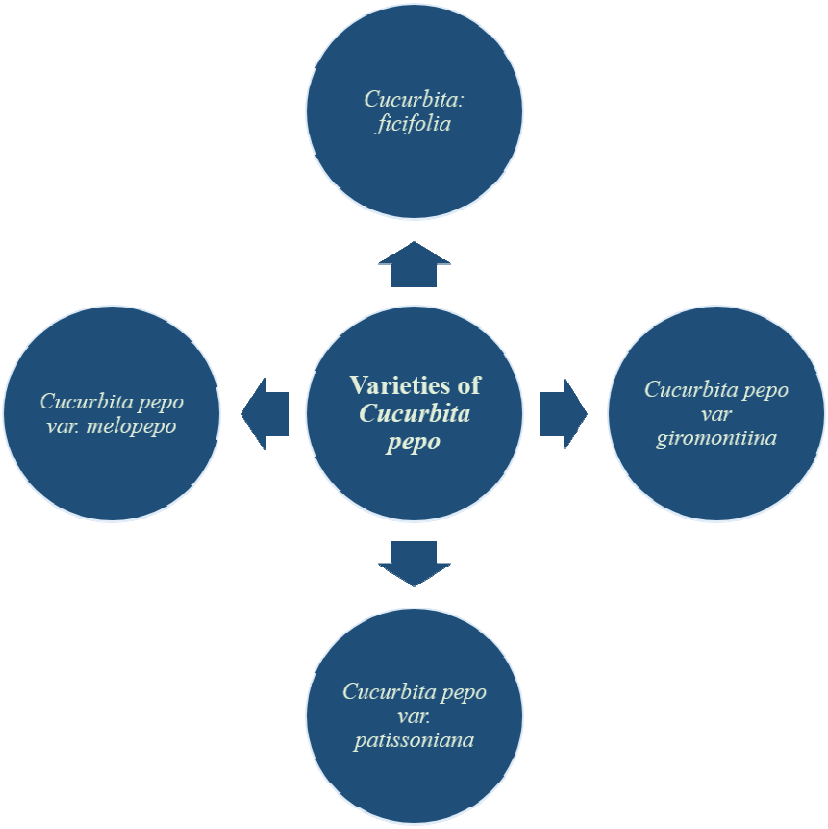

Figure 3: Classification of Various Sub-sp. of *Cucurbita Pepo* in the Pictorial Illustration

Marwa Moumni *et al.* explained their study in a form of a research paper on the detection methods for identifying the framework of "seed-borne fungi in *Cucurbita maxima* and *Cucurbita moschata*" the authors demonstrate the detection method for the identification of "seed-borne fungi" present within the seeds that are obtained from "66 specimens of squash fruit containing both asymptomatic and symptomatic from 2 regions i.e. Italy and Tunisia. The detection of symptoms responsible for the deterioration of fruits. "Blotter test" was used for the detection of disease in seeds [11].

Neelam Ratan *et al.* demonstrated a review study on "*Cucurbita pepo"*. The authors discuss the herbal aspects and chemistry of the species with a brief discussion about the use of fruit in Ayurveda. Further, the study talks about the medicinal uses associated with the fruit and its uses against the activity of cancer [12]

Peter Kusstatscher *et al.* reported their views on "breeding technique assisted with the help of microbiome to interpret the assembly dependent on cultivar in '*Cucurbita pepo'*. "The authors

researched the rhizospheres and the microbiome arrangement of seeds that are associated with pumpkin species and introduced the first step in driving the microbiome breeding method for microbes that are useful to plants. 16S RNA sequencing and the use of ITS1 gene was also evaluated for the microbiome of bacteria and fungi" [7].

Muhsen Abd Ali *et al.* expressed their views on the "diagnosis and extraction of fungi allied with zucchini *Cucurbita* pepo roots and how to use bioagents for the infection control". The authors talk about the detection of *"Fusarium oxysporum*" and the associated strains. The results show the findings of pathogenic strains, the use of biocontrol agents was also reported for controlling the expansion rate of the microorganism [13].

Laura Patricia Valdez-Arjona and Mónica Ramírez-Mella discussed the use of waste obtained from pumpkins as feed for animals and its effect on their health and nutritional value. The researchers emphasize the benefits of plant waste and its employment as food material for feeding the "livestock" [6]

Research Question:

- How to evaluate fungal infection in pumpkin fruit?
- What are the detection methods for the identification of fungal pathogens?

3. METHODOLOGY

3.1.Design

The infected pumpkin seeds with the fungal disease were taken from the agricultural land, cultivators, and the commercial market. The collection was done in a poly bag which was sterilized properly inside out, post the process of collection it was brought to the lab and placed in cool temperature conditions to make sure no further damage occurs to the seeds until the remaining analysis is done. The research method includes the procedure of Incubation, microscopy and PCR detection.

3.2.Sample Collection

Sample collection was done from farmers, cultivators, and commercial markets, a total of 10 varieties of pumpkin seeds were tested after the fungal infection. The seeds were selected from "Malabar gourd, winter squashes, cushaw squash, Long Island Cheese pumpkins, ornamental gourds,Casper pumpkin, pie pumpkin, Cinderella pumpkin, jap pumpkin, and trombone pumpkin"

3.3.Instruments

1. Visual screening
2. Microscopy (microscope under the lens
3. Incubator
4. DNA extraction
5. PCR

3.4.Data Collection

Some of the seeds show symptoms of infection like a color change of the outer shell, whereas some do not. Visual screening is not a reliable method to confirm the presence of the fungal hence we proceed with the other checks. Following the "Standard blotter method (SBM)" seeds were subjected to surface sterilization by adding 0.1% of mercuric chloride for 1 minute and repeating the same process 2- 4 times but with distilled water. A 9cm polypropylene Petri plate was taken, 10 seeds were placed per plate using 3 wet blotter sheets, next proceeding with incubation for 11.5 hours at 25℃ in a pattern of interchanging light and dark cycle for 8 days. The seed specimens after the incubation were observed under 50x magnification in a stereomicroscope. For the isolation of single spores, 10 test tubes were prepared with the "agar slant method using a potato dextrose" with one seed incorporated of each species and incubating the test tubes at 24℃ for 4-7 days. Further the pure fungal colonies were picked and were proceeded with the analysis.

3.5.Data Analysis:

3.5.1. Testing pathogenicity on a liquid agar plate

Microscopy of the seeds confirmed the presence of *Fusarium oxysporum (FO1, FO2) and Fusarium solani (FS1, FS2), Rhizoctonia solani (RS1, RS2*) isolatesin most of the varieties, to check its pathogenic nature the isolates were put inside a Petri plate consisting of 18ml (Agar water). The agar water was prepared and "inoculated with the fungi aforementionedin a 0.5 cm diameter disc from the edge of the fungi colonies at 5 days of age". Incubation of the Petri dishes was done at 24℃ for 4 days, post which the plates were subjected to be "planted with seeds of *Cucurbita pepo* in the presence of 9% sodium hypo chlorate". Testing of the seeds proceeds with their plantation around the verge of growth of the fungus at a "rate of 10 seeds/plate, 4 plates per isolate along with the treatment control by excluding the fungus". Incubation of the plates was done at 24℃. Results observed were recorded after 8 days with the help of "calculation percentage of germination".

$$\text{Percentage of germination} = \frac{\text{Germinated seeds in no}}{\text{whole no of seeds}} \times 100$$

4. RESULT AND DISCUSSION

PCR process begins after the isolation of DNA extracted from the seeds. The method used for the isolation was the phenol-chloroform method followed by precipitation by ethanol. This process often comprises inhibitors in the PCR that are unable to exclude just by washing repeatedly by the use of 70% alcohol. "DNA dilution of the extracts can help in removing the consequences, the only associated problem is that it also minimizes the sensitivity of the PCR". The extraction kits available for DNA can help to reduce the time involved in the extraction of DNA and the isolate handling is easy in comparison to the conventional methods. The resultant DNA was incubated with some quantity of fungal material within a 35µl instant gene matrix at 94℃ for 8 min followed by a short vortex for 15 seconds and centrifugation at 10,000 rpm for 10 minutes. Along with this the primers used for "DNA amplification were ITS1-f and ITS4 to check the presence of fungal DNA". Total reaction volume of 50µl consists of PCR Buffer 1X, MgCl2 (2mM), dNTP mix (0.2mM), bovine serum albumin (1X), forward and reverse primer (1 unit), DNA template, and DNA polymerase (2µl). The temperature conditions for initiating the

reaction were for 10 mins at 94℃ proceeding with 35 cycles for 2 minutes at 94℃, and 2 minutes at 56℃. The final extension was for 10 minutes at 72℃. Gel electrophoresis was set up using 1.5% agarose gel with ethidium bromide for staining (0.1%) in a tris-acetate EDTA buffer separation at 110V for 25 minutes and examination under the UV light. Figure 4 shows the bands under the UV light along with the specification of the species [14].

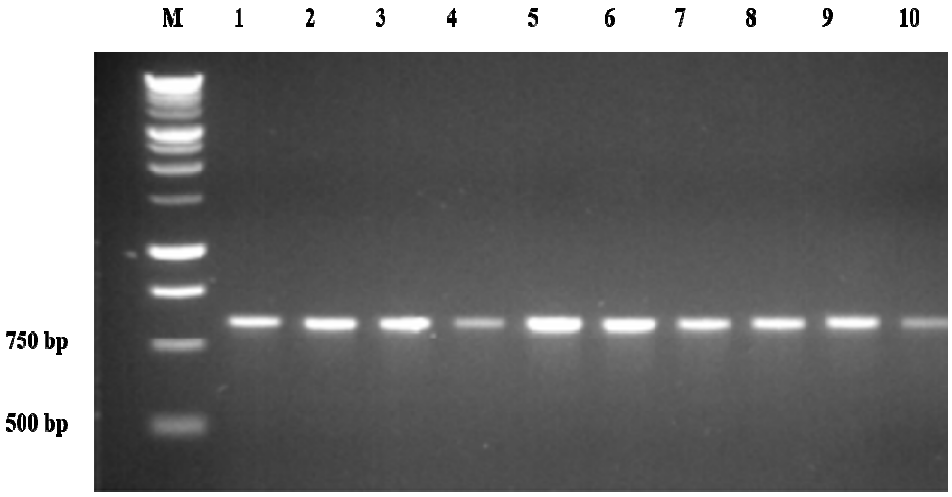

Figure 4: PCR Results with ITS Spacer Primers to Run and Check the Presence of Fungal DNA Column 1 (M) DNA ladder. Column 1-3 (In order, Malabar gourd, winter squashes, cushaw squash) with FS1/FS2 column 4-5 (long island cheese, ornamental gourd) with (FO1/ FO2), Column 6-9 (, Casper, pie pumpkin, Cinderella pumpkin, jap pumpkin, and trombone pumpkin) with (RS1/ RS2), Column 10 (negative control).

5. CONCLUSION

The current research paper concludes different detection methods for the presence of pathogenic fungi in *Cucurbita pepo* seeds and their varieties. The crop seeds are always at the risk of getting infected with the pathogens and the detection becomes difficult because the early or the initial symptoms are usually not visible, in some cases symptoms don't appear hence the diagnosis of the infection becomes extremely difficult. Methods like visual screening could not prove to be reliable and to confirm the invasion of a disease with the identification of a pathogenic agent type we performed other experiments involving DNA extraction, PCR amplification, and gel electrophoresis. ITS primer was used for evaluating DNA sequences. Fungal species of *Fusarium oxysporum* (FO1, FO2), *Fusarium solani* (FS1, FS2), and *Rhizoctonia solani* were diagnosed and identified.

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CHAPTER 12

EXPLORING THE POTENTIAL INHIBITORY ACTIVITY OF NATURAL COMPOUND "VICINE" FROM *MOMORDICA CHARANTIA* AGAINST HIV PROTEASE USING MOLECULAR DOCKING

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ABSTRACT:

The burden of infectious diseases is increasing day by day which is more evident in the case of Human Immunodeficiency Virus (HIV) and the disease caused by it which is referred to as acquired immunodeficiency syndrome (AIDS). Despite the availability of antiretroviral treatments and drugs, there is still a lack of a cure for HIV/AIDS. This is because of their side effects on cardiovascular and cerebrovascular health which can also prove to be life-threatening when used in long-term treatment. In recent years, natural products, especially phytocompounds present in medicinal plants, edible herbs, fruits, and vegetables have received a lot of attention due to their therapeutic properties in folk and traditional medicines. The research aims to assess the binding affinity and the potential interaction of natural compounds from Momordica charantia against the protease enzyme of HIV. The binding energy of -6.12 kcal/mol was obtained with the top-ranked conformation of the ligand molecule when docked with the protease enzyme of HIV. Therefore, the results in terms of good binding energy can be considered a hit compound for future drug development against HIV.

KEYWORDS:

HIV, AIDS, Molecular Docking, Protease, Vicine.

1. INTRODUCTION

The "Human Immunodeficiency Virus"/"Acquired Immunodeficiency Syndrome" (HIV/AIDS) epidemic has grown significantly over the past 30 years, from a relatively minor issue in the 1980s to one of the major causative reasons for death and hardship during the past ten years [1]. HIV/AIDS is a stark outlier to the worldwide trend that sees an increasing proportion of disease burden coming from non-communicable illnesses and injuries. Contrary to the general trend of decline, HIV/AIDS-related mortality and burden climbed continuously until around 2004. Since the first cases of AIDS were documented in 1981, HIV has evolved to be one of the biggest threats to development and global health. Nearly 76 million people have contracted HIV since the epidemic began [2].

Presently, there are about 38 million individuals living with HIV, and since the beginning of the AIDS epidemic, tens of millions of individuals have died from "AIDS-related causes". HIV affects some populations more severely than others despite overall advances in lowering HIV transmission in the U. S. due to established and persistent barriers. Compared to persons of other ethnic backgrounds/races, Black people known as African Americans—are accountable for a

larger percentage of new HIV infections. According to CDC estimates, black Americans made up 13% of the U.S. population but 40% of those living with HIV in 2019. Disparities in HIV treatment must end [3], [4]. These alarming and enduring discrepancies have been exacerbated by residential segregation, racism, persistent systemic inequities, social and economic marginalization, and other ingrained barriers.

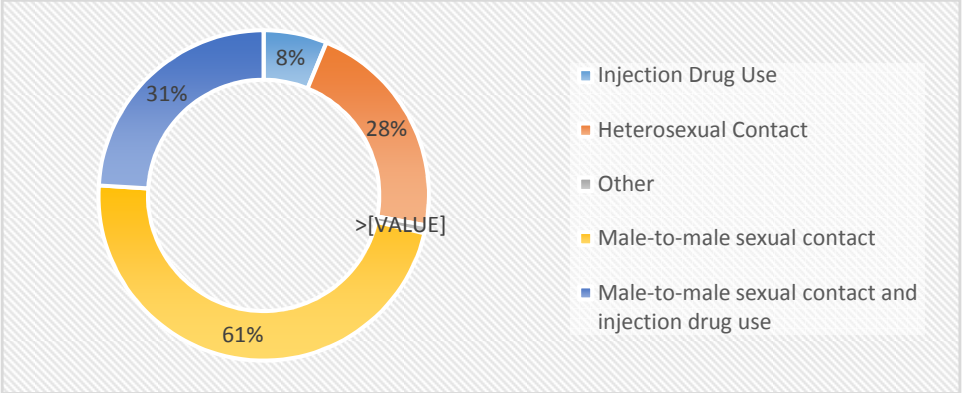

Figure 1: Illustrating the prevalence of HIV infection by Different Transmission Routes.

Particularly over the past 20 years, there have been considerable breakthroughs achieved in the fight against the disease. The frequency of new HIV infections, particularly among children, and AIDS-related mortality have reduced over time, whereas on the other hand, the number of persons with HIV undergoing therapy has risen to 25.4 million in 2019. HIV control efforts are nevertheless made more difficult by persistent problems. There is still no cure for HIV, and also many people who are HIV-positive or at risk of getting the infection do not have access to care, prevention, or therapy. HIV primarily affects people in their prime productive years, and it has an impact on not just the health of individuals but also communities, households, and the economic and social development of entire countries. Owing to food insecurity, other infectious diseases, and other challenges with global health and development, many of the nation's most severely affected by HIV also face significant difficulties. Moreover, as COVID-19 has spread in every part of the globe, its detrimental effects on the "HIV/AIDS" response in middle- and low-income nations have already been noted. These include delays in the provision of antiretroviral medications and prophylactic measures. [5].

Antiretroviral therapy (ART) has decreased the number of AIDS-related fatalities, but not everyone has access to it. Additionally, there is still uncertainty regarding the development of curative treatments and a reliable vaccine. It has been suggested that preventative and awareness campaigns may prove to be a more effective strategy [6]–[8]. These hopes, however, did not come true because of the high HIV prevalence and restricted access to antiretroviral therapy among important demographics. Social factors played a significant part in the multifaceted spread of HIV. HIV testing has to go by the ethical standards safeguarding patient privacy, and this, combined with discrimination and stigma, may have concealed the disease in many settings and still does so today. Prevention programs including using condoms, voluntary male medical circumcision, community awareness campaigns, and preventing mother-to-child transmission haven't been as effective as they may have been perhaps because of unresolved systemic failures.

1.1. Structure of *HIV Virus:*

Gp120, Gp41, Viral envelope, P17, P24, protease, integrase, reverse transcriptase, and RNA. The designation GP120 refers to its molecular weight. It is required for viral entrance into cells because it is involved in the attachment to particular cell surface receptors. Gp41 is a component of the envelope protein complex found in retroviruses such as HIV [9]. It is a kind of enveloped virus that replicates in the host cell via reverse transcriptase. A viral envelope is an envelope structure by which a virus binds. P17 protein is used to construct the viral core. It's in the form of a bullet. Reverse transcription, integrase, and protease are three enzymes needed for HIV replication. P24 is a part of the HIV capsid. In addition to that, retroviral aspartyl protease is required for HIV, to complete its life cycle. This enzyme cleaves newly generated polyproteins at the proper location to produce the natural protein components of the infectious HIV virion. A retrovirus-produced enzyme that allows its genetic information to be incorporated into the DNA of infected cells. Figure 2 provides a clear insight into the structure of HIV[10].

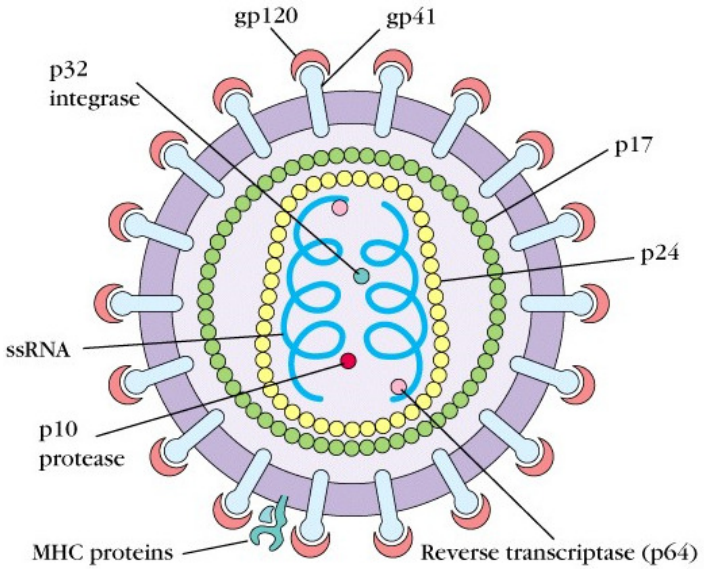

Figure 2: Illustrating the Structure of Human Immunodeficiency Virus (HIV).

1.2. Protease and its importance for Drug targets:

A catalytic Asp is located at position 25 in each monomer of the homodimeric aspartyl protease that makes up the HIV protease. Nine processing sites on the HIV-1 protease cleave the polyprotein precursors “Gag” and “Gag-Pol” that are produced by the HIV-1 virus to create mature, functional proteins. Before being subsequently processed into RNase H (p15), protease, reverse transcriptase (p51), and integrase, the Pol polyproteins must first be separated from the Gag-Pol polyproteins. Since two flexible "hairpin" flaps are covering the active location, it is not completely exposed. To provide the entry of the substrate to the active site, the flaps must be opened. By blocking access to the active site of the protease, the enzyme activity that causes HIV-1 can be reduced. Therefore, this protein is a primary therapeutic target for the treatment of AIDS since the activity of this enzyme is crucial for the ability of the virus to cause infection.

HIV protease is an attractive target for drug development because of its crucial function in the maturation of viruses. Designing new and better inhibitors has been made significantly simpler

because of the enormous number of HIV protease protein structures that have been solved. Following are the HIV protease inhibitors that have been given FDA approval: indinavir, fosamprenavir, amprenavir, ritonavir, saquinavir, nelfinavir, lopinavir, tipranavir, atazanavir, and darunavir. Unfortunately, negative effects occur with the majority of inhibitors when used over an extended period. The most frequent adverse effects of HIV protease inhibitors include cerebrovascular and cardiovascular problems, as well as metabolic disturbances carried on by them, including insulin resistance, dyslipidemia, and lipodystrophy/lipoatrophy.

Therefore, the above issue of side effects brought by available protease inhibitors calls for a revision of the natural products present in medicinal plants and other edible fruits and vegetables.

1.3. Momordica charantia L. (MC) and its important chemical constituent

Momordica charantia L. (MC), often referred to as "bitter gourd" or "bitter melon", is a plant that thrives in subtropical and tropical regions and is a member of the Cucurbitaceae family. Because they include nutraceutical and nutritional elements, the leaves, and fruits of Momordica species are high in phytochemicals and could have a variety of health-promoting benefits [11]. The plant has long been utilized in folk medicine for a variety of medicinal purposes, including the treatment of T2DM, bacterial infections, obesity, hypertension, cancer, and viral infections [11], [12].

Therefore, this paper aims to evaluate Vicine, one of the important compounds present in Momordica against the protease enzyme of HIV using a Molecular docking tool. In this research paper, the first section provides a fundamental for HIV/AIDS burden as well as the importance of targeting protease enzyme for the development of the inhibitors. The second section provides a review of related work which has been published. The third section provides the methodology that is used to perform the study. The fourth section of this paper provides the result and the discussion and the concluding remark in section 5.

2. LITERATURE REVIEW

Panda *et al.* carried out a study taking virtual screening into the consideration followed by the ADMET profiling, which was then followed by the molecular simulations on protease enzyme of HIV-1. They performed a virtual screening process and then the affinity of the screened compounds including "Afzelechin", "Epigallocatechol, "D-Catechin", "Afformosin", and Epicatechin. Were employed for the process of docking followed by the calculation of the binding energy using the MM-GBSA method. The results of their study revealed that the compounds like Epigallocatecho9l demonstrated promising binding energy which can be further used as a hit compound and discovery of natural protease inhibitor.

Another study by Vora *et al.* investigated mulberroside C as well as the endophytes of *Morus alba* against HIV. First, they carried out a HPLC for the screening of the compounds, followed by the assessment of the cellular toxicity and the assessment of the anti-HIV activity with the help of cell-free assay and cell-line assay. The results of their study revealed that out of the extracted compounds, mulberroside C has the promising inhibitory activity against HIV which acts as a potential inhibitor of protease [13].

Another study by Vora *et al.* studies the binding insights of some phytocompounds against the most potential targets of HIV with the help of the molecular dynamics simulations analysis. The prime target involved in their study were Gp120, integrase, ribonuclease, and protease. They

performed molecular dynamics simulation studies for the top-ranked compounds. The results of their study revealed that the compounds namely curcumin. Chebulic acid and Mulberroside C can be used as potent inhibitors of the established target of HIV including reverse transcriptase, Gp120 protein, and protease [14].

3. METHODOLOGY

3.1. Design:

In this research, molecular docking is used as a tool to find the possible interaction between the selected protein target of HIV and the ligand for future drug development. The present study makes use of Autodock4, a bioinformatics tool for the process of molecular docking. The structure of the protease enzyme was downloaded in .pdb format, in parallel with the retrieval of the compound "Vicine". XML format which was then converted into .pdb format using freely available conversion software. Autodock was then used for the docking procedure between the protein and ligand which resulted in the output file with the file format of DLG. The DLG file was then used to check, analyse and visualize the complex.

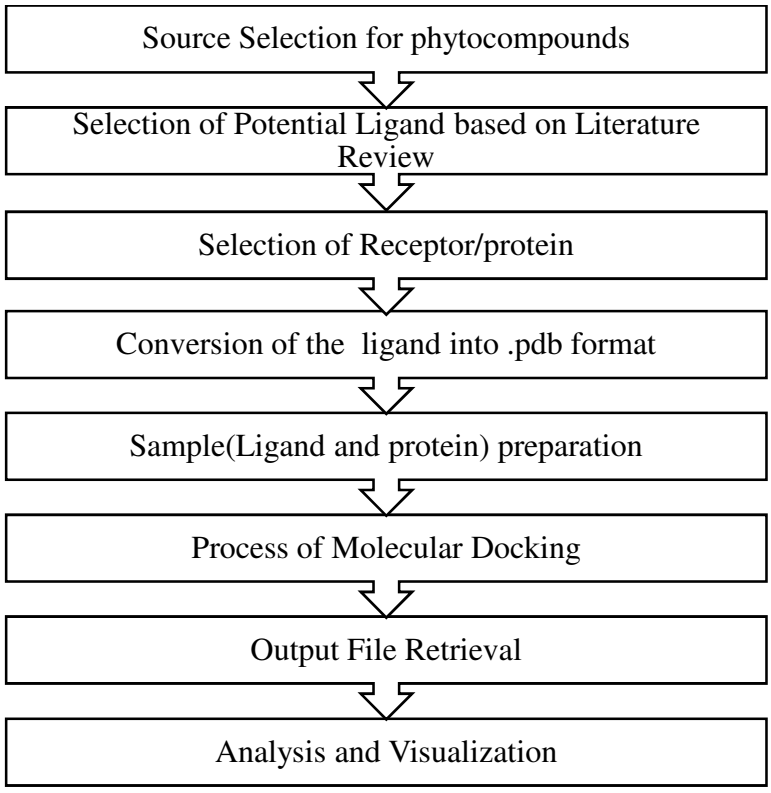

Figure 3: Flow Diagram of Methodology

3.2. Instrumentation:

The structure of the protease enzyme of HIV was retrieved from RCSB PDB. PDB stands for Protein Data Bank, while RCSB refers to "Research Collaboratory for Structural Bioinformatics Protein Data". Access to information on the three-dimensional structures of proteins that are present in a range of animals, including humans, plants, and microorganisms, is made possible by PDB RCSB. By providing links to relevant research articles that help in determining the

likelihood of interactions between the protein and ligand, this website also assists in acquiring crucial information on proteins.

A National Institute of Health (NIH) open chemical database called PubChem was used to obtain the structure of the ligand "vicine". Chemical structures can be found in two-dimensional, three-dimensional, and crystallographic forms in the database PubChem. The results can then be applied to a wide variety of studies and research initiatives. Displaying compounds in a variety of patterns, including wire-frame, sticks, ball and stick, and many more, is also made simple by this database. Additionally, it is crucial in the extraction of interesting compounds for additional computational and molecular modeling investigations.

Later, Autodock4 [15] was employed to determine how the protein and ligand structure interacted. In computational methods to identify novel compounds that bind to target proteins or to investigate protein-protein interactions, the bioinformatics tool Autodock4 is frequently used. This tool or application, as a result, guesses, without the need for wet laboratory experiments, how a small molecule or selected ligand will bind to and interact with a protein in a live biological system. After protein-ligand docking, the complex was examined and visualized using the BIOVIA Drug Discovery studio. This tool allows for the identification of the specific amino acids of proteins involved in the interaction with the ligand to create a stable complex which is a prerequisite for drug development.

3.3. Sample Collection and Preparation:

3.3.1. Protein Retrieval and Preparation:

The structure of the protease enzyme with PDB ID: 1OHR and PDB DOI: 10.2210/pdb1OHR/PDB, obtained from the X-ray diffraction method was downloaded from RCSB PDB. The structure of receptor/protein was bound with an inhibitor which was then eliminated with the help of pymol. In addition to that water, molecules were also removed and the protein was then exported to the Autodock tool for further preparation. The water molecules were deleted and then the polar hydrogen was added into the protein structure followed by saving the file in .pdbqt format for further docking process. The structure of protein after eliminating the unnecessary compounds is shown in Figure 3 below.

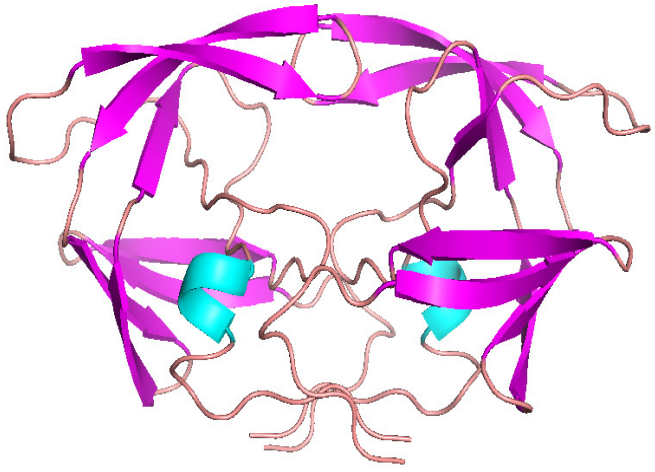

Figure 3: Illustrating the three-dimensional structure of the Protease Enzyme of Human Immunodeficiency Virus (HIV).

3.3.2. Ligand Retrieval and Preparation

The 3-D structure of ligand "Vicine" was obtained from PubChem in the format of. XML which was then converted into .pdb format using the Open Babel tool which is easily accessible and freely available. The .pdb file was then taken into the Autodock tool for the further preparation of the ligand molecule. After successful preparation, the file was then saved in .pdbqt format for further docking process. The ball and stick structure of the ligand is provided in Figure 4 below where different elements are represented by different colors.

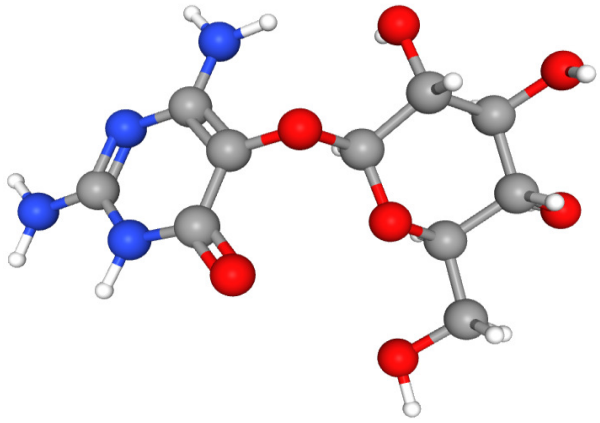

Figure 4: Illustrating the 3-D Ball and stick structure of Ligand "Vicine".

3.4. Data Collection:

Using docking, a variety of potential ligand positions and conformations are generated for the different binding sites. Rigid molecular docking into the protease enzyme was used to conduct a computational study. The macromolecule "protein/receptor" was put rigid during molecular docking while the ligand molecule which was taken as "Vicine" was made flexible. The tool was used to retrieve the binding energy table from the DLG file following the analysis of Docked log file in the Autodock tool. The molecular interactions between protein-ligand complexes, including different types of bonds and bond lengths, were examined using the most desirable conformation of the ligand with the lowest binding energy. The extra information on the interactions between the protein and ligand was subsequently confirmed using different visualization tools utilizing the DLG output file (Figure 5).

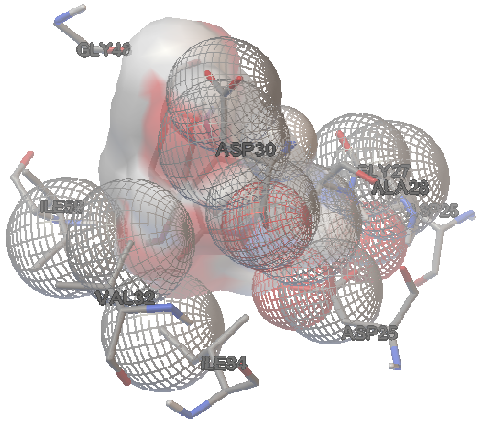

Figure 5: Illustrating the Docked Complex of HIV Protease and Vicine.

3.5. Data Analysis:

The 2-D figure shown in Figure 6 was constructed to exclude any surrounding chains of amino acids that were not contributing to the development of the protein-ligand complex to acquire a clearer picture of all the amino acids participating in the interaction with the ligand molecule. We have observed a total of 9 hydrogen bonds interacting with the amino acids; ASP25, GLY48, ASP25, ASP25, ASP25, GLY48, ASP30, ASP30, and GLY49 of protein. Table 1 enlisting the bond categories as well as the amino acids of a protein involved in the interaction with the ligand is provided below. In addition to that, the 2-D structure is also provided for better clarification about the precise amino acids demonstrating bond formation with a ligand molecule.

Table 1: Enlisting the Amino Acids Involved in the Interaction with Ligand Molecule with Respective Distances and Bond Category.

Sr. no.	**Protein: Ligand**	**Distance(Å)**	**Bond Category**
1.	UNL1 - ASP25	2.13007	H-Bond
2.	UNL1 - GLY48	2.85817	H-Bond
3.	UNL1 - ASP25	2.41566	H-Bond
4.	UNL1 - ASP25	1.96534	H-Bond
5.	UNL1 - ASP25	1.81068	H-Bond
6.	UNL1 - GLY48	1.95998	H-Bond
7.	UNL1 - ASP30	2.05948	H-Bond
8.	UNL1 - ASP30	1.95863	H-Bond
9.	UNL1 - GLY49	3.7272	H-Bond

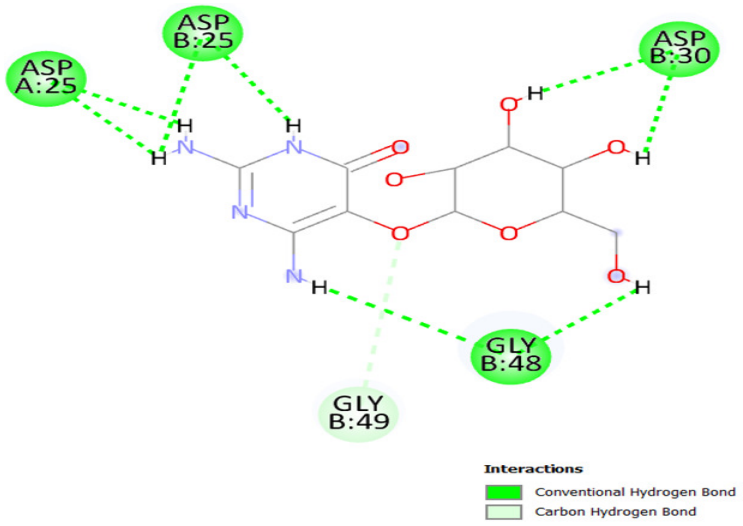

Figure 6: Illustrating the 2-D Docked Structure of Protein and Ligand.

4. RESULTS AND DISCUSSION

The “binding free energy” is equal to the sum of the "polar", "non-polar", and "non-bonded interaction energies". “Total Intermolecular Energy”, “Final Total Internal Energy”, and “Torsional Free Energy” are summed to provide Binding Energy, which is then subtracted from the energy of the Unbound System. In a DLG format file containing a list of the binding energies, the results of docking were retrieved. The more negatively the energy suggests, the larger the Ligand: Protein Stabilization, it is often seen. The graphical representation below in Figure 7 depicts the binding energy along with the run from 1 to 10 obtained from the DLG file in the form of an RMSD table.

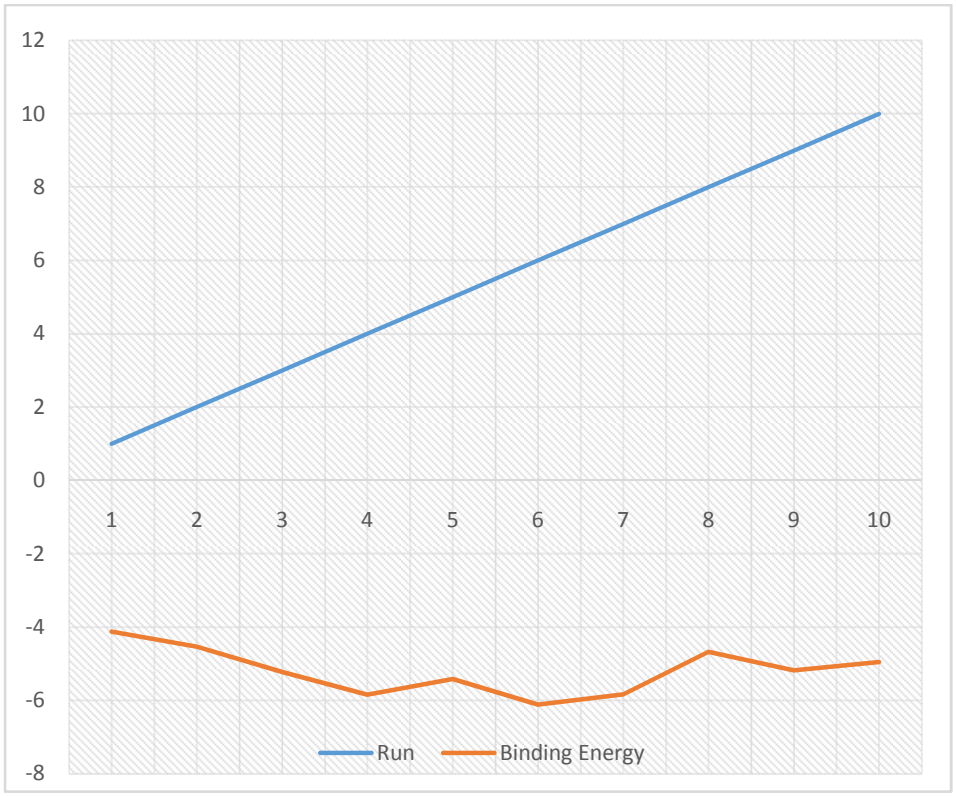

Figure 7: A Graphical Representation of Binding Energy obtained from RMSB Docked Log File.

5. CONCLUSION

Overall, the vast majority of scientific research point to the fact that frequent use of bitter gourd can prevent or treat a number of health-related problems including infectious diseases. We have discovered a possible antiviral inhibitor “vicine” using an *In silico* method, which can be used as a starting point to halt the replication of HIV. This inhibitor is from plant that is widely used as medicines. This research study thus can help future researchers to identify the compounds from bitter guard for its potential use in current era of alternative medicine.

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CHAPTER 13

A COMPREHENSIVE STUDY FOR DRUG TARGETING IN CANCEROUS CELLS USING NANOTECHNOLOGY APPROACHES

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ABSTRACT:

The effectiveness of cancer therapy has significantly improved as a consequence of scientific and technological innovation. It is possible to immediately treat malignant cells by using particles that are made to be targeted to cancer cells. Drug delivery systems may regulate the pace and area of a medicine's release. Conventional chemotherapeutics have some serious side effects, such as the impairment of the immune system or other different types of organ systems with rapidly dividing cells, which all contribute to their ineffectiveness due to their lack of specific targeting, low solubility, and inability to penetrate the tumor core. Ineffective therapy with a lower dosage or a lower likelihood of survival result from this. This study mainly examined several strategies for targeting cancer cells. There are several successful cancer therapy studies presented, as well as the use of nanoparticles to deliver specific medications to cells. Additionally, by delivering specific medications to the cells, nanoparticles may lessen the side effects of conventional cancer therapies, as shown by a number of effective study.

KEYWORDS:

Active Targeting, Chemotherapy, Drug Delivery, Drug Targeting, Nanotechnology, Passive Targeting.

1. INTRODUCTION

Cancer refers to several diseases that are defined by the formation of abnormal cells and have the possibility of spreading to other organs [1].Cancer patients now have access tothere are several treatment options available, including radiation therapy, surgery, and chemotherapy; yet, due to the side effects of these therapies, they are not considered to be the most successful.It is essential to ensure that the medications used in therapy could distinguish between cancerous and healthy cells. So in the current period, polymer-based cancer treatment strategies are employed to target particular cancer cells in the body. A lack of specificity in conventional chemotherapy medications prevents them from focusing on cancerous cells.The explanations listed below offer a basic summary of why standard chemotherapy medicines fail:

1. Chemotherapy has low selectivity and dose-limiting toxicity because the active cancer medicines reach the tumor cells.
2. Drugs administered orally may cause disordered pharmacokinetics that leads to higher-than-necessary dosages and eventually increased toxicity because of the exposure to active drug compounds [2].

3. Because macrophages quickly consume traditional chemotherapeutic medicines, which linger in the bloodstream for just a short period before being flushed out, these drugs have no impact on malignant cells, rendering chemotherapy useless.
4. A lack of medication solubility.
5. In many cases, chemotherapeutic medicines are unable to reach the malignant cells at the core of solid tumors.

A drug is any molecule with the potential to alter the chemical and physiological processes of cells, tissues, organs, or even the entire body. Drugs may also be classified as psychoactive substances. They have the potential to eradicate infectious agents such as bacteria, viruses, and fungi. This is a fundamental principle that underlies every medication. The term "active ingredient" refers to the specific chemical composition of the medicine that would be in charge of eliciting the preferred physiological reaction in the patient [3]. Active compounds in medication are often available in only minute quantities and are surrounded by inert fillers, while inactive ingredients serve as excipients, fillers, binders, or lubricants and do not have any impact on the body's physiology [4].

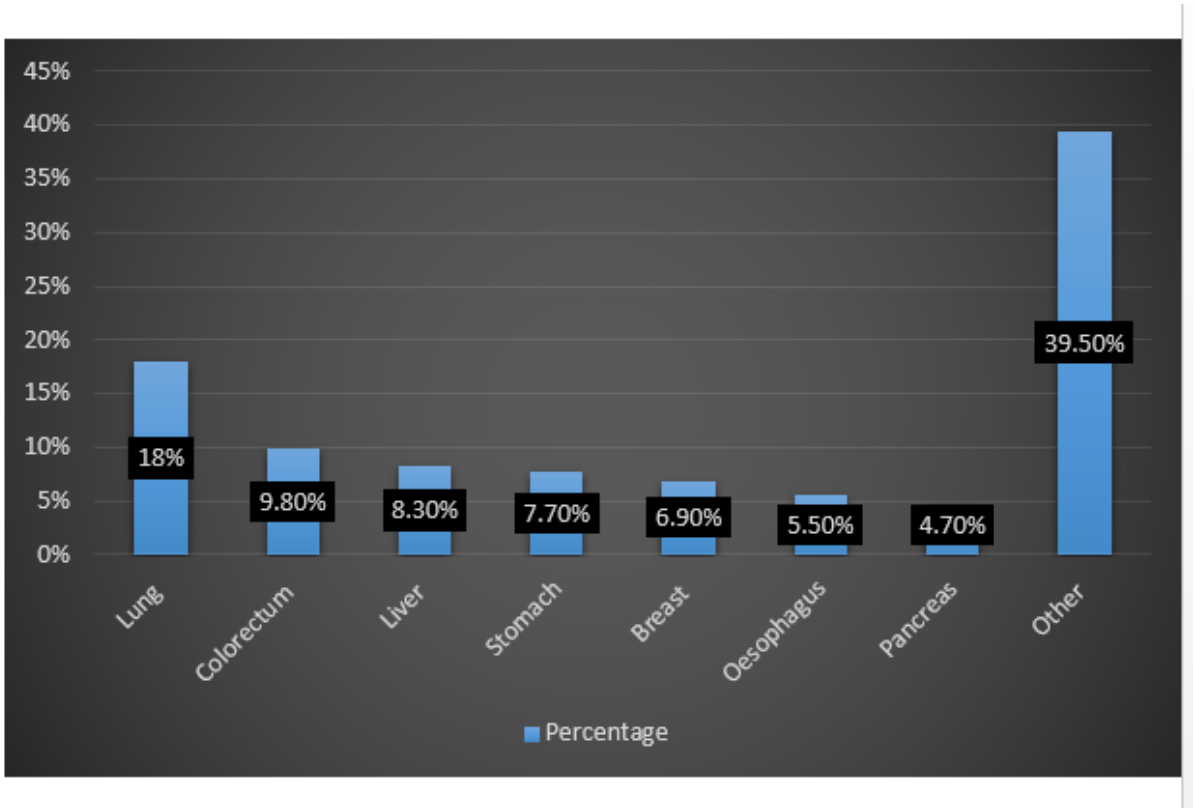

Figure 1: Displays the Percentage of cancer-related fatalities globally in 2020. (*Source: State Health)*

The majority of medications work by attaching to and blocking certain receptors or enzymes and altering their functions. To be successful, a medicine must be long-lasting in the body and not affect biomolecules other than its intended target molecules.Herbal drugs were the primary source of pharmaceuticals until they were chemically produced. They have been crucial in the

battle against infectious illnesses and epidemics since they have been employed to treat almost every disease and abnormality [5].Modern pharmacological dosage forms have several challenges, including inefficiency, inefficacy, bioavailability, toxicity, biocompatibility, adverse effects, and incompatibility with other drugs.Especially in nanomedicine and also the nano-based drug delivery system, nanotechnology's use of nanostructures and nanophases has been shown to fill the gap between the physical and medical sciences. Nanotechnology refers to any application of materials or devices at molecular or macromolecular sizes. Typically, a nanoparticle's diameter spans from one to 100 nanometers (nm). The use of nanoscale assays in screening campaigns may reduce screening campaign costs.Nanoparticles used as drug carriers offer numerous significant technical advantages, including, high carrier capacity, high stability, the flexibility to containsubstances that are both hydrophilic and hydrophobic, and also the ability to be administered by oral or inhalation routes.

The use of nanotechnology in cancer is actively being investigated for application in cancer screening, diagnosis, and therapy. It contributes significantly to eliminating many of the difficulties that conventional techniques face in the treatment, diagnostics, as well as detection of cancer, which demonstrates the importance of this method. Diverse lines of investigation are now being pursued with the goals of locating a nanotechnology-based cancer therapy that is more precise and reducing the unpleasant side effects of more traditional methods of therapy [6].

The targeted drug delivery technologies for cancer treatment enhance therapeutic outcomes in comparison to the present convectional treatment while simultaneously reducing the undesirable effects of the conventional treatment. Therefore, the systematic and nonspecific target drug delivery systems contribute to the quick clearance of the medication at the greatest acceptable dosage, which in turn reduces the toxicity of the drug. Particles made of inorganic or organic material may, in principle, be used to approach the goal of a targeted medication delivery system. The use of organic particles, such as nanogels, polymers, liposomes, dendrimers, and micelles, as target drug delivery systems are highlighted.

2. LITERATURE REVIEW

M. L. Goncalez *et al.* stated in their study that to produce and characterize In vitro skin penetration and retention of Kojic acid (KA)-Loaded Liquid Crystalline Systems (LCS) comprised of PPG-5-CETETH-20 (surfactant-S), Water (W), and Cetostearyl Isononanoate (oil-O). A (35% O, 50% S, 15% W), B (30% O, 50% S, 20% W), and C (20% O, 50% S, 30% W) are the three options were identified using a 2% KA. Polarized light microscopy revealed a hexagonal mesophase. According to the results, products created may influence KA penetration and retention in the skin according to data from in vitro KA permeation as well as retention studies. The author concludes that both KA-unloaded and KA-loaded LCS are nontoxic based on in vitro cytotoxic testing. Skin application of KA using PPG-5-CETETH-20 may be possible [7].

Bing Cai *et al.* conducted a comparison study of a new method for evaluating patch drug release to the standard USP apparatus. The effectiveness of 29, 57, and 198 L cm2 of Duragesic patches were tested on a synthetic skin simulator. Hydrochloric acid with a pH of 1.0 was used todissolve after 1, 2, 3, 4, 6, and 24 hours, disintegrating the artificial skin simulators.Medication concentrations were evaluated using High-pressure isocratic reverse-phase liquid chromatography. Increasing synthetic skin simulator wetness enhanced medication release rate.The medication release rate increases as the synthetic skin simulator's moisture level increases. Using this innovative technology, the author found that transdermal drug delivery

systems could be differentiated among pharmaceutical concentrations in the initial phases of development [8].

Erik Brewer *et al.* discussed in their study the inert delivery systems and a lack of rapid medicine release at the point of administration that typical nanomaterial formulations use. A few "smart" technologies have been highlighted in this research that would further boost the advantages of nanomaterials. Methodologies for initiating the release of encapsulated drugs, such as pH and temperature drug delivery devices, were examined, whereas the aptamer, as well as ligand conjugation, have been mentioned as strategies for intracellular and targeted delivery, with a focus on both experimental data from the lab and real-world outcomes [9].

Pablo G. Argudo *et al.* stated in their study that the microemulsion was used as a template to generatenanoparticles formed from agarose gel that was both homogeneous and of uniform size.Water-based dispersions of monodisperse agarose gel nanoparticles were created using the template approach in conjunction with temperature-induced gelling or a solvent exchange methodology. The resulting particles had an apparent hydrodynamic size of 150 nm on average. A synthetic pesticide (azamethiphos) was examined to see whether the hydrogel particles could encapsulate and release it. Findings demonstrate that pesticide molecules enclosed in the nanoparticles are delivered in a diffusion-controlled method. The author concluded that Agarose's biodegradability and these findings make it possible to build a novel vector for parasitic management in reservoirs [10].

O. Yassine *et al.* discussed in their study that when exposed to an alternating magnetic field, a nanocomposite gel made of PNIPAM as well as magnetic iron oxide nanobeads releases liquids (such as pharmaceuticals) in a regulated manner. By using a simple and affordable microfluidic technology, nanocomposite microparticles may be manufactured quickly and effectively with a monodisperse size distribution having diameters between 20 and 500 micrometers.Rhodamine B is used as a liquid drug model to evaluate microparticles for controlled drug release. Continuous mode released 7% while pulsatile model released 80%. Magnetic actuation offers targeted heating and also is straightforward to integrate with the microfluidic manufacturing process [11].

Oral ingestion or intravenous injection are two methods that are included in the conventional medication development and delivery system procedure. The distribution of medications throughout the body is mostly the responsibility of systemic blood circulation. As a consequence, the organs only get a trace amount of active pharmaceutical substances [12].Sometimes drugs have side effects because they impact organs other than the ones they were designed to treat. In addition, the process of developing a novel medicinal molecule is both very costly and time intensive. The present issues and difficulties that pharmaceutical firms are encountering in connection with drug delivery and the development of drugs are shown in Figure 2 and discussed below:

1. *Low Solubility:*

During the process of developing a particular formulation of the medicine, one of the most significant challenges that arose was the drug's poor solubility in water. The drug's bioavailability is negatively impacted by its poor solubility. As a result, this presents a significant obstacle for any new chemical entity that may be found by industry or scientific research [13].

1. *Low Bioavailability:*

The amount of a drug's dosage that is accessible for absorption by the body is referred to as its bioavailability. It is one of the most important pharmacokinetic characteristics of medicine. A drug's bioavailability is 100 percent when supplied intravenously, but it reduces owing to partial absorption when administered via other methods (such as orally). When administering medicine other than intravenously, bioavailability must be taken into account [14].

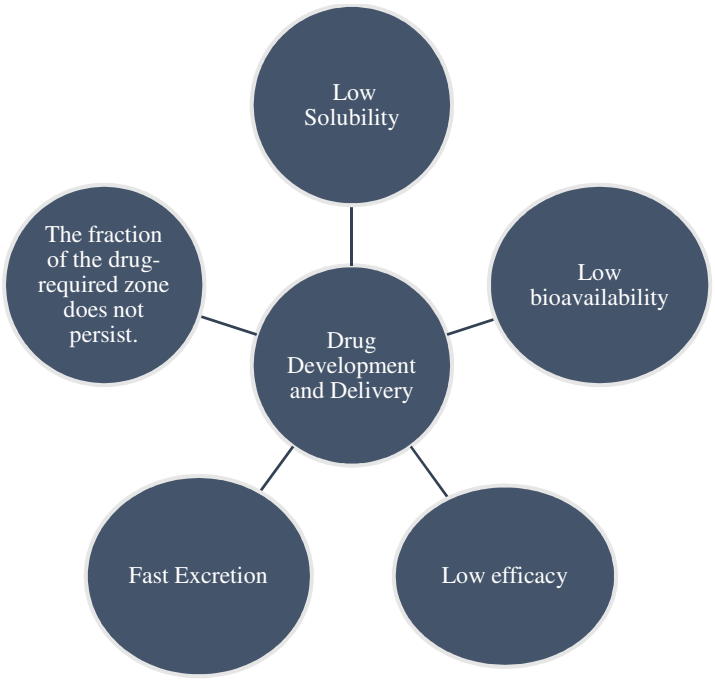

Figure 2: Displays the Pharmaceutical industry's Obstacles to the Development and Delivery of the Drug.

2. *Low Efficacy:*

A drug's efficacy is measured by how well it works after a certain dosage has been administered. The medicine must have a high affinity for the target to be highly effective. This is termed the molecule's affinity. Low affinity for the target chemical means that maximal response will be lowered. The medicine molecule's low effectiveness is one of the main issues that cause the treatment of serious illnesses to take a long period [15].

3. Fast Excretion:

The process by which excretory organs like the kidneys remove waste and toxins from the body is known as excretion or elimination. Because the required quantity of drug molecules does not reach the target organs because of the rapid excretion of the drug molecule, the medicine's effectiveness is greatly reduced.

4. Fraction of drug required zone is not persist:

For optimal therapy, some parts of the organ need an accumulation of the medication in a certain quantity. For example, the concentration of the drug has to begreater in malignant cells than in normal cells. This is necessary for correct and effective treatment. Chemotherapeutic drugs used

in the treatment of cancer are characterized by suboptimal drug accumulation. The idea of a drug delivery system was developed so that problems like these, which are connected to the formulation of medicine, might be solved. The term "drug delivery system" refers to both the formulation approach and the mechanism that is used to administer pharmaceutical substances in sufficient quantities inside the body to provide the desired therapeutic effect without risk to the patient. It's a method to provide medication to a patient where the dosage is altered so that certain parts of their bodies have a higher concentration of the drug than others.

There are essentially two strategies that may be used to deliver the medication to the cancer cells, and these are actively targeting and passive targeting shown in Figure 3. The targeted treatment of cancer has been greatly advanced by nanotechnology's revolutionary contributions.To drive nanoparticles toward the cells of interest, only little modifications to their size, shape, physical and chemical characteristics, and so on are necessary, and so on are required. This would enable the nanoparticles to be "programmed." Based on their choice, they may aggressively or passively approach the neoplastic cells.

3. METHODOLOGY

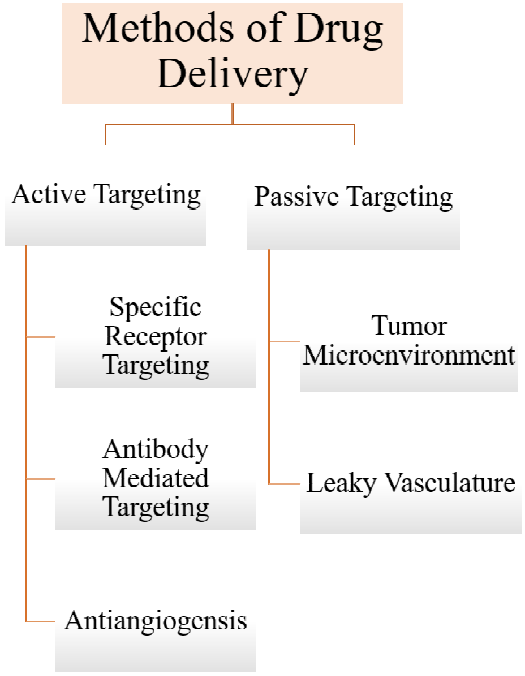

Figure 3: Active and Passive Techniques for Drug Targeting.

4. DISCUSSION

4.1. Active Targeting for Drug Delivery :

Nanoparticles used to transport chemotherapeutic medicines are generally designed to have a synergistic effect on diseased cells.Linking the nanoparticle to a targeting moiety is a common method of active targeting. Functional properties are possessed by nanoparticles in a variety of ways.To attach to cancer cells' receptors, nanoparticles must have an important functional group on the surface of the particle itself. Active targeting nanoparticles are designed to precisely target malignant cells by ligand-receptor interaction or antibody-antigen identification [16]. In active

targeting, the chemotherapeutic drugs are contained in nanoparticles that are specifically tailored to contact with cancer cells. Molecular recognition is the basis for active targeting. The surfaces of nanoparticles have been modified to particularly target cancerous cells. Targeted compounds are often applied to the surface of nanoparticles for molecular recognition. By ligand-receptor or antibody-antigen recognition, nanoparticles can target malignant cells.There are three major components to a nanotechnology-based targeted delivery system:

5. An Agent Capable Of Causing Death (Anticancer Drug).
6. A Moiety-Targeting Enhancer of Molecular Penetration.
7. A carrier.

Nanoparticles may now be manufactured from a wide range of materials, including polymers, lipids, ceramics, or metals. Drug delivery vectors generally consist of polymers and lipids, both organic and manufactured. The phagocytes and macrophages in our bodies absorb the drug-carrying nanoparticles because of the immune system's invasion nature. The polymer coating technique was created to prevent the engulfment of these nanoparticles. The polymer has either hydrophilic or hydrophobic areas in its structure. To avoid being opsonized or eliminated, the nanoparticle has a hydrophilic polymer covering its surface that resists plasma proteins. As a result, it's referred to as a "cloud" [17].

At the molecular level, cancerous cells vary from healthy cells in several ways. The distinctive trait is that certain receptors are overexpressed on the surface of them.By fusing complementary ligands to the surface of nanoparticles, cancerous cells may be specifically targeted. Once the drug-encapsulated nanoparticles bind to the receptors, they are quickly ingested by the cells through receptor-mediated endocytosis or phagocytosis. For therapeutic usage, these ligand-receptor interactions are now being studied in several ways.

4.1.1. Targeting Specific Receptors:

- *"Folate Receptor":*

Overexpression of folate receptors in malignant cells provides an anticancer therapeutic target. Scientists are using the technique to build the nanoparticles' surfaces with folic acid [18].Folic acid was investigated by Russell-Jones *et al.* in four mouse tumor models to see whether it might beutilized as a targeted agent for poly(N-(2-hydroxypropyl) methacrylamide) (pHPMA) conjugated daunomycin administration. Using folic acid-specific daunomycin-HPMA conjugates, more tumor-bearing mice survived and lived longer. Folate may enhance the effectiveness of other polymer-bound mycotoxins, according to the results [19].In immunodeficient athymic female mice, foliate-linked methotrexate dendrimers were evaluated by Kukowska-Latalloto and colleagues. The nanoconjugates were administered multiple times a week into the tail veins of the mice.At the same dose levels, dendrimer-conjugated methotrexate was 10 times more efficacious than free methotrexate, with far lower cytotoxicity. Since this resulted in longer-lived mice [20].

- *Transferrin (Tf) Receptor:*

Certain tumor cells over-express transferrin receptors, which are being studied for their ability to attach and enter cells, to improve their iron absorption by nanoparticles. A variety of materials may be added to transferrin for tumor targeting such as chemotherapeutic agents, toxic protein

conjugates, RNase conjugates, antibody conjugates, and peptide conjugates.The tf-lytic hybrid peptide was identified by Kawamoto *et al.* to target only malignant cells. They used an athymic mouse model withHuman Mammary Carcinoma (MDA-MB-231) cells to give Tf-lytic peptide.Nanoparticles with little transferrin modification stay stable at physiologic salt concentrations and are more effective at transfecting leukemia cells, according to Bellocq *et al.* findings that therapeutic nucleic acid therapies may be delivered systemically using nanoparticles modified with transferrin (TF) [21].

Angiogenesis in hepatocellular carcinoma is suppressed by a dual-particle tumor targeting strategy. Nanoparticles enclosing ganciclovir and galactosamine andthe dual-particle tumor targeting system was made up of EPR-mediated targeting nanoparticles harboring an HSV TK gene. Thymidine kinase digests ganciclovir, killing cancer cells, once both nanoparticles are taken up by the cells at once. Cancerous cells are eliminated as a result.

4.1.2. Targeting through Antibody:

Antigens expressed by tumor cells are typically mismatched to the cell type, microenvironment, or developmental stage in that they are located because of genetic abnormalities. Tumor antigens don't elicit as robust an immune response as they should since the immune system recognizes them as its cells. These monoclonal antibodies (mAbs) may be utilized to boost the immune system's anti-tumor capability and enhance the body's ability to fight cancer. In cancer cells, proteins that are abnormally produced and necessary for proliferation are targeted by these antibodies. For targeted medication delivery, nanoparticles coupled with antibodies against particular tumor antigens are being created.

Phage display libraries allow for the rapid selection of antibodies or monoclonal antibodies that bind to and enter cancerous cells. Different epitopes of the same target cell may be targeted by this strategy, which results in a collection including potentially effective antibodies. A single receptor epitope is therefore identified by numerous antibodies, which results in more precise and selective activity.Leukemia cells have an increased number of Fas receptors on their surfaces, nanoparticles may be engineered in such a way as to boost the production of the Fas ligand which, when bound to its receptors, functions as a type-II transmembrane protein,commences the process of death in the cell. The immune system's modulation, as well as the beginning of cancer both depend to a great extent on the interactions that take place among ligands of the Fas family with their respective receptors.

4.1.3. Antiangiogenesis:

Angiogenesis refers to the process by which new blood vessels grow from old ones. Having angiogenesis is critical for growth and development, and also forhealing a wound. It also contributes to the production of granulation tissue. The excessively high quantities of angiogenic growth factors generated by cancer cells overpower natural angiogenesis inhibitors, leading to abnormally leaking or convoluted blood vessels that are chronically inflammatory in the patient. Most angiogenesis research today focuses on inhibiting vascular endothelial growth factor (VEGF). A high VEGF level is seen in 60% of malignant tumors. Angiogenesis stimulators beat angiogenesis inhibitors in the initial phases of tumor formation, allowing for more uncontrolled blood vessel growth and production.

4.2. "Passive Targeting":

Nanoparticles tend to concentrate in malignant cells during the increased permeability and retention (EPR) phenomenon during the process of passive targeting. The EPR effects may be seen as an overarching principle that applies to all nanocarriers. According to experts, the EPR effect is now the gold standard for developing cancer-targeting drugs. Passive targeting is accomplished by introducing nanocarriers and cells into the tumor interstitium using leaky tumor capillary openings that move them by convection or passive diffusion (a collagen network and gel-like fluid).

For example, nanoparticles may be used to target cancer by simply scanning the environment for signs of the illness. However, cancerous cells continue to degrade nutrients by blood vessels in an abnormal manner, which results in angiogenesis-induced leaky and wide blood arteries around the cells. As a result of basement membrane anomalies and a lack of pericytes, this occurs that line develops rapidly endothelial cells.During passive delivery of the medicine to cancer cells, there may be several limitations are:

i. The level of tumor vascularization and angiogenesis affects passive targeting.

ii. As a consequence of this, the nano-carrier's extravasation might look quite different depending on the kind of tumor or where it is located anatomically.

iii. As a result of elevated interstitial fluid pressure in a tumor, medication absorption and distribution are hindered.

4.3. Preventing the breakdown of lysosomes:

Enzymes found in the lysosomes may degrade nanoparticles and pharmaceuticals found within cells. Once within the lysosome, hydrolytic enzymes can break down the nanocarriers and the medicine it is carrying, which is why nano tools often reach this compartment first. This means that the carrier's intracellular distribution is altered whenever the medicine enclosed is a nucleic acid.

4.4. Traditional chemotherapy has some drawbacks:

To combat cancer, chemotherapeutic medications now in use target cancerous cells that are rapidly proliferating. Because chemotherapy damages healthy cells that reproduce quickly, such as bone marrow macrophages, digestive tract cells, and hair follicle cells, it is employed in cancer treatment. Chemotherapy using traditional methods has the major flaw of not being able to target just malignant cells with its effects.Because of this,most chemotherapy treatments cause Myelosuppression (increased production of white blood cells, leading to immunosuppression), mucositis (inflammation of the digestive system lining), alopecia (hair loss), organ damage, or even anemia or thrombocytopenia. Dose reduction, treatment delays, and even therapy discontinuation may be necessary due to these adverse effects.Chemotherapeutic drugs are rendered ineffectivebecause cell development is essentially inhibited at the tumor's center in solid tumors. Because chemotherapy can't get to the core of the tumor, cancerous cells are often left uninfected.

Chemotherapeutic medicines often have a solubility problem. Solubility may be improved by nanoparticles. Adding micelles to hydrophobic medications might increase their solubility. Dendrimers feature many binding sites, making it feasible to bind both hydrophilic and

hydrophobic compounds. Liposomes may be used to swiftly transfer hydrophobic medications to the target region following the administration.

5. CONCLUSION

Cancer treatment has benefited greatly from nanotechnology, and the field is seeing fundamental shifts as a result. It has greatly impacted the ability to identify cancer cells selectively, deliver drugs precisely where they need to go and succeed where conventional chemotherapies have failed.An important conclusion drawn by the authors of this research is that new ways of medication administration employing nanotechnology approaches are improving cancer therapy. Cancerous cells may be identified, targeted medication delivery can be achieved, and traditional chemotherapies could be overcome by the method. Research and clinical trials are now being conducted on a variety of items that are based on nanotechnology.Because cancer is one of the deadliest and most serious illnesses, nanotechnology could be able to make a positive contribution to a change in clinical practice that might lead to the development of an approach that saves lives.

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CHAPTER 14

A COMPREHENSIVE ANALYSIS OF DISEASE DIAGNOSIS FOR ECONOMICALLY IMPORTANT CASH CROPS

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ABSTRACT:

Loss of food owing to the pest attack and evasion in crops by various microorganisms for example viruses, fungi, bacteria, and nematodes have become common in the population and resistant against the chemicals at the same time. To reduce the frequent encounter of virulent microorganisms and host plants and minimize the risks associated with it, detection of the pathogen and study of different aspects associated with it is extremely important. Plants and even seeds sometimes don't show the symptoms and continue to get re-infected with other pathogens. Farmers and cultivators all around the globe suffer high economic losses and time wastage in mending the consequences of the symptom. This review article focuses on the detection of pathogens by techniques feasible in research labs, these techniques are Enzyme-linked immunosorbent assay (ELISA), Polymerase chain reaction (PCR), Flow cytometry, and immunofluorescence (IF). Further, the study points out the advantages of the early and accurate detection of the infection, which will also lead to modifications in the existing techniques to help enhance precision and accuracy.

KEYWORDS:

Disease Detection, Enzyme-Linked Immunosorbent Assay (ELISA), Flow Cytometry, Immunofluorescence (IF), Polymerase Chain Reaction (PCR), Pathogen, Virulent.

1. INTRODUCTION

The growing demand for food and food insecurity are the world's top problems that need immediate attention both internationally and nationally. The year 2008 reported price hikes on food products which created a crack in the economic and political sectors of the country. According to a survey, the consumption of food will rise double the existing number to meet the requirements of billions of people. With the growing demand and lack of food supply, the majority of the population suffers from malnutrition, rickets, and other health issues. Issues like this contribute to the deterioration of crop fields. The reason for the damage to crops is a large number of pests and microorganisms with pathogenic nature causing harm to the vegetation all across the world. Figure 1 shows other reasons contributing to crop losses. An estimation of 25-50% of crops is lost annually on a global level due to pathogenic diseases.

Majorly crops such as rice, barley, soybean, and corn are severely affected by frequent encounters with diseases and contagious infections [1]. Farmers in past used conventional approaches for managing the microorganism and the associated diseases. Application wise the approaches turned out to be inefficient in terms of result understanding and inaccurate identification of microorganisms. Moreover, detection methods in current times are efficient in

all these aspects. Treatment of pathogens presently is done by chemical and biological agents like "pesticides" and "fungicides". The categorization of diseases prevalent in the plant is grouped according to plant pathology. Table 1 suggests common diseases and their causing agents. Treating pathogens and managing the consequences does not suffice the root cause of the problem, to reduce the chances of reencounter infections it is necessary to conduct deep research in inventing new scientific solutions for better treatment [2]. The link between the microorganism and host plant for a longer time leads to survival susceptibly and a powerful resistance mechanism which also relates to the widespread of the diseases in other regions. Several countries have imposed regulations to restrict the entry of non-native pathogens into new premises that are usually backed by various identification methods.

Table 1: List of Pathogenic Agents and the Diseases Caused by them in their Target Host plants.

Pathogenic Agents	Diseases	Target Host
Alternaria solani	Early blight	Potato and Tomato
Xanthomonas campestris	Black rot	Brassicas
Pseudomonas solanacearum	Granville wilt	Eggplant and Tobacco
Alternaria triticina	Leaf blight	Wheat
Pseudomonas syringae	Wildfire of tobacco	Tobacco

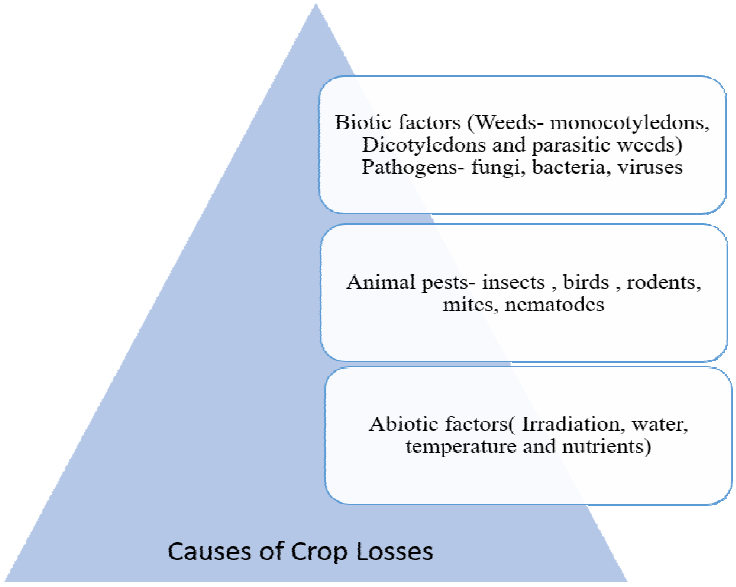

Figure 1: Pictorial Representation of Other Factors Responsible for Crop Losses.

1.1.Classification of pathogens targeting vegetation

1.1.1. Fungi

Belongs to the kingdom of eukaryotes, often known as the decomposer in the environment. Moulds, mushrooms, and yeast are an example of it. They are non-vascular, unicellular with both sexual and asexual modes of reproduction. Fungi are different from other species because of their characteristic features like the presence of chitin in the cell wall. They are heterotrophic by nature, the digestive enzymes in the organism facilitate the absorption of food nutrients from the surrounding habitat. Fungi organism is not dependent on the process of photosynthesis for the production of food rather their mobility act as a mechanism against the food hunt. They grow best in moist, hot climates even at room temp (25℃) [3].

1.1.2. Bacteria

Bacteria belong to the kingdom of prokaryotes, an organism that lacks a nucleus. Usually, less than 10µm in size are most widely found in the environment. Identification of bacteria can be done based on their shape and size. Bacteria can be classified as vibrio, bacillus, Coccus, and spirillum. It can be easily grown on Mannitol salt agar, tryptic soy agar, and eosin methylene blue agar [4].

1.1.3. Viruses

Viruses are not categorized under any kingdom among the 5 kingdom classification because they are neither alive nor dead. They are composed of either DNA or RNA and are dependent on host body machinery for multiplication. The structure of the virus consists of an outer protective protein coat called capsid in which the genetic material is intact.

1.2.Lab-based detection techniques for pathogens and disease diagnosis in crops.

Techniques based in the lab provide comparatively accurate and precise results in comparison to traditional methods used for the identification of the pathogenic agent and disease diagnosis. Techniques like PCR, ELISA, Flow cytometry, and IF help in the rapid detection and identification of a microbe. The recent advancements in molecular technology tools especially in detecting foodborne infections have provided a major breakout inrefining the existing applications of biotechnology.

2. LITERATURE REVIEW

Yi Fang and Ramaraja P. Ramasamy documented their views on Methods used for the detection of plant disease. The authors discuss the seriousness of the present issue which is related to crop damage due to pest and pathogen attacks resulting in economic and political crisis. The paper explains the use of “Polymerase chain reaction (PCR), Enzyme-linked immune sorbent assay (ELISA)” and other techniques and their employment in disease detection [1].

Jodi Woan- Fei Law et al. reported a paper on ‘Quick detection techniques for foodborne bacterial pathogens. The authors explain the sudden increase in cases comprising foodborne infections and how it is now been considered a global health concern, further the study emphasizes the principles used behind techniques like “PCR, nucleic acid sequence-based amplification (NASBA) and Loop-mediated isothermal amplification (LAMP)” in the detection of pathogenic agents and diseases in plants [5].Ajay Kumar Gautam and Shashank Kumar explicated a research study on methods used for the identification and detection of diseases

prevalent in crop fields. The authors describe different organisms from the 5 kingdom classification and a brief discussion on the use of "scanning electron microscopy (SEM), Nuclear magnetic resonance (NMR), and visible and infrared spectroscopy" for examining the diseases [2].

Syed Mahyar Mirmajlessi et al. illustrated a research paper on the detection of pathogens in strawberries by employing the PCR method. The authors discuss various pathogens responsible for limiting strawberry production and the use of PCR in the detection and diagnosis of disease. The researchers also introduced a new method which is a DNA sensitive technique that has applications in rapid identification of the pathogenic agent [6].

Syed Mahyar Mirmajlessi et al. explained their views on 'Detection and Diagnosis of pathogens targeting plants by the use of real-time PCR'. The authors explain the employment of q-PCR as a fundamental molecular technique for the detection of infection and how the fluorescence "signal measures specific reaction kinetics" [7].

M.L. Edwards and J.J.Cooper demonstrated a research paper on "detection of plant diseases by the use of In-direct ELISA" the paper discusses the detection of plant viruses by indirect-ELISA. "The technique employs protein A as an agent in sandwich antibody-antigen-antibody sandwich. The primary layer of A- protein makes the surface for the coating layer of antibody and associated A- protein with an enzyme which detects the second layer of antibody [8].

Liesbet D' Hondt et al demonstrated the use of "flow cytometry in studying different aspects of studying genome of the plant". Flow cytometry helps in the characterization of the fungal genome, the study also talks about the past researchers and associated benefits and consequences with the addition of prospects of the technique. Flow cytometry is a fundamental tool used in both research and detection tools [9].

B.Ben Bohlool and Edwin L. Schmidt explained a "Research study on studying the microbial biology by the use of Immunofluorescence". The authors discuss the principle working of the IF technique and the use of the same basics in the determination of microorganisms present in the tissue specimen of the animal along with the process of experiment with a detailed description of every step in the method [10].

3. DISCUSSION

To provide an effort in minimizing the chances of crop exploitation by pests and other microorganisms it is necessary to introduce substitute and beneficial tactics in managing the consequences. One of the important aspects of disease management is the early detection or timely identification of pathogen causing infection in the host plant. There are different symptoms associated with the pathogen invasion and sometimes the symptoms don't appear therefore a reliable diagnosis of the disease is needed in eliminating the further chances of reinfection by developing diagnostics techniques. PCR detection is a molecular tool that is easy to operate and provides reliable results.

3.1.PCR Detection for pathogenic microbes

The process of PCR starts with the isolation of microbes or fungal spores from the infected part of the leaves. In the case of a fungal spore, the isolation process will proceed with pooling the fungal spores and culturing them on a petri dish with the liquid media of sabouraud dextrose

followed by picking the fungal colonies and proceeding with the DNA extraction method. DNA extraction can be performed by the conventional method of phenol-chloroform method or by the use of any commercial DNA extraction kit. The next step involves DNA purification by using a (Commercial kit). To check the presence of genomic DNA in the isolate, the Sample DNA is run through an agarose gel electrophoresis. After the completion of electrophoresis, PCR is set up. PCR involves the use of template DNA, forward and reverse primer, deionized water, dNTPs, and Taq polymerase enzyme. The process is completed in three steps, starting with denaturation of the DNA template into a single strand then annealing of the primers to the original strand, and synthesizing a new strand the last step includes an extension of the newly formed DNA strand from the primers. Figure 2 shows the stages of PCR [11].

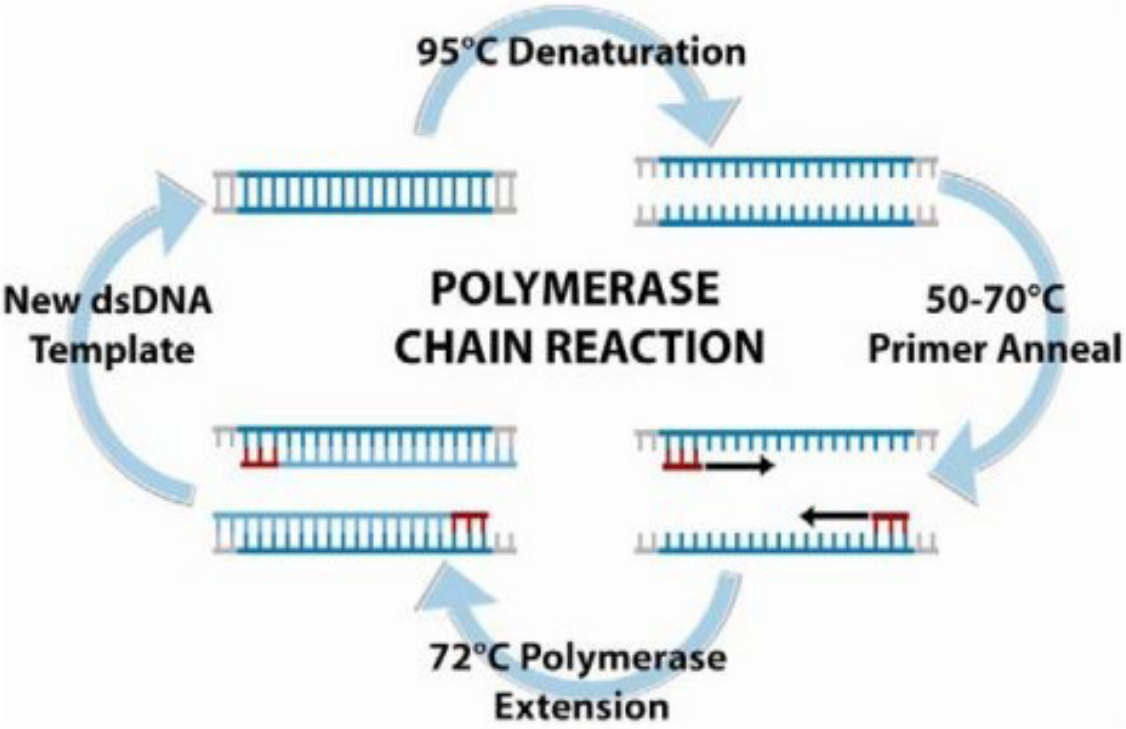

Figure 2. Stages of PCR Cycle Starting with Denaturation of DNA Template, Annealing of the Primers, and Extension of Newly Formed DNA.

3.2.Application of Flow Cytometry in Detection of Pathogen in Crop Fields

The technique measures and counts the small particles in the stream of fluid. The flow cytometry unit consists of 3 sub-sections i.e. optics, electronics, and fluidics. The system works when all the particles get excited and jump from a lower state of energy to a higher state of energy by the action of light. "It can identify various factors such as "sideward scatter (SSC) and forward scatter (FSC) along with wavelengths of fluorescence which is dependent upon the source of excitation and the handling of the instrument. FSC along with the SSC provides results about the size, shape, and complexity of the cell. Flow cytometry has an application in determining the plant genome size because it is comparatively simple to use and rapid. The process involves the use of a blade for the nucleus to get suspended in the solution that can later be analyzed by the flow cytometer single parameter. The results are in the form of a fluorescence intensity histogram and G1 as the peak, whereas G2 is considered as the second peak. The results are then

interpreted with the content of DNA and mitotic cells. Figure 3 explains the process of detection inside a flow cytometry arrangement" [9].

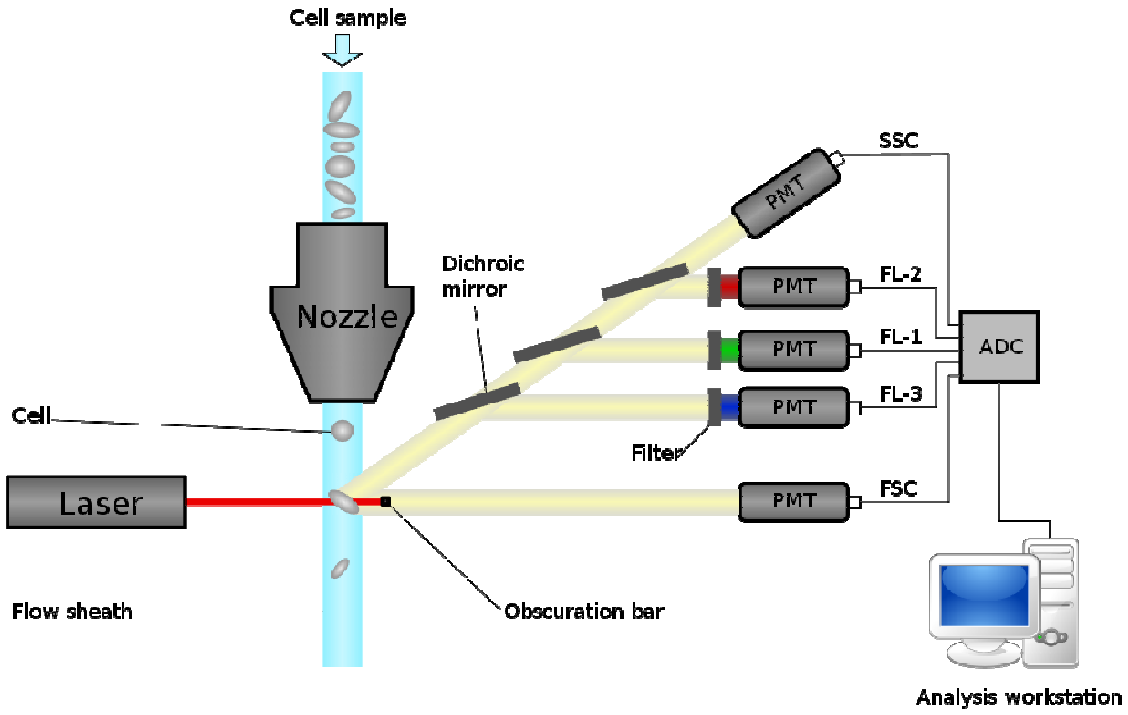

Figure 3. Diagrammatic Representation of Arrangement of a Flow Cytometry Machine

3.3.Pathogen Detection by ELISA

ELISA is a diagnostic tool with applications in the identification of a particular antigen against the antibody that is particularly specific for it. The assay works on binding the target antigen with the antibody that is present in the test sample which is already set around the walls of a microtiter plate. Antigen identification is made possible by a visible color reaction that is processed by an enzyme. The whole assay reaction is performed inside a plate of 96 wells. The reaction of color change is done by a spectrophotometer to quantitate the pathogens. ELISA uses polyclonal and monoclonal antibodies for detection. Figure 4 shows the procedure of ELISA.

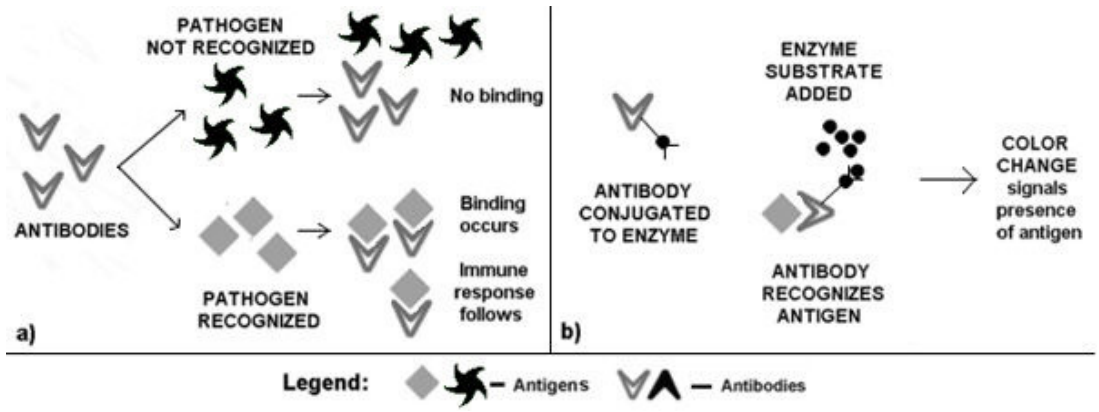

Figure: 4 Systematic Representation of ELISA Assay for Detection of Antigen Causing Plant Infection.

3.4.Immunofluorescence in the detection of Plant Pathogen

Use of Immunofluorescence in quick identification and detection of microbial infections in the plant host. It involves staining plant tissues and detecting the antibodies in vivo that are attached to an antigen of the tissue by the use of a labeled single antibody along with fluorophore for the tissue or cell staining. The Method detects the target molecule and attaches to it, this whole process is also known as direct immunofluorescence. Figure 6 shows the process of immunofluorescence. Fluorescein isothiocyanate is the most commonly used dye for detection. It belongs to the class of xanthene dye. Figure 5 describes the chemical structure of the dye [12].

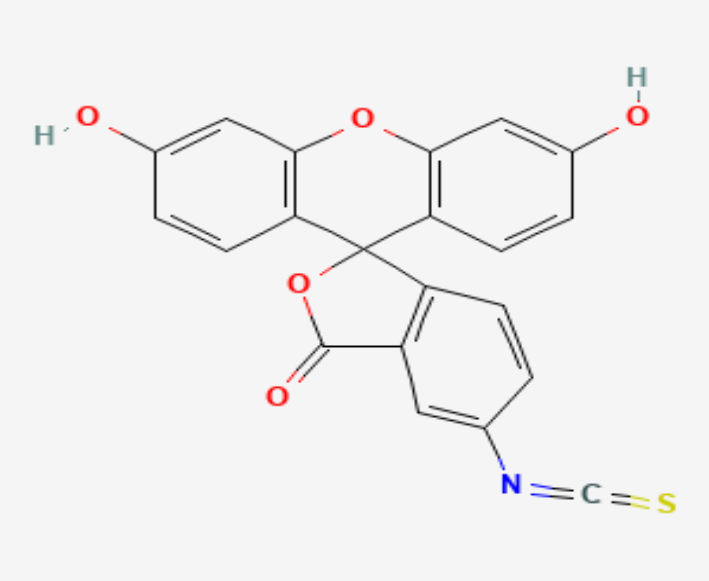

Figure 5. Chemical Structure Representation of Fluorescein Isothiocyanate (Commonly Used Dye in Immunofluorescence).

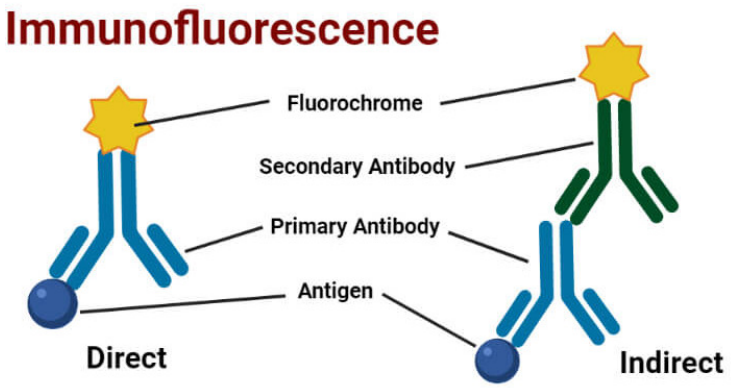

Figure 6. Pictorial Representation of Immunofluorescence Showing both Direct and Indirect methods.

4. CONCLUSION

Crop wastage is an issue that creates economic problems worldwide. With pathogens and pests infecting plants and becoming resistant to the treatment of most of the chemicals. Detection of such pathogens is important to develop new diagnostic tools with advanced technology and high accuracy. The present review study contains different detection and diagnostics tools for diagnosing pathogens and microorganisms in an infected plant. This paper reports the detection of diseases by PCR assay, Flow cytometry, ELISA, and immunofluorescence.

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CHAPTER 15

A PROSPECTIVE STUDY ON BIOMARKERS FOR PRECISION MEDICINE AND CLINICAL TRIAL VALIDATION

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ABSTRACT:

Markers used for pathological, biological, or pharmacological processes or applications are recognized as biomarkers. It has applications in cancer diagnosis, prognosis, risk assessment, therapy response, and tracking disease progression. Medical decision-making and therapeutic guidance using biomarkers aims to improve patient outcomes while decreasing potentially harmful treatment. In present study an attempt for evaluation of biomarkers from a clinical standpoint and to find out that how a biomarker impacts individualized treatment plans and care for patients. In order to develop and validate genomic biomarkers or signatures, a specific framework is required. This paper includes various types of clinical trials that have been conducted using biomarkers for the purpose of determining the therapeutic value of a biomarker or a novel medicine by using a matching biomarker. According to the results of this study, precision medicine holds great potential for advancing healthcare in the future. Despite the fact that oncology is where precision medicine is now at its most advanced level of research, other fields, such as those dealing with rare and genetic illnesses and COVID-19, have exciting possibilities.

KEYWORDS:

Biomarkers, Genomic Data, Precision Medicine, Predictive Biomarkers, Prognostic Biomarkers.

1. INTRODUCTION

The term "biomarker," which is a "biological marker,"(BM) is used to denote the presence or absence of a particular biological event or activity in the human body. As per the general perception for biomarkers to be used for diagnosis and “medication," although some are used to reveal that the body has been exposed to a chemical, toxin, or other environmental influence. The molecular diversity across diseases with the same diagnosis has been steadily exposed to advancements in biotechnology and genomics. Treatment strategies may be developed when researchers have a better grasp of disease biology, and useful biomarkers or screening procedures could be created when researchers better comprehend disease heterogeneity [1].

Any material, activity, or structure inside the body that may be monitored or measured and whose outcome might influence or prognosticate the development of sickness or its effects is considered a biomarker by the World Health Organization (WHO) [2]. Any objective and subjective assessments of a patient's health are included, as well as all diagnostic tests. A biomolecule in tissues, whole blood, and other bodily fluids which can be used for diagnosis is called an oncological biomarker (oncomarker). It is a unique assayable trait that might be used to detect abnormalities, monitor physiological processes, or evaluate pharmaceutical effects [3].

There are several medical applications for biomarkers, and their usage will become much more common as customized medical care becomes the norm. Biomarkers may be used to determine what will occur if patients take or would not use a specific medication, as well as their risk of getting specific medical diseases. The recent advancement of sophisticated molecular assay technologies, including SNP arrays, Protein Arrays, and Gene Expression Microarrays, has enabled the identification of possible novel biomarkers in addition to the creation of composite genetic profiles for gene therapy [4].

The parallel growth of high technology has encouraged the creation oftrial-based data-driven analytical techniques for high-dimensional genomic data.The parallel growth of high technology has encouraged the creation of approaches for data-driven analysis of high-dimensional genomic data employing trials, meaning that information about a specific clinical characteristic, such as responsiveness to a therapy or survival outcomes after therapies, is used in evaluating genetic data. In particular, two significant statistical methods are highlighted:

i. Genetic Characteristic Screening for Further Research.

ii. Genetic predictor/classifier construction for a clinical outcome. However, there are unique difficulties associated with selecting a subset of meaningful signals from the genomic data in the presence of a significant number of noise factors due to the data's high-dimensional.

iii. In the case of developing genetic biomarkers, several bio statistical or bioinformatics-based strategies have been presented [5].

Significant advances in genomics and proteomics over the past several decades, as well as impressive advances in the usage of genome expression assessment to study molecular data gathered from the patient, have entirely altered the field of personalized medicine.Biomarkers may be used in a variety of settings. Biomarkers have several potential uses, including as diagnostic tools, for patient stratification/triage, for evaluating therapy response, measuring disease staging, and for deciding safety by indicating toxicity and adverse effects shown in Figure 1 [6].

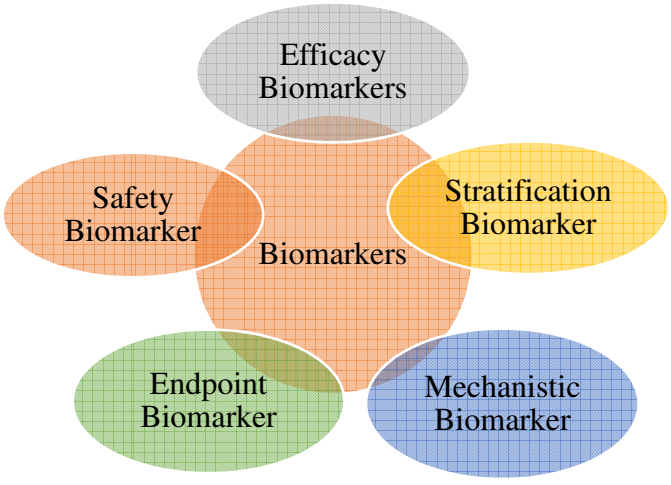

Figure 1: Types of Biomarkers and their Possible Applications.

Using biomarkers for diagnosis and prognosis is nothing new; in fact, it dates back millennia. The concept of employing diagnostic testing to guide treatment is now well-established in medical practice, thanks to the widespread availability of such testing via standard laboratory procedures. The pathologists diagnose malignancies according to phenotypic histological or immunochemical markers and guide prognoses and treatment plans. Finally, individuals who are experiencing adverse effects of statin therapy may be identified by testing their liver function enzymes [7].

This study is organized in the following manner. After defining an important group of biomarkers for individualized healthcare and outlining key standards for validating those indicators, Biomarkers, Personalized Medicine, Translational Research, and the Future of Healthcare, Analysis of gene expression data for use in developing genomics biomarkers and Clinical Medicine of Utilization Trends and Barriers to Implementation discuss in Section.

2. LITERATURE REVIEW

Razvan Cristescu *et al.* stated in their study that a cancer genomes dataset from four “Merck pembrolizumab” clinical studies. This website evaluates 300 patient samples from 22 tumor categories. Immunotherapy response is predicted byTumor mutational load and an inflamed "hot" T cell microenvironment. Therapeutic utility predictions for two candidate biomarkers are determined in this investigation. Inflammatory biomarkers (“PD-L1 expression” and T cell-inflamed GEP) and “Tumor Mutational Burden” (TMB) may effectively classify human cancers with different health outcomes to “Pembrolizumab Monotherapy”, the author concludes [8].

Enrico Glaab *et al.* discussed in a study that clinically relevant FDA-cleared diagnostic or lab-developed assays to uncover common characteristics or recommend future biomarkers study emerged fromomics-based biomarker discovery investigations for patient stratification. In all, 352 items passed. The study of confirmed biomarker signatures revealed similar conceptual and practical elements which may justify appropriate testing success or inform future biomarker studies.According to the author, the first non-cancer applications demonstrate the promise of multidimensional omics biomarkers identification for other complicated disorders.Early filtering and robust discovery techniques, continuous assay design advancements, empirical measurement technologies, or thorough multicolored verification all point to improved research [9].

Kim van der Eecke *et al.* conducted a study to summarize the genetic alterations assess their clinical repercussions in liquid and clinical specimens, and compare them across illness steps or clinical characteristics.There were 11 different studies discussed. The author studied 1682 individuals with “Metastatic Hormone-Sensitive Prostate Cancer (mHSPC)”. Aberrations in the Wnt pathway, DNA repair, and TP53 were all associated with the high-intensity disease. However, constraints in sequencing data, solutes (tissue or liquid), alteration calling thresholds, or targeted patient groups with under-representation of recurring metastatic sickness need confirmation in biomarker-driven controlled randomized clinical trials before any therapeutic use. The ability to scale quickly is dependent on standardized testing procedures and comprehensive documentation [10].

Harald Hampel *et al.* stated in their study that Alzheimer's disease (AD) treatments haven't been found.The first systematic look for therapeutic biomarkers of Alzheimer's disease across the entire genome. Both exomes and transcriptomes were sequenced from 21 patients. In the Knowledge Extraction and Management (KEM) technique, unsupervised Formal Concept

Analysis (FCA) is used was used to investigatethe link between patient information and effectiveness outcome measures. These patient selection indicators are now being studied in Phase 2b/3 clinical trials. This FCA/KEM research serves as a model for identifying patient screening signals in the initial stages of neurologic developing drugs [11].

Ana Paula Alarcon-Zendejas *et al.* conducted a study that this meta-analysis seeks to offer a summary of recent breakthroughs in the identification of novel diagnostic markers using transcriptomes, genomics, and artificial intelligence which are likely to enhance prostate cancer patient clinical care. Connected Paper,Google Scholar and PubMed performed an extensive investigation using search terms related to genomic biomarkers.Researchers investigate prostate cancer's novel molecular markers for diagnosis, prognosis, or treatment prognosis, and how bioinformatics methods like machine learning and artificial intelligence might benefit the clinical. Spatial Transcriptomes, Exomes Sequencing, and Whole-Genome Sequencing are also included. The author concludes that transcriptomic or genomic analyses have contributed to the study of prostate cancer by revealing fresh details concerning coding and non-coding genes as indicators.

3. DISCUSSION

Clinical investigations are necessary for determining a biomarker's validity and clinical value before it can be used in clinical practice for precision medicine. The gold standard for determining a biomarker's or a novel treatment's therapeutic value is the results of randomized clinical trials. Different biomarker-based randomized clinical trial approaches have been suggested and used in recent years.

3.1. Validation Criteria for Personalized Medicine Biomarkers:

3.1.1. Prognostic and Predictive Molecular markers:

In contrast to predictive biomarkers, which offer insight into the impact of treatment, prognostic biomarkers provide information about the entire cancer prognosis for a patient independent of therapy.A predictive biomarker can be a target for therapy.To determine who is most positioned to gain from a certain therapy, clinicians might use predictive biomarkers that are measures taken either before therapy or as a baseline. Predictive biomarkers are typically chosen to work with a newly developed therapy. Prognostic biomarkers are measures taken before therapy begins that would provide data about the prognosis for both untreated and standard-of-care-treated patients. Prognostic biomarkers are useful because they may guide patients to receive routine care, but unlike predictive biomarkers, they cannot guarantee a positive outcome from therapy. More aggressive therapy would be necessary for individuals with a bad prognosis, whereas those with a fair outlook would not benefit from any further therapies.

3.1.2. Validation of Biomarkers Criteria:

The purpose for which a biomarker is being developed must inform the criteria used to validate it. Prognostic and predictive biomarkers have been suggested to undergo three distinct forms of validation: analytical validation, clinical validation, or clinical relevance [12]. The term "analytical validation" is used to describe the process through which an assay's reliability, repeatability, and measurement accuracy (including sensitivity and specificity) are established to a gold standard test [13].

The capacity of a biomarker to forecast prognosis and clinical outcomes for particular individuals is what we mean when we talk about its clinical validity. There could be therapeutic accuracy for a diagnostic indicator if there is a connection between both the biomarker's state as well as a clinical outcome (like survival rates).A controlled randomized trial is needed to measure treatment effects (of a new medication relative to a control treatment) and evaluate whether treatment variability differs depending on the state of the biomarker, i.e., a diagnosing interplay, in a straightforward screening verification of factors that influence a clinical endpoint. Lastly, clinical usefulness requires that the biomarker be validated and may be used to enhance patient outcomes and provide tangible benefits to patients. Therapy effects linked with the usage of the created predictive biomarker would be assessed during the development of a novel treatment as well as a companion predictive biomarker [14].

3.2.The Role of Biomarkers, Personalized Medicine, or Translational Research in the Development of the Future of Healthcare:

Biological macromolecules and physiological characteristics that are objectively assessed to function as a marker or indication of a normal or pathological cascade are referred to as Biomarkers (BMs). Validation or detection of illness-specific biomarkers is very helpful in the process of developing personalized treatment strategies [15].The notion of customized healthcare, particularly as it pertains to medical intervention based on new biomarkers, is believed to have a substantial impact on the future of healthcare. It represents a recurrent expansion of development in the area of medicine toward a more differentiated assessment of both individuals and illnesses based on using biomarkers with unique properties that are increasingly available due to recent advances in "omics" technology [16].The recent developments in biomarker analysis, medical informatics, or Biocomputing, in conjunction with advances in biotechnology, have increased new possibilities in the rapidly expanding disciplines of personalized medicine or predictive medicines.

When correctly implemented, translational medicine seeks to increase communication across different areas of research and development to highlight the benefits of sharing knowledge from "bench to bedside" and "bedside to bench." The most successful tailored patient care choices may be made when information freely flows between the clinical and preclinical contexts. By using cutting-edge molecular research methods for individual patients, "precision medicine" aims to reduce their risk of developing the disease [17].The goal of this discipline is to help patients and doctors choose the most effective methods for treating illness, taking into account each person's genetic makeup and the specifics of their living conditions. Precision medicine has the potential to significantly impact the healthcare system over the next several years. Numerous new prognostic or diagnostic technologies would improve our ability to predict the likely outcomes of pharmacological treatment. Moreover, expanding the use of biomarkers during medication development might result in positive therapeutic results.Health outcomes may be enhanced, and healthcare may become more cost-effective if this is implemented.

3.3.Clinical Trial Designs Based on Biomarkers to Evaluate Their Value:

Different randomized clinical trial approaches depending on biomarkers are described in this section. One way to do this is to compare the standards for the treatment without the use of the biomarker, therefore establishing the biomarker's clinical value. Such an aim is inherent in the biomarker-strategy designs. Another category is using a predictive biomarker in conjunction with

a novel therapy under investigation to prove the treatment's therapeutic value. This is the goal of both the enrichment and also the randomize-all approaches.

1. *Biomarker Strategies:*

Patients in a study utilizing a biomarkers approach design are assigned at random to one of two groups: those who will have their treatment decisions informed by the biomarker, or those who will have their treatment decisions uninformed by the biomarker. Therefore, comparing two approaches with and without the use of the biomarker in deciding on therapy is the major goal. For example, in a randomized study for recurrent ovarian cancer, a strategy of deciding treatment based on tumor chemosensitivity (predictive) screening is compared to a technique of employing a physician's selection of chemotherapy using usual practice [18].

Due to the fundamental structure of the strategy-based designs, patients from both biomarker-based and non-biomarker-based studies halves of the research get the same treatment.As a result, a significant number of people would have to be chosen to test a precise, often tiny difference in results between the two groups.One modification would be to randomly allocate individuals from the two procedures to therapy that differ from one another. When selecting whether or not to take chemotherapy, Women who had estrogen receptor-positive breast cancer but no lymph nodes were split into two groups to either a biomarker-based analysis focusing on the Mamma Print predictive signals or a strategy based on standard conventional prognostic factors.

2. *Designs for Enrichment:*

An enriched or focused design uses a predictive biomarker to assess the effectiveness of an investigational drug against a standard of care in those who have a good chance of responding well to the treatment.As a consequence, the enrichment approach assesses the efficacy of treatment instead of the total patient population, a biomarker-based subgroup of patients. Patients should be screened to identify the existence of biomarkers in this technique.The effectiveness of the enrichment design in contrast to the traditional strategy of randomly allocating all patients without using biomarkers isbased on the number of biomarker-positive individuals as well as the new medication's efficacy in biomarker-negative patients.When there is strong scientific evidence suggesting that patients without the desired biomarkers would not take advantage of the new therapy and when the inclusion of these individuals would raise ethical problems, the enrichment design should be used. Furthermore, the enrichment biomarker must have been analytically verified with known assay precision, reproducibility, or robustness before the study could begin [19].

3.4.Implications for Clinical Medicine of Usage Trends or Implementation challenges:

Biomarker studies often consist of many phases, from initial discovery to eventual clinical use. Typically, there are four distinct stages in the biomarker development process. Clinical examination of a biomarker often includes examining hundreds to thousands of samples after an initial screening phase in which a smaller number of samples is evaluated. A well-established assay, sample handling protocol, as well as data analysis/assessment; (ii) a properly designed and executed clinical trial; and (iii) a convincing hypothesis based on a strong rationale supported by evidence-based research are all necessary for successful integration of biomarker studies in cancer clinical studies.

Translation, as well as precision treatment, are the two primary areas where biomarkers are finding therapeutic use at the moment. Targeted medications may be developed by translating the findings of previous studies into information about intracellular signaling and related signaling networks. On the other hand, precision medicine and disease stratification make it possible to specify which therapy, in which dosage, and at what time, must be administered to a certain patient. However, in the field of biomarkers, there has been a shift toward a focus on the roles of scientists in the workplace, diagnostic tools, and cutting-edge technology. The tendency in biomarker research nowadays is to outsource it to countries where labor is cheaper, such as China, Russia, India, and Brazil.The developments and tendencies in precision medicine are not without their critics. Multiple obstacles thus stand in the way of the biomarkers reaching their full potential. To begin, there is a deficiency of reliable, widely-accepted assays for detecting biomarkers in clinical settings that are now available via medical service labs. In addition, data analyses of studied biomarkers are not yet well-developed to fulfill the user-friendly requirements required by most doctors and scientists [20].

3.5.Using Already Existing Frameworks to Develop New Treatments and Tests:

The following are examples of the potential use of clinical biomarkers in oncology:

1. Scanning the general population for presymptomatic cases and diagnosing individuals with symptoms as soon as possible;
2. Use as a treatment-independent predictive biomarker to carefully analyze treatment outcomes;
3. To assess how well a drug reaches its intended recipient or how effective it is against a malignancy;
4. To evaluate the influence of the medication on the patient;
5. A biomarker that could be used to predict the result of a therapy treatment or to evaluate the efficacy of several treatments;
6. To stand in for the true measure of effectiveness.

3.6.Biomarker Testing in Clinical Research: A Tool for Providing Adaptive Protocols Based on New Data:

There are obstacles to conducting precision medicine tests to test a biomarker-targeted treatment, especially for uncommon illnesses or polygenic disorders like schizophrenia, which need a large number of patients. Mechanistic techniques of answering more questions in less time add to the complexity. One new approach to dealing with such complicated problems is the use of international consortiums to coordinate their efforts. They use an approach including master encompassing procedures to answer several questions in the least amount of time, while still recruiting thousands of patients and healthy controls.

Taking this into account, a recently developed methodological improvement is to compare several therapies in different patient populations using the same overarching clinical trial design. The master protocol is a term used to describe this kind of planning, and it refers to a single, comprehensive protocol that is meant to address several issues. Using this strategy, researchers may examine the efficacy of several targeted therapies for a single sickness, a single focused therapeutic for multiple illnesses, or a wide range of target treatments for a wide range of disease features.

4. CONCLUSION

The incorporation of biomarkers as the main outcomes in clinical studies is now almost universally regarded as standard practice, reflecting the widespread adoption of biomarkers in bothbasic and clinical research, as well as clinical practice.Biomarkers are critical to the improvement of the process of developing drugs and the area of biomedical research in general. For the sake of improving our treatment options for all illnesses and gaining a deeper knowledge of normal, healthy physiology, it is crucial to comprehend the connection between observable biological processes and clinical results. Biomarkers are helpful in the diagnosis, prognosis, or clinical management of cancer, including the use of targeted medicines.Recent breakthroughs in biotechnology and genetics have sparked more study into bioinformatics and computational approaches for the creation and validation of novel genetic markers or diagnostic procedures that may be used to match the correct medicines to the correct patient. The documented disease diversity based on genetic biomarkers thus necessitates the creation of new methodologies of clinical study analysis and design for assessing the efficacy and therapeutic usefulness of novel medicines and associated biomarkers towards effective personalized or predicted therapy.

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CHAPTER 16

AN OVERVIEW ON RAPID IDENTIFICATION AND MOLECULAR DETECTION OF FOODBORNE PATHOGENS

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ABSTRACT:

Testing for foodborne pathogens is necessary to ensure a safe food supply and lower the risk of food poisoning since they may be found in a wide range of foods. Whether it happens during the actual operation or on the work surface, cross-contamination or from handling of raw materials, must be avoided at all costs. To ensure the contaminants free food products, quick identification become essential and crucial also. Guideline prescribed for "Good Manufacturing Practices" (GMP) and "Hazard Analysis Critical Control Points (HACCP)" may reduce the likelihood of contaminated goods during their preparation, processing and production. The emphasis of this research is on the fundamentals and applications of recently developed techniques for quick testing of microbial contamination in food products. Molecular detection techniques like Simple 'polymerase chain reactions (PCR)', 'nucleic acid sequence-based amplification, 'loop-mediated isothermal amplification, 'biosensor-based approaches', 'enzyme-linked immunosorbent assays (ELISAs)', 'lateral flow immunoassays', and other 'immunological-based' techniques are all used for detection of food contamination. The findings of this evaluation study shows that rapid and sensitive detection methods are more sensitive and more specific in comparison to conventional biochemical identification. If the detection method becomes rapid, the chances for food contamination and foodborne diseases may be avoided.

KEYWORDS:

Biosensors, Food Borne Pathogens, Good Manufacturing Practices (GMP), Hazard Analysis Critical Control Points (HACCP), Loop-Mediated Isothermal Amplification (LAMP), Polymerase Chain Reaction (PCR).

1. INTRODUCTION

Infections that are caused by food have become a significant threat to the public's health around the globe owing to a rise in foodborne disease prevalence over the last two decades [1].As some instances, particularly in underdeveloped countries having no valid data, it is difficult to assess the worldwide prevalence of foodborne illnesses. Nevertheless, a rise in the frequency of foodborne diseases has been recorded in many regions of the globe [2]Although the causes of foodborne illness have been steadily growing since year 1996, the number of outbreaks has climbed from one hundred twenty one in year 1995 to one hundred seventy seven in year 1996 [3].It's estimated that forty eight million Americans will become sick this year, with 128,000 of those people requiring hospitalization, and 3000 deaths occur each year from foodborne infections even though the United States has the world's most secure food supply [1].

Large-scale outbreaks of foodborne illnesses continue to be a persistent danger to the general population's health, and this is especially true for persons who are extremely young or very elderly, as well as pregnant women and persons with impaired immune systems [4].It is difficult to offer an exact estimate of foodborne disease prevalencethroughout the world; nonetheless, it has been stated that foodborne pathogens are responsible for millions of cases of infection and poisoning as well as thousands of fatalities each year. In addition, epidemics cause billions of dollars' worth of harm, along with issues relating to public health and the loss of agricultural products [5].

Food is a common route of transmission for a wide range of disease-causing microorganisms. Food-borne illness costs billions of dollars every year because of increased morbidity and death, which results in time off from work and decreased production [6].The number of instances of gastroenteritis that is attributed to food is estimated to range anywhere from 68 million to 275 million every single year. This is because outbreaks of food-borne diseases may be underreported by around a factor of 30. Even at the lowest end of this spectrum,food-borne infections continue to pose a seriousendangering both the environment and human health.In most cases, illnesses that are transmitted via food are brought on by eating or drinking anything that has been tainted with microorganisms or the poisons that they produce. Bacteria, viruses, fungi, and parasites are all examples of foodborne pathogens. Foodborne infections are caused by pathogens that enter the body via contaminated food or water [7].

"Escherichia coli O157:H7", "Listeria monocytogenes", "Bacillus cereus", "Vibrio spp"., "Campylobacter jejuni", "Clostridium perfringens", "Staphylococcus aureus, "Salmonella enterica", and "Shiga toxin-producing Escherichia coli (STEC)" are a few of the most common foodborne bacteria that cause the overwhelming majority of outbreaks of food-borne illnesses.Concerns about the food safety assurance system have been raised by public health organizations in response to the growing availability of fast food and the rising demand for items that undergo minimal processing before being packaged and sold [8].Human health relies on the effectiveness and maintenance of the food people eat. Both *E. coli* and *Salmonella* are major contributors to food-borne diseases. Multiple subcultures, biotype/serotype detection, or other traditional microbiological detection procedures are time-consuming as well as labor-intensive [9].Consumers may become sick from, parasites, fungi, and the presence of germs and viruses in the environment, and these pathogens can readily infect food items that are intended for human consumption. Food-borne infections are a category of infectious diseases that are acquired by the ingestion of tainted foods or food products [10].However, there are benefits and drawbacks to each speedy procedure. Most quick detection technologies fall into three broad categories: nucleic acid, biosensor, and immunological. This study addresses the merits and disadvantages of the most current fast detection technologies for foodborne bacterial infections.

2. LITERATURE REVIEW

Aseer Manilal *et al.* stated thatHIV/AIDS epidemic, lack of hygiene, overpopulation, antimicrobial resistance, and microbiological infections are common in underdeveloped nations.In their study Tender Rosmarinus officinalis foliage was isolated in ethanol (EtOH) and tested for antibacterial activity against 10 Multiple Drug Resistant (MDR) strains isolated, human-type culture viruses, or meat-borne isolated bacteria.R. officinal EtOH reported inhibiting MDR clinical isolates' development to varying degrees. The most susceptible bacterial isolates

were *Salmonella* and *Staphylococcus aureus.R. officinalis*, is reported as a promising source of antibacterial chemicals for drug-resistant bacteria and meat-borne illnesses [11].

Mariateresa Ferone *et al.* conducted a study in which newer and developing techniques for bacterial diagnosis and characterization in food materials are compared to more classic benchtop methodologies. A wide range of bacterial identification techniques are available, and also the information is designed to provide researchers with an overview of the advantages and disadvantages of each one. They found that some of these developing methodologies, such as the untapped potential of spectroscopic methods and hyperspectral imaging, are also argued for incorporation into the mainstream [12].

Zhang *et al.* created liquid-phase microfluidic nucleic acid purification chips for sample DNA or RNA extraction as small as 500 microliters down to individual bacterial cells in a fraction of the time. It simply takes around 30 minutes for a chip to immediately measure the volume of the sample. The compact size of these devices also means that they use significantly fewer reagents and samples, which lowers the detection costs and makes it possible to conduct tests quickly and cheaply [13].

The decimal decrease times of bacteria found on chicken fillets while boiling water was studied by, Aarieke E.I. de Jong *et al. Salmonella, Campylobacter jejuni,* and *Escherichia coli* were all used in research. The bacteria were injected into whole chicken breast fillets, which were then kept and cooked the following day. The temperature on the surface was attained in less than 30 seconds and was maintained for almost a minute. *C. jejuni, E. coli,* and *S. Typhimurium* had total computation times of 1.90, 1.97, or 2.20 minutes, correspondingly.

Refrigerated storage before cooking chicken flesh made foodborne bacteria more heat resistant. The amount of heat resistance was also influenced by a high-challenge temperature or a rapid pace of temperature rise Campylobacteriosis (a sickness caused by eating chicken fillets) was assessed using data from the study. This study's findings suggest that the amount of time spent cooking is far more important than previously thought [14].

3. DISCUSSION

Public health agencies in industrialized countries have adopted stringent regulations and standard requirements for food control systems, like “Good Manufacturing Practice (GMP)” and Hazard Analysis Critical Control Point System (HACCP), to combat the spread of these diseases at the food processing and distribution levels. The HACCP is an approach to food safety that emphasizes the use of appropriate sanitation procedures. The HACCP technique acknowledges any extra or more specific standard precautions required for food operations. It also emphasizes proper hygiene standards and anticipates remedial procedures until monitoring findings suggest a lack of command, which necessitates further education and accountability on the part of operators [15].

Preventive and corrective action at certain risky points in the food supply chain may be made much easier with the identification of foodborne pathogenic microorganisms. As a consequence, many academics have shifted their focus to the development of quick procedures that may provide analytical findings in the lowest amount of time possible. Pathogen detection technologies now used in the food business as a preventative precaution against foodborne illness are being compared in this chapter. A thorough understanding of pathogen detection

procedures is critical, but it's just as important to learn about the probable causes of contamination as it is.

3.1.Food that has been contaminated by various sources:

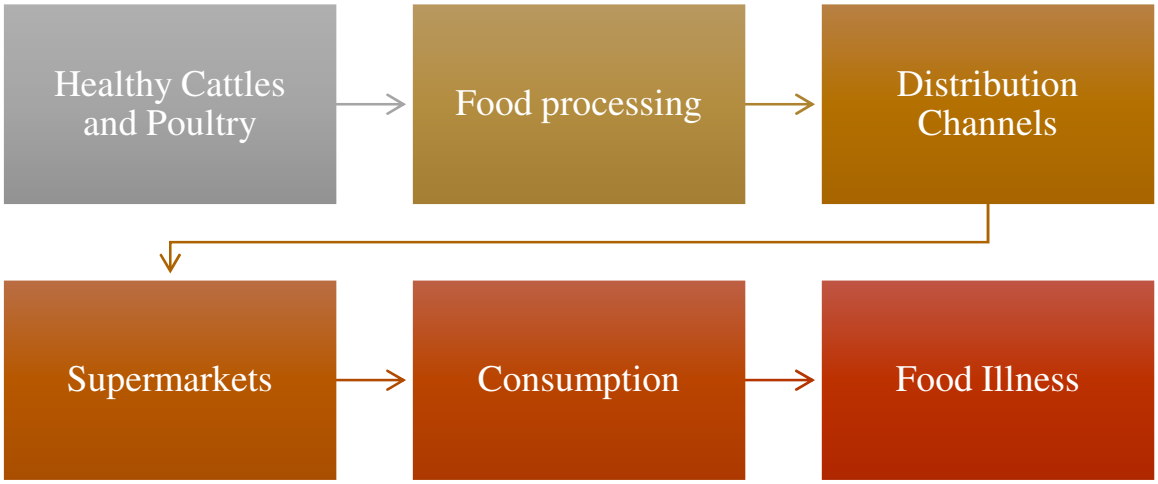

Figure 1: Pathway of Foodborne infections.

Consuming food or water that has been contaminated by bacteria may lead to foodborne illness.As can be seen in Figure 1, several common foods may be a source of foodborne illnesses. There is a variety of fresh produce items to choose from foods as meats and dairy products in addition to fruits, vegetables, herbs, seeds, and nuts [16].After the pre-harvest period, the majority of these items either go via a local distribution system where they are sold whether products are sold from the farm to the consumer, or through a bigger distribution network, where they are sold to the industry. Consumers in developed nations acquire the raw ingredients they need for their homes from grocery stores and other retail establishments. Not only is food a fantastic form of energy and nourishment for humans and other animals, but it is also an excellent supply of fuel for the growth of microbes.

Because there are so many components that are tied to one another, the potential for contamination is increased.As a first step, tracing potential points of contamination in raw materials through to their final destination in the supply chain is necessary. The use of proper sanitation and hygiene measures may help achieve this goal,to increase the number of cutting-edge detecting technologies.Agar plate culture and routine biochemical identification are the traditional techniques for finding foodborne bacterial pathogens in food [17].Pre-enrichment media, selective enrichment media, and selective plating media are some approaches that may be time-consuming since their success is dependent on the microorganisms'capacity to thrive under a broad range of cultural situations. Preliminary identification of infections often takes two to three days using traditional procedures, and verification of species usually takes a week or more with these approaches.

To address the limits of current foodborne pathogen detection techniques, and new approachesin recent years, there has been a great degree of sensitivity and selectivity. Scientists are also constantly innovating more efficient methods for in situ examination and differentiation of living cells [7].Because they can show the rapid multiplication of harmful bacteria in both unprocessed

and cooked meals, rapid detection technologies are an essential part of the food industry. Rapid processes that are accurate and sufficient to identify infections at low concentrations can be used to identify foodborne pathogens.Infectious microorganisms in food may be identified using these methods. The ability to detect even a single virus is essential since eating contaminated food may make you sick. Rapid methods are thosethat need less effort and time to complete, in addition to being free of mistakes.

3.2.Techniques For The Diagnosis Of Food-Borne Infectious Diseases:

To circumvent the constraints imposed by traditional procedures, several speedy methods have been created and are now available for purchase to fulfill the requirements of the food business.Rapid approaches, when applied to industrial settings, must have precision, great sensitivity, and rapid performance are excellent characteristics. To reduce the risk of illness, modern fast techniques may detect bacteria in tiny amounts in raw and prepared meals. These techniques save time, and effort, and prevent human errors more than previous, more labor-intensive alternatives [17].

Current quick detection technologies have a range of detection times that fall between a few minutes and many hours. The specificity and sensitivity of evaluating food samples without the requirement to pre-enrich them before analysis must be enhanced, though. Aside from being regarded as a major constraint in most approaches, enrichment is crucial for reviving or healing damaged or stressed cells, separating viable from non-culturable cells, or diluting the inhibitor in food samples [18].

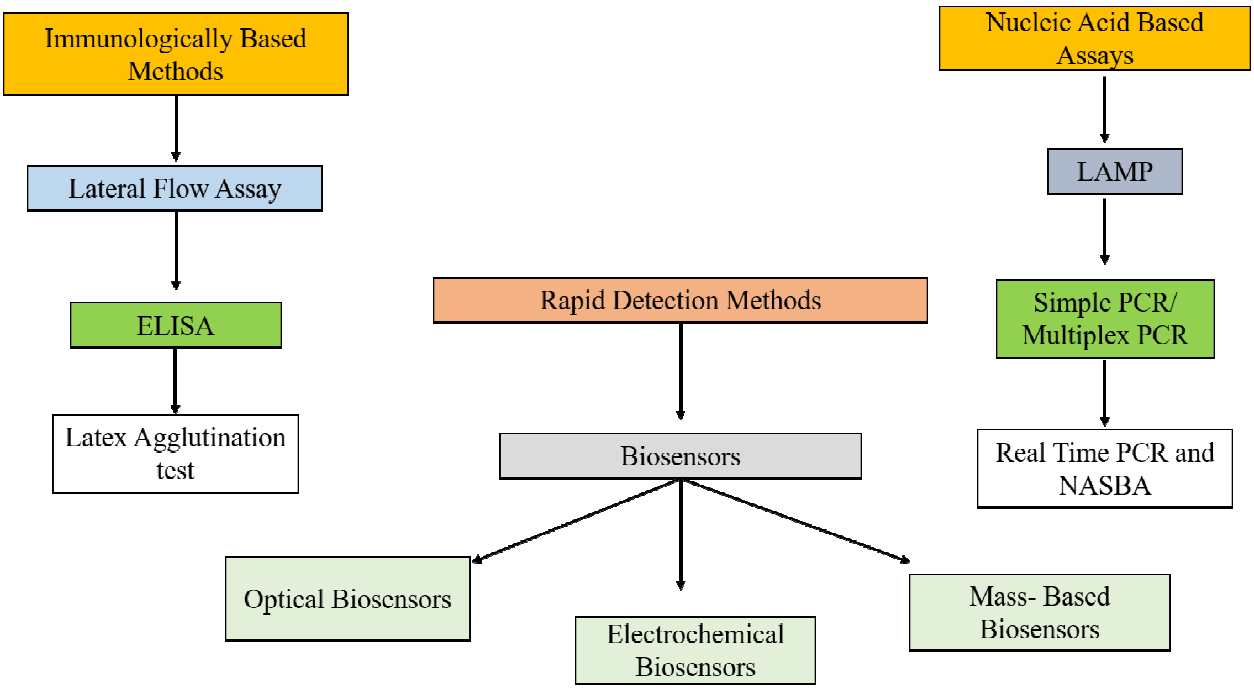

Figure 2: Detection Methods for Toxic Microorganisms of Foodborne Infections.

Figure 2 depicts quick detection techniques such as biosensors, immunological procedures, and nucleic acid-based technology.Multiplex polymerase chain reaction, real-time polymerase chain reaction, loop-mediated isothermal polymerase chain reaction, and oligonucleotide DNA

microarray are all technologies based on nucleic acids. Optical, electrochemical, and mass-based biosensors are all examples of biosensor-based methods.

3.3.“Nucleic acid-based methods”:

Nucleic acid-based methods rely on the identification of specific DNA or RNA sequences in invading pathogens.It does this by causing a hybridization between a synthetic oligonucleotide and the desired nucleic acid sequences (probes or primers), which have a sequence that is similar to the sequence of the target.Foodborne infections may be caused by *Clostridium botulinum*, *Vibrio cholerae*, “*Escherichia coli O157*” and “*Staphylococcus aureus*” [19].Nucleic acid-based methods might be used to identify the genes involved in toxin production in these diseases. Several pathogens, like *Campylobacter jejuni* that have confusing features, may be detected and validated using nucleic acid-based approaches.To avoid misinterpretations or unclear findings, nascent infections are detected using nucleic acid-based approaches.Among the most recent developments in the field of nucleic acid-based technology are amplification techniques such as “Microarrays”, “Quantitative Polymerase Chain Reaction (qPCR)”, “Nucleic Acid Sequence-Based Amplification (NASBA)”, and “Loop-Mediated Isothermal Amplification (LAMP)”.

3.3.1. “*Polymerase chain reaction” (PCR)*:

Improved methods of identification and quantitative measurements, such as multiplex polymerase chain reaction (PCR) and oligonucleotide DNA microarrays, have allowed for the simultaneous detection of five or more diseases. The first step is to convert the double-stranded DNA into single-stranded DNA by heating it to very high temperatures. Primers, both forward and reverse are created by joining single-stranded synthetic oligonucleotides or specialized primers to the DNA. Primers, which are single-stranded DNA analogs, are extended using deoxyribonucleotides and a thermally stable DNA polymerase. Ethidium bromide-stained electrophoresis gels display the PCR amplification products as distinct bands.

3.3.2. Nucleic acid sequence-based amplification (NASBA):

It was Compton (1991) who invented NASBA, which uses isothermal conditions to amplify nucleic acids, as opposed to the thermocycling mechanism required by PCR. Amplification of RNA via NASBA involves the reverse transcriptase convertingthe replication of a DNA molecule using RNA as a template (cDNA). T7 polymerase, RNase H, and AMV reverse transcriptase are the three enzymes involved in the NASBA process, which takes place at a temperature of roughly 41°C. By using agarose gel electrophoresis, NASBA amplicons may be identified [20].After the NASBA regulation change in 2010, product identification techniques including agarose gel electrophoresis and enzyme-linked gel test are now time-consuming and expensive. Because of this, a novel real-time NASBA assay has been developed, which uses probes with fluorescent tags to locate single-stranded DNA amplicons (ssDNA). Some examples of foodborne pathogens that have been identified in real-time utilizing NASBA include, “*Campylobacter jejuni*”, ‘*Campylobacter coli*”, “*Salmonella enterica*”, “*Vibrio cholera*” and “*Staphylococcus aureus*”.

3.3.3. Loop-mediated isothermal amplification (LAMP):

Notomi *et al.* designed LAMP, a revolutionary nucleic acid amplification technology, to rapidly, sensitively, and specifically identify foodborne microorganisms. For 60 minutes at 60 °C, Bst DNA polymerase big fragment continuously relocating strands Synthesis of DNA from the big

subunit [21].To selectively target six distinct sections of DNA, LAMP uses two inner primers and two outside primers. LAMP yields DNA structures like cauliflowers, each with numerous loops and stem-loop DNAs of varying diameters. LAMP may create a large number of amplicons in 60 minutes, which is typically 103 times or more than simple PCR. Since then, because of its speed and accuracy where pathogens that can be spread via food have been detected using LAMP. LAMP has been employed to identify a variety of foodborne pathogens.LAMP outperforms PCR in detecting food-borne infections because of its superior sensitivity and specificity.Furthermore, many kinds of LAMP assays were developed to determinecontamination with disease-causing organisms.Additional kinds of LAMPs include in-situ LAMPs, multiplex LAMPs, reverse-transcription LAMPs, and real-time LAMPs. Real-time monitoring of LAMP amplification products by the observation of turbidity or fluorescence in the samples has rendered Ethidium bromide staining and gel electrophoresis obsolete. Thus, this allows for a high-throughput study to be conducted without sacrificing accuracy or precision.

3.4.Immunology-based methods:

Immunological approaches are now the most widely used and widely effectiveinnovation in the field of microbiological pathogen identification. This technique can identify contaminated organisms as well as the biotoxins that they produce, and it is both quicker and more resilient than previous methods.However, detection based on nucleic acids is both more specific and sensitive than the alternatives.Although antibody-based techniques shorten test times, their usefulness is limited by the difficulty of identifying infections in "real-time" when the number of pathogens to be detected is small. Low sensitivity of tests, poor antibody affinity to a pathogen or other analytes, and possible contamination from pollutants are all possible issues that arise.

ELISA or lateral flow immunoassay are two more immunological procedures often employed to identify foodborne microorganisms. As a method for detectingthe risks of germs, and working with a large number of samples, ELISA is precise and labor-saving.Moreover, this technique has a few drawbacks, such as the requirement for highly trained personnel and the potential for erroneous negative results caused by reactions to unrelated antigens.Immunomagnetic separation on magnetic beads combinedwith "*Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF)"* to detect staphylococcal enterotoxin B and immunomagnetic separation on magnetic beads combined with flow cytometry to detect Listeria monocytogenes are two examples of novel antibody-based methods combined with other pathogen detection equipment.

*3.5.Biosensor*s:

Strategies based on RNA, DNA, and antibodies, which are considered typical for detecting food pathogens, are increasingly being supplemented with biosensors. Detection of harmful microorganisms using biosensors has been a major focus in recent years. The real-time reaction of biosensors makes them preferable to typical techniques for food safety checks throughout the manufacturing process. A biosensor is an analytical instrument that is made up of two primary components, which are a bioreceptor and a transducer. There are two possible types of receptors that might be responsible for identifying the target analytes:

1. Nucleic acids as well as cell receptors are examples of biological stuff.
2. Aptamers and recombinant antibodies are examples of biologically generated materials.

3. Synthetic biomimicry: imprinted polymers and catalysts.

Since pathogens don't need to be enriched in advance of detection, biosensors have an advantage over nucleic acid-based technologies and immunological processes. Biosensors may be either optical, electrochemical, or mass-based and are some of the more contemporary technologies routinely employed to identify foodborne microorganisms.For food pathogen detection, optical biosensors are ideal since they don't need additional sample preparation even in complicated matrices, as well as reduced interference and lower signal loss. According to, optical biosensors use light amplitude, phase, frequency, or polarization changes to determine the presence or absence of disease.The tiny nature of optical devices makes them less intrusive while providing more sensitivity and specificity than other types of biosensors. Immobilized bio components, on the other hand, remain an issue. For the time being, the commercialization of these biosensors will have to wait. This is owing to some variables, including the high price of their quality assurance and equipment designs.

There are two types of electrochemical biosensors: the first is label-free and can handle huge quantities of samples; the second is label-freenonetheless, due to the extensive washing steps required, it is not practical for samples with low microbial counts. A final consideration is the widespread use of mass-produced biosensors. These devices are low in price but high in convenience and do not require the use of labels. However, they have low sensitivity and specificity due to long incubation times for bacteria and the numerous washing/drying steps that must be performed.

4. CONCLUSION

Rapid foodborne pathogen detection and identification techniqueshave emerged in recent years as a solution for the issues that are not resolved through conventional biochemical methods.Various researchers has reported various aspects of detection methods like biosensors, immunological techniques, and other nucleic-based technologies. Conventional pathogen detection techniques are good enough for sensitive, but sometimes they are not specific. However, this technique requires longer time to identify pathogens than analytical approaches. Eventually cost and sensitivity factor is also a reason to replace conventional detection method. Though optical and electrochemical detection methods have their drawbacks but these techniques can quantify the digital value to express the extent of infection. As a result, new speedy procedures are developed, optimized and adopted to improve the sensitivity and efficacy. In terms of sensitivity, optical approaches may be superior, but they are costly and difficult to use. Electrochemical approaches, on the other hand, demand a higher level of performance to identify infections.

Different pathogens and food samples required for different strategies. The food business has several challenges to overcome foodborne infections. Technology now on the market that can rapidly detect viruses. Howevercurrent approaches are regarded to be more time- and labor-efficient, as well as less prone to human mistake. These techniques are pricey and need skilled technicians.For most of the food products, new detection technologies are being introduced often, but their acceptability by the industry relies on more than just speed. Recent progress has converged to address the need for more rapid, specific, and easy techniques to identify pathogenic bacteria in food products.

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CHAPTER 17

DETECTION OF INFECTIOUS PATHOGENS THROUGH BIOLIMINESCENT ENZYME EXTRACTED FROM *PANELLUS STIPTICUS*

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ABSTRACT:

Panellus stipticus also known as bitter oyster is one of the "brightest glowing mushrooms" with bioluminescent properties. In recent times bioluminescence has captured the attention of the medical community because it could be used in the conversion of chemical energy to light energy. Though it is already known in insects like fireflies, still ongoing research is discovering new species of different species with the presence of bio luminesce. This research paper reports the extraction of the luminiferous enzyme from the mushroom species, the purification of the substance based on the chemical composition, and its application in the detection of infectious pathogens. The medicinal field is continuously striving hard to invent new diagnostic tools for several diseases, and the detection of some diseases early can help in saving lives. One such is the correct and early diagnosis of tuberculosis. The disease is still a major concern as lakhs of cases are registered every year.

KEYWORDS:

Bioluminescence, Detection, Luminiferous, Mushroom, Panellus stipticus, Tuberculosis (TB).

1. INTRODUCTION

Panellus stipitus is the brightest glowing mushroom in the world. The fungi belong to the family of Mycenaceae, from the genus Panellus. The normal color of mushrooms is light yellow more cream side during daylight but appears to be bright in dark during the night, they are usually bitter, acidic, and non-poisonous. Figure 1 shows the pictorial representation of the enzyme bioluminescence has gathered attention from the medical and biophysicist society for the use of transforming chemical energy into visible light energy. "The kingdom Fungi", includes various species such as rusts, molds, mildews along with mushrooms. Organisms similar to fungus like are also known as slime molds but still, they don't fit in the "kingdom of fungi". Fungi are the most abundant scattered organism with unique applications in the ecological and medicinal field, "some of them are free-living in and around soil and water. Whereas some develop symbiotic relationships with plants and animals".

Mushrooms are fungi that belong to the class of eukaryotes. Eukaryotes are classified as organisms with membrane-bound organelles and well-organized nuclei. Chlorophyll is absent in fungi though they are incorporated in the kingdom of Plantae. The organism is differentiated from other oxygen-bearing organisms based on "vegetative growth" and the mode of intake of

nutrients. The structure of fungi consists of a thread-like long structure called hyphae. Mycelium is formed by the collective arrangement of hyphae in a construct giving a mesh-like shape. The cell wall of fungi is made up of polysaccharides and chitin. Biology as a subject has different branches of study, mycology is a study concerned with the understanding of fungi which comprises different aspects such as biochemical characteristics genetic evolution, and its wide uses in medicine and the food industry. The discovery of penicillin is still used in many drug preparation and the discovery of penicillin led to the unveiling of other noble fungal species [1]

The level of understanding at the molecular level has been studied thoroughly in insects like fireflies and some bacteria, while few studies have also been conducted on luminous fungi. With such extensive knowledge and experimental data, the information regarding the subject is still not sufficient. The lack of a database is mainly because almost every mushroom that is luminous produces "yellow-green light at nm 525". The functioning involved in producing light differs from species to species. Particularly some taxa do go through a luminous reaction with the employment of the enzyme luciferin, the whole reaction is carried by the luciferase enzyme that acts as catalysis. Investigations into bioluminescent mushrooms are still going to understand the complexity and other aspects at the molecular level. Figure 1 shows the pictorial representation of Panellus stipticus. The reason behind the light-producing phenomenon in mushrooms is because of the presence of enzymes that allows the emission of photons in form of light under a pitch-black dark environment. Past recorded studies on light-producing mushrooms have reported several hypotheses in understanding the purpose behind the role of light production, one of the possible reasons could be a technique in attracting insects and other small organisms for the dispersion of spores or to protect themselves against the animals in causing them harm or restricting them from getting consumed because of the glowing appearance.

Figure 1: Pictorial Representation of *Panellus stipticus*.

Bio luminesce is a natural process that involves respiring organisms to emit light. The phenomenon comprises small molecule oxidation which is catalyzed by the enzyme luciferase to construct a species of excited energy level that will further cause the emission of light. Over 30 species of bioluminescent are reported, while the search for discovering novel species is still in process.

The enzyme luciferase which is responsible for the production of light or bioluminescence belongs to an oxidative enzyme class and is generally differentiated based on the presence of photo-protein. Figure 2 explains the chemical structure of luciferin. Enzyme luciferase has

important applications in the field of biotechnology. One of the major applications is "imaging microscopy and as a reporter gene which is the same as "protein fluorescent". Moreover, the protein fluorescent does not need any other substrate along with the luciferin [2].

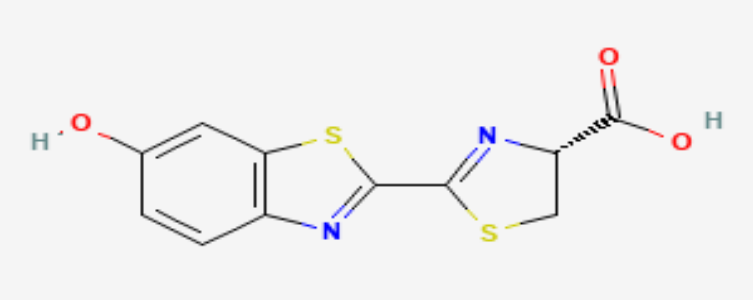

Figure 2: Representation of the Chemical Structure of Luciferin Enzyme along with the Functional Groups

With the advancement in biotechnology and the genetic engineering field, the enzyme luciferase can now be made in a research lab through the tools of genetic engineering in the use of different approaches. Genes associated with the enzyme luciferase can be easily produced and transferred into the recipient cell. By now animal models like silkworms and mice have already been genetically modified to synthesize protein, on the other hand, the potato has also been modified for the same. The reaction of luciferase includes the emission of light only when the enzyme luciferase act on the selective "luciferin substrate". "The discharge of photon can be sensed by a light sensor system such as luminometer". The reaction does not require the excitation of light particles for the bioluminescence luciferase. However, as less as "0.02 pg can be employed for measuring a scintillation counter". The research related to the study of biological study usually makes use of luciferase as a reporter gene to monitor the transcriptional mechanism inside a cell that is destined to be transformed with the use of a genetic tool having gene luciferase which is under the influence of "promoter of interest". Figure 3 describes various applications of bioluminescence.

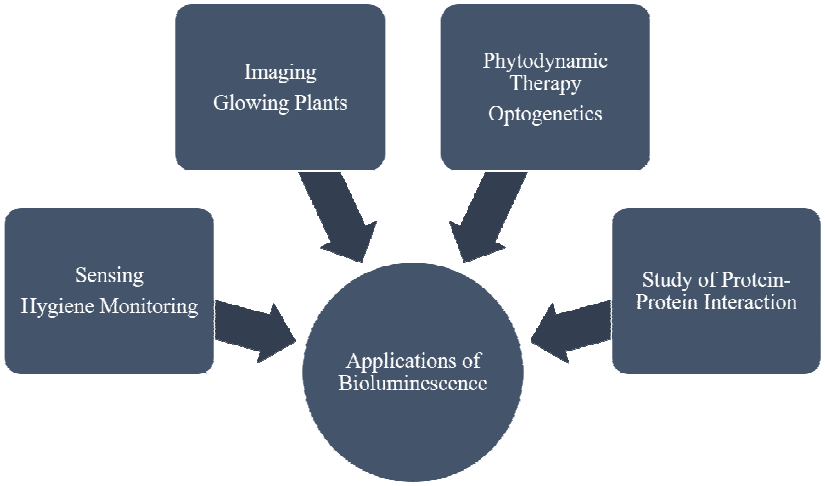

Figure 3: Diagrammatic Representation of Applications of Bioluminescence in Different Sectors of Science.

The extraction of bioluminescent enzymes and their use in medical diagnosis has created a new approach for simplifying detection methods. The growing number of Tuberculosis (TB) cases has always been an issue of concern. Figure 4 shows the ratio of tuberculosis cases around the world. Mycobacterium tuberculosis causes tuberculosis in human beings by attacking the spine, kidney, or brain. The three stages involved in the progression of TB start with exposure to the bacteria followed by the latent and active stages. People with HIV infection with weak immunity or with major diabetes and kidney infection can lead the infection. Early detection of it in human beings can prevent the progression of the disease, which ultimately helps in saving lives [3].

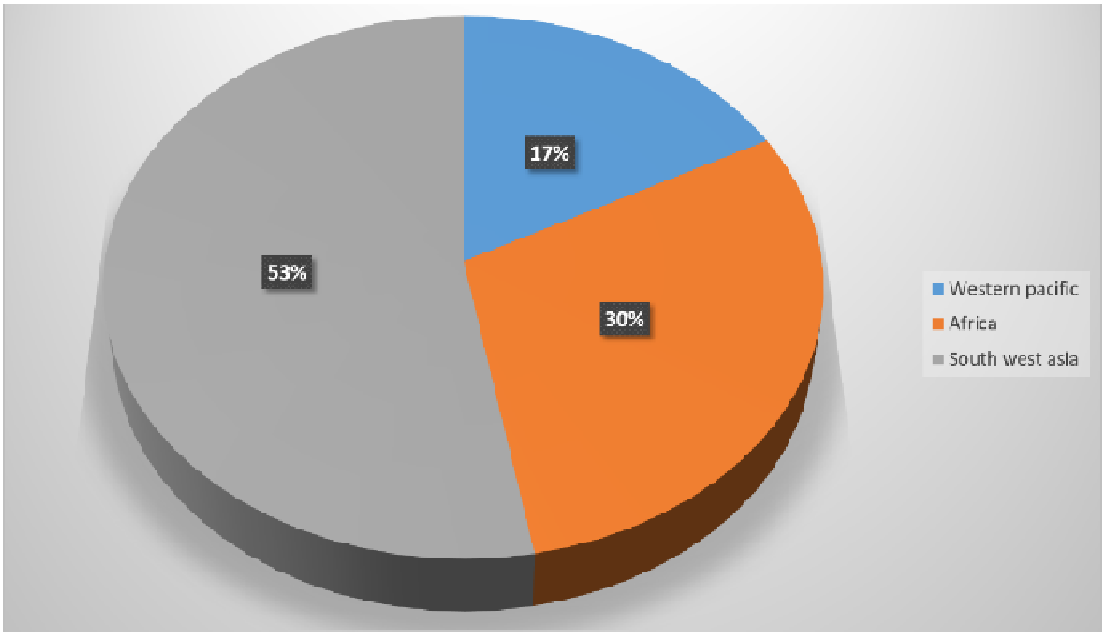

Figure 4: Pie chart showing the Percentage of Cases of Tuberculosis (TB) all around the World.

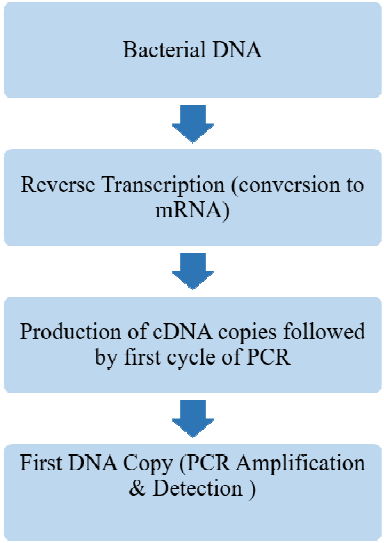

Figure 5: Diagrammatic Representation of the Stages of Steps during the Process of RT-PCR Assay.

Tuberculosis is a dangerous disease that has a chronic effect on the patient's health sometimes with life-threatening effects. The use of molecular biology and the tools employed in it provide effective diagnosis in identifying the presence of bacteria. Moreover, the molecular tools focus on "polymorphic genetic sequences". Reverse transcriptase polymerase chain reaction (RT-PCR) is similar to a polymerase chain reaction (PCR) but uses an additional step in detecting the disease-causing pathogen by first performing transcription from RNA to DNA and then allowing the amplification of the template DNA. Figure 5 shows the process of RT-PCR [4].

2. LITERATURE REVIEW

Yuichi oba *et al.* demonstrated their research on the "synthesis of luciferin from firefly in adult lantern using L-cysteine for the formation of benzothiazole". The authors explain the reaction mechanism that is responsible for producing "luciferase-luciferin". Furthermore, the study also discusses the use of cysteine and hydroquinone as biosynthetic along with the first experiment performed in 1970 [5]. Vernon C. Bode and J. woodland Hastings explicated research on '*Gonyaulax polyhedra* purification' of its bioluminescence. The author discusses the importance of bioluminescence that is sourced from *Gonyaulax species* and a brief discussion on the molecular level. Later the paper also reports on the purification method used for enzyme purification and the properties of a "bioluminescent system" [6].Nae- cherng yang *et al.* explained the research on the extraction of "ATP by the use of boiling water" the researchers talk about the extraction and measurement of bioluminescence. The paper discusses the method of extraction, the first method they used was Tris-borate another one was with perchloric acid. The results of both methods hindered the "luciferin-luciferase system" therefore a new procedure was put forth which is a single-step boiling method for ATP extraction [7]. Aisha J. Syed and James C. Anderson discussed their research on the advantages of bioluminescence and its use in the biotechnology field. The researchers explain the use of bioluminescence in gene assays, screening high throughput, protein-protein interaction, and analysis of pollutants in the environment. The paper also talks about a comparative study stating past present and future opportunities associated with it [8].

Ruxana T. Sadikot and Timothy S. Blackwell described their views on Imaging used for Bioluminescence. The authors elaborate on the process of bioluminescence and its use in molecular biology, especially in the imaging section. The technique also has applications in research related to biological activity in vivo. The research article further discusses the technology used in imaging and its application in "bacterial pneumonia, lung inflammation" [9].Frank McCapra elaborates on their view on the chemical reaction involved in bioluminescence. The paper discusses the chemistry and biological reactions involved in the enzyme. The research study briefly describes the characteristics and chemical reactions associated with the enzyme and its presence in different organisms [10]. E.A. Meighen expressed her views on the bioluminescence produced by bacteria and its molecular biology. The paper talks about the cloning and the expression mechanism of lux genes in various bacteria producing light which comprises organisms both from marine and terrestrial communities. Moreover, the paper also discusses the arrangement of the Lux gene which encodes for the enzyme luciferase [11].

Catharina C. Boehme *et al.* delineated a research study on quick detection techniques for tuberculosis infection. The paper describes the delay in diagnosing tuberculosis and the importance of early diagnosis in the infected patient. The research paper tests the method of

Xpert MTB/RIF a molecular test for Mycobacterium tuberculosis (MTB) and its resistance to the medicine rifampin [12].

3. METHODOLOGY

3.1.Design

The methodology involves extraction as well as purification of the substance luminiferous from the *Panellus stipticus* (Cryopreserved at -85°C). The methodology also involves the use of bioluminescence in the determination of tuberculosis in nucleic acid hybridization.

3.2.Sample Collection

Panellus stipticus was taken from its natural habitat and later the fruiting body was harvested. Hyphae of the fungi *Panellus stipticus* were subjected to inoculation in a media containing sawdust. Rice bran was introduced in the media for the promotion of mycelia extension.

3.3.Data Collection

The whole experiment was sustained in incubation conditions for 30 days at 25 °C. After the incubation of the media, the media was transferred to "Mizugoke" which belongs to the sphagnum genus. The incubation was continued for another 30 days at room temperature under dark conditions with 100% humidity. The experimental conditions lead to the formation of a fruiting body. It was cultured and frozen under low-temperature conditions. Figure 6 shows the process for extraction of luminiferous substance.

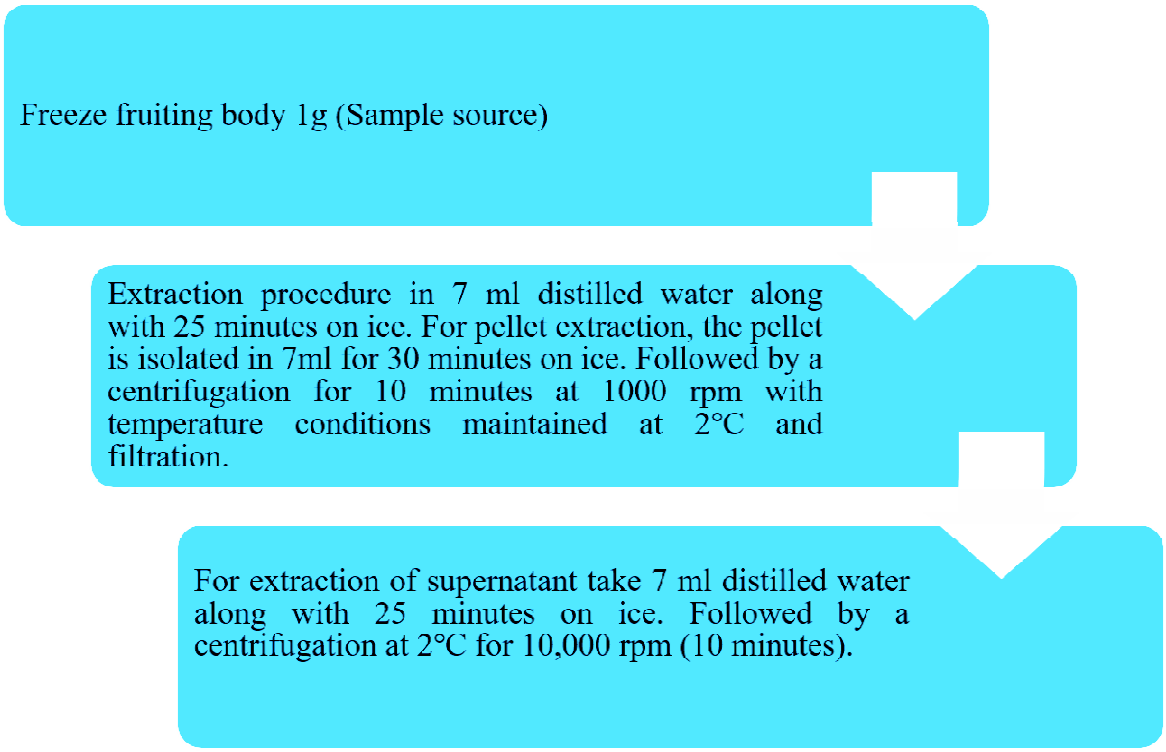

Figure 6: Shows the Diagrammatic Representation of the Extraction Process for a Luminiferous Substance.

3.4.Data Analysis

3.4.1. Purification of the extracted Isolate

The isolate further proceeded with the purification method which involves absorption on the solid phase column. The elution will be done under the presence of 12% MeOH with a 5% concentration. During every fraction of evaporation and the process of dissolving takes place in distilled water.

3.4.2. Detection of Tuberculosis by Bioluminescent

The use of bioluminescence has various uses in assays like DNA/RNA. The excitation of photoprotein is not required hence assays with bioluminometric applications provide high accuracy with detection and easy handling in comparison to fluorometric procedures. The use of Ca^{2+} as a photoprotein reporter.

4. RESULTS AND DISCUSSION

Panellus stipticus is a fungi species that illuminates in dark. The bioluminescent mechanism makes the fungi glow in the dark environment, the gill edges and the gill junction attached to the stem and cap of the fungi are the sites of luminescence the current research study talks about the use of Bioluminescence from*Panellus stipticus* obtained from its natural habitat and its use in the detection of tuberculosis disease. The study comprises of extraction and purification process of luciferase enzyme from the bright glowing mushroom. The luminiferous substance was extracted and stored at -85℃ for further research investigation. Furthermore, the research study also talks about the use of bioluminescence in the early identification of infectious pathogens for better treatment and preventing further progression in the patient's body. For tuberculosis detection, the method used is the "conventional plating procedure" that involves a time of 2-4 weeks for the effective growth of the cells in a sterilized environment before proceeding with the introduction of "colony forming units". The RT –PCR method helps in the quick identification of causing bacteria responsible for TB diagnosis in the infected tissues under the influence of primer-specific sequence. The whole detection procedure is dependent on the bioluminescence accuracy.

5. CONCLUSION

The research paper focuses on the extraction and purification method for the isolation of luciferase enzyme from *Panellus stipticus*, a species of illuminating mushroom from their natural environment, and later employs it in the detection of tuberculosis disease in samples of infected patients. The technique involves RT-PCR assay along with the treatment of deep freezing using low temperatures for storage of isolated enzymes and further proceeding with the detection assay.

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CHAPTER 18

MANAGEMENT OF ENVIRONMENTAL THREAT BY USING MICROBIAL AGENTS FOR BIODEGRADATION OF PLASTICS

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ABSTRACT:

Plastics are widely used in packaging, construction, healthcare, and consumer goods. Plastic is a synthetic polymer and non-biodegradable material. Plastic play a vital role in every sector of the world economy. Since they are used to make a wide range of goods, including defense materials, tiles, faux leather, sanitary ware, plastic bottles, and many other household items, they form the basis of many enterprises. The current state of the steadily growing plastic pollution, it becomes a major problem of solid waste and environmental burden. The phthalates used to make flexible plastic is very toxic and has the ability to solubilize in fat. It is a major environmental threat in which phthalate accumulates in body tissues and enters into ecosystem. Conventional methods of removing plastic from the environment (such as landfilling and burning) are ineffective owing to toxic consequences and are confined to recycle. Therefore, the biodegradation process is now getting more and more attention from research communities for an effective and eco-friendly approach to degrading different types of plastic waste. Hence, this review aims to provide fundamental into the type of plastic, the need for its biodegradation, and the future potential of fungal and bacterial agents. This review may provide the platform for further research on biodegradation. Evidence based on the review of existing literature supporting the ability of different bacterial and fungal strains capable of effectively degrading polyethylene, polyurethane, polystyrene, and propylene. After reviewing the existing literature revealed that the bacillus, pseudomonas, and other cyanobacteria contain the most ability to degrade plastic waste whereas, in the case of fungi, the most ability is attributed to Aspergillus and Penicillium.

KEYWORDS:

Biodegradation, Bacillus, Phthalate, Plastic, Polymer, Pseudomonas.

1. INTRODUCTION

Plastics are synthetic polymeric materials with long chains that find widespread use in many facets of life due to their exceptional qualities, including high flexibility, plasticity, lightweight, electrical and thermal insulation, cheap cost, and corrosion resistance. The output of plastics increased globally from 1.3 million tonnes in 1950 to 359 million tonnes in 2018 (including PP fibers). However, there are little production data for man-made fabrics made of fibers such as nylon, cellulosic, polyester, acrylic, cellulosic, and PP. The first cellulosic fiber to be made commercially was nylon in 1892 [1].

Because of the properties of plastic, such as its adaptability, durability, inertness, and flexibility, people have become more and more reliant on its use daily. Although it was expected that

plastics would be durable and have a broad range of applications, along with the widespread usage of disposable products, it was not predicted that there would be issues with waste management and plastic debris. Plastic has some unique qualities, such as high heat combustion, water content that is significantly lower than that of biomass, less moisture absorption, and growing availability in the area. Without plastic, modern life would undoubtedly look quite different [2]. The applications of plastic in medicine and public health are its most significant benefits. Plastics are affordable, consume minimal energy during production, and are biocompatible and lightweight. Numerous varieties of plastic, which can be soft, translucent, biodegradable, or flexible, serve as cutting-edge materials for prosthetics, synthetic tissues, absorbable sutures, and other medical applications. Plastics do, however, have several drawbacks, such as toxic materials that might seep out and hurt people and other living things. The packaging uses over 40% of all plastic, by far the most. This is the situation at least in Europe, thus the rest of the world must also have this problem. The automobile and construction sectors are ranked second and third, respectively as illustrated in Figure 1 below.

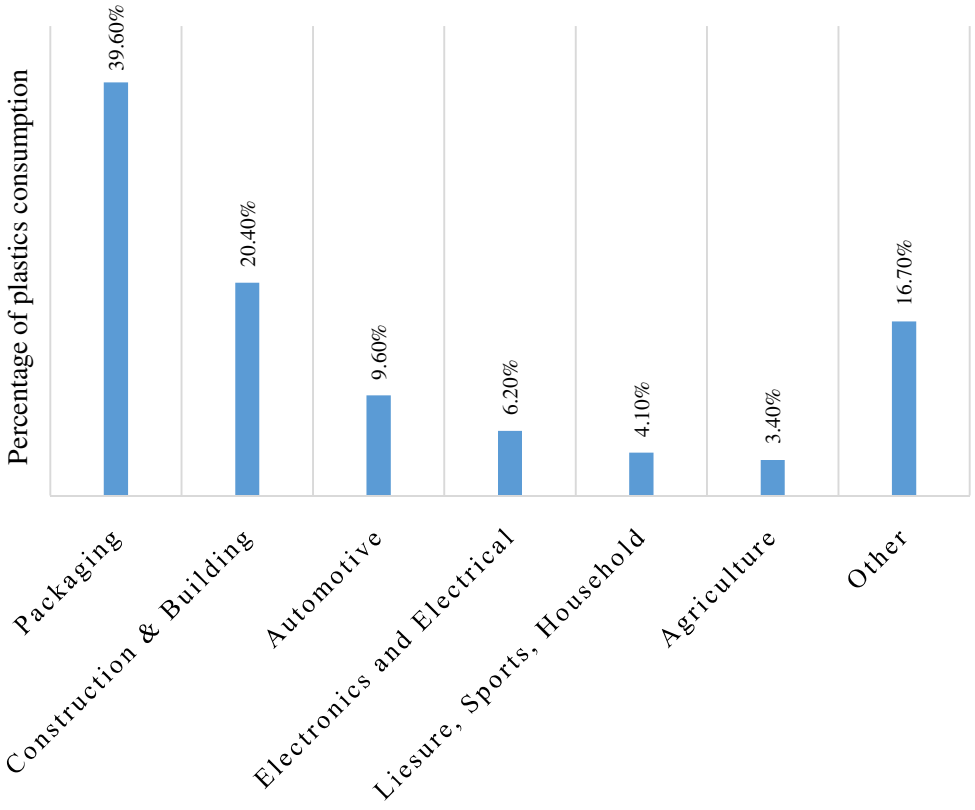

Figure 5: Plastic Consumption by Different Sectors in Europe 2019.

This study aims to provide a platform for plastic based polymer technology advancement and significantly biodegradation of plastic waste which is one the focus area of environmental research. The ineffectiveness of conventional methods including chemical, physical, and a combination of both ort thermos-oxidation. In this review, the first section discusses the fundamentals behind the agenda and some of the statistics associated with it. The second section intensively reviews the fungi and bacteria having the ability or the potential to degrade plastic materials with evidence from existing literature followed by the discussion on the potential

mechanism of action and the further exploration of the research agenda which was then carried forward with the conclusion.

2. LITERATURE REVIEW

2.1 Origin of the term "Plastic"

The term "Plastic" is derived from the "Greek" word "plastikos", which means "able to be molded into various shapes." It is described as a polymer that becomes mobile when heated and can therefore be cast into molds. Oxygen, chloride, nitrogen, silicon, carbon, and hydrogen are all components of plastic. Natural gas, tar, and coal are utilized to extract the fundamental components of polymers. Plastics are created by chemically joining monomers altogether. Polythene, which represents 64% of total plastics, is a "linear hydrocarbon polymer" comprised of long chains of monomers called "ethylene". Polyethylene has the formula "CnH2n", where 'n' is the number of carbon atoms present [3].

Plastic is recalcitrant because of its hydrophobic property, large molecular mass, and intricate three-dimensional structure, all of which limit the accessibility of the substance to microorganisms. Plastics include polyurethane, nylon, polystyrene, propylene, polythene, and others as illustrated in Figure 2 below. "Low-density polyethylene (LDPE)" and "high-density polyethylene (HDPE)", both thermoplastic polymers manufactured from ethylene monomers, are mostly employed as thin films and packaging sheets. Among all types of plastics, strong, lightweight, and long-lasting LDPE materials have a variety of purposes.

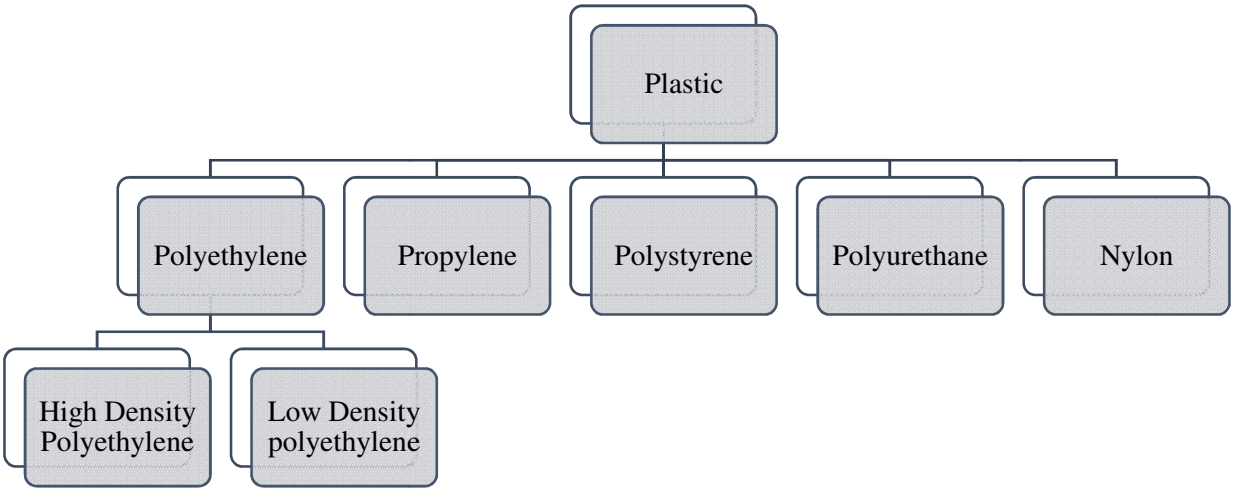

Figure 6: Types of Plastic polymers.

A significant amount of plastic waste gets into the environment by multiple paths as a result of inadequate management and disposal techniques, producing substantial environmental contamination concerns that are now growing by the day. When they enter the environment, plastic items can gradually break down and produce a substantial amount of microscopic plastic debris owing to biological, physical, and biochemical changes. The particles were named "microplastics", and microplastic contamination has developed as a worldwide topic of growing concern [4].

2.2 Impact of Plastic Toxicity on Human Health and Environment

Sheeting and packing plastics are frequently thrown after use; nevertheless, due to their longevity, these plastics may be found anywhere and persist in the environment. While research on the monitoring and consequences of plastic waste remains in its early phases, the preliminary findings are concerning. Plastics derived from petroleum are abundant in human residential as well as occupational environments. When these plastics reach the end of their useful life, they are often landfilled alongside solid wastes. Plastics include several harmful constituents, including polyfluorinated compounds, brominated flame retardants, phthalates, and bisphenol A (BPA), which can seep/leach out and have negative effects on the surrounding environment with danger to human health [5]. Due to their enormous manufacturing volume and the absence of adequate management regulations in many countries, plastics in electronic waste (e-waste) are becoming a significant environmental and public health hazard. According to studies from Nigeria, China, and India, plastic harmful chemicals from e-waste can travel past processing facilities and into the environment.

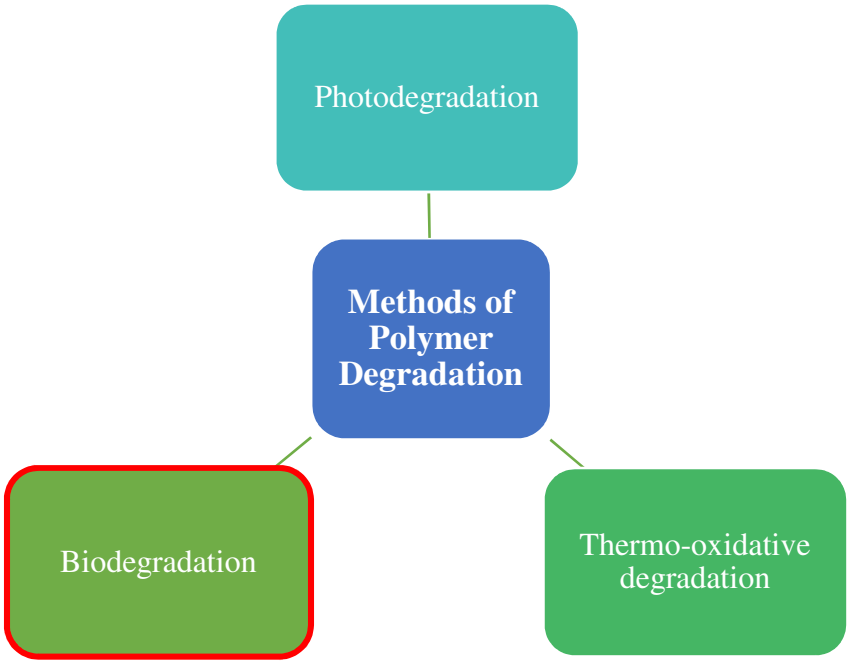

Figure 7: Methods for the Degradation of Polymeric Materials.

Human populations and the distribution of plastic waste are related. The need for plastics as well as their derivative products has risen as the human population has increased. Inconsiderate plastic waste disposal can lead to pollution, which can be observed in a number of ways, such as the deterioration of the beautiful surroundings, the trapping and consequences for aquatic life forms, the blocking up of sewage systems, particularly in low-income countries, that also causes an environment that is ideal for the reproduction of mosquito vectors as well as other disease-carrying vectors, in addition to the production of unpleasant smell and a decline in air quality.

Polymer degradation is described as any change in its chemical or physical characteristics caused by environmental variables such as heat, moisture, and light as well as bioactivity [6]. According to the underlying factors, there seem to be three types of polymer degradation processes: and

biodegradation, thermo-oxidative degradation, photodegradation as illustrated in Figure 3. Biodegradation by microorganisms, mostly bacterial and fungal agents, is gaining popularity because of its low cost, long-term ecological sustainability, and lack of human intervention.

Researchers have looked into and identified microorganisms from the environment that can break down polymer compounds, such as polypropylene and polyethylene. Microorganisms that break down polymers and are linked to degradation include *Klebsiella, Staphylococcus, Micrococcus, Streptococcus*, and *Pseudomonas*. By combining polyethylene with various additives, auto-oxidation of the polymer is increased, resulting in molecular weight reduction of the polymer, which microorganisms are then able to break down with ease [7], [8].

2.1. Plastic degradation by fungi

Eukaryotic organisms classed morphologically as yeasts, filamentous fungi, or dimorphic fungi make up the large and diverse realm of fungi. Such organisms could be obligatory, opportunistic (mutualists, pathogens, or decomposers), or saprotrophs (decomposing dead matter). Fungi may be found in a variety of habitats, but some of them have developed to be able to adapt and survive in extreme marine and terrestrial settings. They are now being isolated and investigated for the biodegradation of plastic and its different derivatives. Several researchers have carried out different experimental studies for evaluating the activity of fungi plastic degradation.

Sarmah *et al.* investigated cyanobacteria that were isolated from submerged polyethylene surfaces in household sewage water. The findings of their investigation on the degradation of polyethylene were evaluated using "SEM", "FT-IR", "NMR", "CHN content", "heat", and "tensile strength" of polyethylene. Their research showed that *Oscillatoria subbrevis* and *Phormidium lucidum*, two cyanobacteria, could degrade 30% weight of the evaluated polyethylene during 42 days [9].

Another research by Taghavi *et al.* examined the potential of several strains obtained from farm sludge, activated sludge, soil, and worm excreta to biodegrade polypropylene, polystyrene foam, and high-density polyethylene, and polyethylene terephthalate. The fungi with the highest potential to degrade plastic were identified as three fungi. The results of their study revealed that Out of three fungi, *Aspergillus flavus*, a fungus, was shown to be able to degrade 5.5% of HDPE after 100 days [10].

Rodrigo *et al.* undertook a study in which they investigated the biodegradation of polystyrene, polyethylene, and polyurethane samples in liquid media by three filamentous fungi obtained from Antarctica. Their findings showed that *Penicillium spp*. had the highest breakdown % in old plastics among the three fungal strains, with a value of 28.3 % in polyurethane and values of 3.53% and 8.39% in polystyrene and low-density polyethylene, respectively [11].

2.2.Plastic degradation by Bacteria

Maroof *et al.* developed a novel named "*B. siamensis"* bacterial strain that can breakdown 8.46% of LDPE following 90 days incubation. FESEM studies indicated that the LDPE films had a little surface disturbance. The modest degradation rates, on the other hand, revealed that LDPE degradation process is a relatively slow, continuing, and process dependent on time [12].

As per the study by Bollinger *et al.*, a new "PET hydrolyzing enzyme (PE-H)" was discovered in the genomic sequence of the marine bacteria "*Pseudomonas aestusnigri"*. The results of their study revealed that at 30°C, PE-H breaks down amorphous PET. A "Y250S variant" was developed that displays increased hydrolytic activity against PET as a result of the restructuring of the active site conformation brought about by mutagenesis and structural modelling studies, offering a unique insight into the structural characteristics required for efficient degradation of polyester [13].

Cárdenas Espinosa *et al.* carried out isolation of "*Pseudomonas sp. TDA1"* from an "oligomeric PUR diol solution" as well as "PUR-derived 2,4-diaminotoluene" was found to have great biodegradative activity [14].

Auta *et al.* studied the growth performance and mode of "polypropylene (PP)" degradation by "*Rhodococcus sp. strain 36*" and "*Bacillus sp. strain 27*" isolated from a mangrove environment after contact with PP microplastics. " *Bacillus sp. strain 27*" and "*Rhodococcus sp. strain 36*" both lost weight after incubation of 40 days, demonstrating that the strains may modify, colonize, and absorb PP microplastics as a source of carbon [15].

Skariyachan *et al.* studied the plastic-degrading microorganisms in dung (cow) samples obtained from highly plastic-acclimated areas using conventional procedures. The zone of clearance approach was used to understand degradation ability, and the weight loss procedure was used to evaluate the degree of degradation.

Their findings revealed new isolates such as Bacillus vallismortisbt-dsce01, which has been shown to degrade LDPE up to 75% after 120 days [16].

The above studies have investigated the different bacterial and fungal agents capable of degrading plastic polymers in extreme conditions as well as from other locations. However, the present study aims to provide a critical review of the recent research investigations investigating different microbial consortiums to identify the most capable bacterial, and fungal agent for plastic biodegradation.

Plastic waste is very harmful for the environment. As a result, its eradication from the planet is very critical. Biodegradation is regarded as the best option for plastic waste degradation among thermic, photo-oxidative, mechano-chemical, and catalytic degradation processes due to the low effort required and its eco-friendly nature. However, a detailed characterization of capable plastic-degrading bacteria and microbial compounds is required. Various studies show that different isolates of biodegradable plastic are obtained from cow dung samples from plastic accumulation locations and other areas such as the neighborhood of the plastics manufacturing industry.

In addition, plastic-degrading bacteria and fungi from extreme environments are discussed in this work.

Investigations on the biodegradation of polymeric substances made from petroleum have been a ground-breaking attempt to reduce environmental plastic pollution.

The bacteria and enzymes that have been claimed to break down these synthetic polymers have been covered in this review. Numerous Bacillus, Pseudomonas, and cyanobacterial strains have been shown to partially break down a variety of petro-plastics, including polyethylene,

Polystyrene, Propylene, and ester-based PU, as well as complex, recalcitrant materials such as polyaromatic hydrocarbons.

Microorganisms, such as bacteria and fungi, begin the process of biodegradation. Generally, this microbial degradation of plastics includes the development of fungi the bacterial agents on a plastic surface, in which the fungi utilize the plastic waste as a source of food under the pressure of external factors such as pH and temperature.

Such fungi or bacteria will release extracellular enzymes including proteases, lipase, and cutinase, as well as lignocellulolytic enzymes, esterases, carboxylesterases, and certain pro-oxidant ions, which will breakdown the plastic material. The hydrolysis/oxidation enzyme enhances polymer hydrophilicity and thereby degrades complex molecules into simpler molecules.

3. METHODOLOGY

Primarily the above-reviewed studies have carried out the isolation of the microorganisms followed by molecular identification which was then followed by the screening. After performing the screening of bacteria having the plastic degrading ability, optimization of the potential isolates was performed followed by the critical analysis of the weight loss of plastic by a microbial consortium selected out of the microbial population. After performing the weight loss analysis, the characterization of the biodegraded bead with the help of methods like SEM, FTIR, and many others is illustrated in Figure 4.

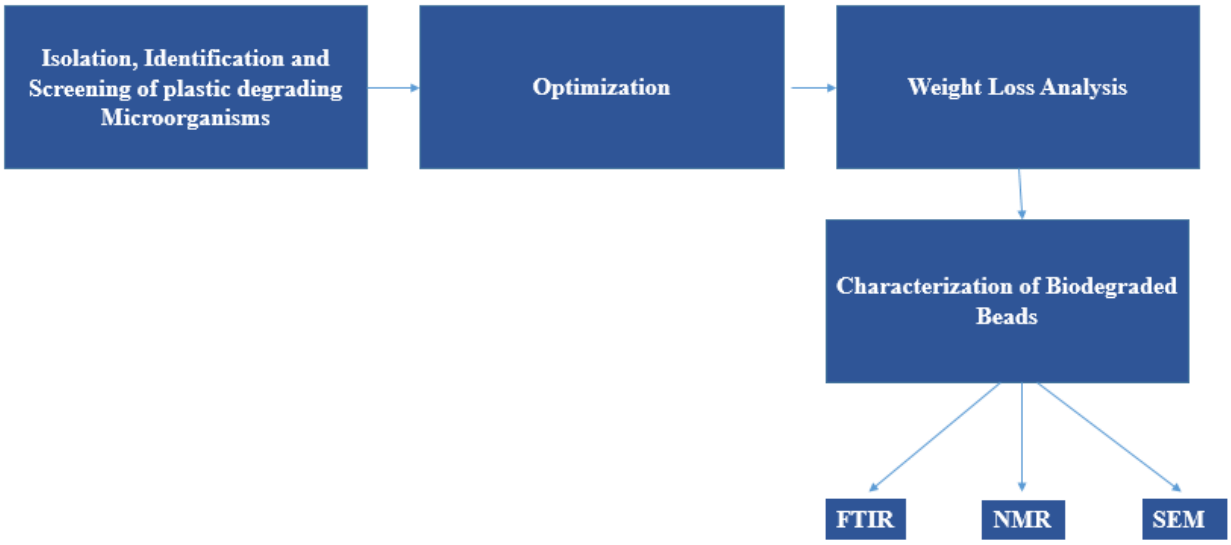

Figure 8: Methodology to Find Out the Microbial Agent for Plastic Biodegradation.

3.1. Mechanism of Biodegradation of polymers

The research studies investigating the microbial degradation of the plastic compound have already gone into the mechanistic insight of microorganisms which specify the production and secretion of different extracellular enzymes. However, the basic mechanisms of biodegradation remain the basic same as illustrated in Figure 5 below.

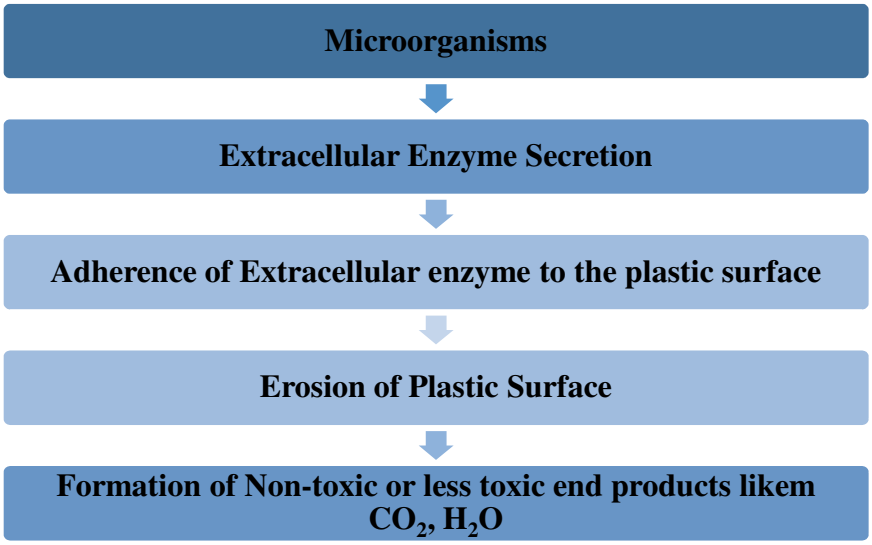

Figure 9: Mechanism of Action Involved in the Biodegradation of Plastic by Microorganisms.

4. CONCLUSION

We cannot carry out our daily needs without plastic, but due to its negative effects, it is necessary to establish effective procedures for its proper disposal and investigate alternatives such as starch-based and mixed plastic. Due to its hazardous effects, traditional ways of removing plastic from the environment (including burning and landfilling) are unsuccessful and limited to recycling. As a result, research communities are paying more and more attention to the biodegradation process as a practical and environmentally benign method for breaking down various forms of plastic trash. The potential of plastic-degrading microbial species has been the subject of numerous reports, but none of them have been found to have any real-world applications. Therefore, it is imperative to find effective organisms and create technology that can efficiently digest plastic without damaging the environment.

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CHAPTER 19

AN ASSESSMENT OF EXISTING AND EMERGING NOVEL APPROACHES FOR WASTEWATER TREATMENT

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ABSTRACT:

Many contaminants and their derivatives are being dumped into the aquatic environment as a result of urbanization and industrialization. The majority of pollution is generated by nutrients, low-concentration organics, and pollutants that are very harmful to humans and freshwater habitats. Because of urbanization and inefficient traditional wastewater treatment methods, the quality and quantity of fresh water, particularly for home and industrial reasons, are decreasing. For decades, standard discharge requirements have been met with just some success by treating effluents using conventional wastewater treatment methods. But for treated water to be used again in the home, commercial, and agriculture domains, breakthroughs in wastewater treatment are necessary. Hence, the aim of this study is to the recent advances and emerging trends with novel approaches for the treatment of wastewater generated from different sources ranging from agricultural to industrial. This review also provides a critical discussion on the limitations of developed approaches which can further be analyzed for their real implementation and establishment for future wastewater treatment.

KEYWORDS:

Biological Oxygen Demand (BOD), Novel Approach, Microalgae, Wastewater, Water Treatment.

1. INTRODUCTION

Clean water is essential for ecological health, social and economic development, and human wellness. Nevertheless, as people grow and natural ecosystems deteriorate, it is increasingly more difficult to ensure that there are enough reliable water supplies for everyone. Two essential elements of the strategy are better wastewater management and decreased pollution production. If we want an economy that is more circular and hence more sustainable, we must appreciate wastewater for its potential rather than discard it or ignore it. Safe wastewater treatment may provide us with more than just a fresh source of water; it might also offer us nutrients, energy, and other salvageable materials[1]. Throughout the whole water cycle, from the abstractions of fresh water through its pre-treatment, distribution, utilization, storage, and post-treatment, as well as its use for treating the wastewater before its release into the environment to begin the cycle all over again, water must be managed correctly [2], [3]. Worldwide, the amount of wastewater produced and its total pollutant load are growing as a result of population increase, faster urbanization, and economical growth.

The availability of sufficient and clean water supplies directly depends on how wastewater is managed. Increasing untreated sewage, agricultural runoff, and industrial discharge, water quality has been declining and water supplies have been degraded all over the globe s[4]. Approximately 1.8 billion individuals consume polluted water, placing them in danger of contracting diseases including typhoid, dysentery, cholera, and polio [5], [6]. This is because wastewater worldwide runs back into the ecosystem. Wastewater will play a significant part in supplying the rising water need in quickly increasing cities, boosting energy production and industrial growth, and assisting sustainable agriculture, far from being something to trash or disregard.

The world is dumping wastewater, according to a new study on wastewater treatment that was released by the UN. This is particularly true in emerging and undeveloped nations where less than 8% of the wastewater gets treated. The biggest challenge currently is how to shift perspectives so that wastewater is seen as an opportunity rather than as a problem. Untreated wastewater and improper sewage water from industry continue to degrade water quality around the world. Water from wastewater can be used as a substitute for fresh water, but the process must begin with how the wastewater is collected, handled, and disposed of. Pollutants and toxins are frequently present in wastewater. Organic pollutants, plant nutrients, Heavy metals, pathogens, and micropollutants are a few of them. All of the above quality concerns may have an adverse effect on the environment and public health, which may have a negative influence on economic impacts on society. [7]

1.1. An effective approach for Wastewater treatment

As illustrated in Figure 1 and Figure 2, numerous chemical, physical, and biological procedures, including precipitation, oxidation, carbon adsorption, flotation, evaporation, solvent extraction, biodegradation, membrane filtration, ion exchange, electrochemistry, and phytoremediation, have been documented over the past three decades. Which approach is ideal? Since each treatment has unique benefits and drawbacks in terms of cost, efficiency, practicability, and environmental effect, there is no simple solution to this query. In general, chemical, physical, and biological techniques are used to remove pollutant compounds. Because industrial effluents are so complex, there is currently no one approach that can treat them effectively. In reality, a variety of techniques are frequently used to provide the required water quality most cost-effectively [8]–[11].

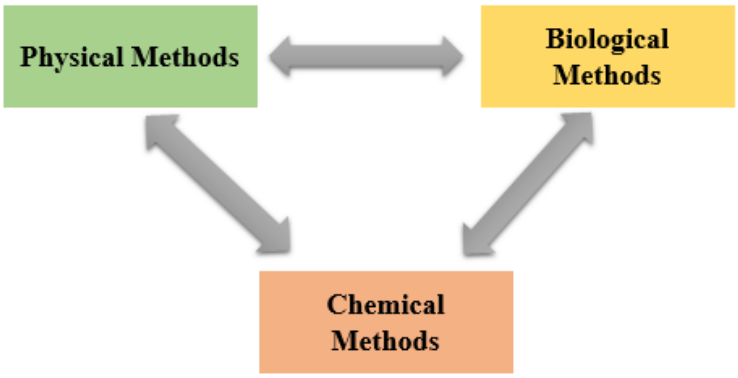

Figure 10: Illustrating the three methods of wastewater treatment that are harnessed in their combination.

However, the development of alternative waste treatment procedures has become necessary as a result of the establishment and implementation of strict regulations for the discharge of waste into the ecosystem. Numerous microorganisms have been identified to be essential in wastewater treatment processes. Their enzymes can target and remove resistant contaminants through precipitation and product transformation. They may also alter the features of a particular waste to make it more accessible for treatment or support the conversion of waste material into value-added goods. In addition to microbes like bacteria and other agents, microalgae have also been investigated for their efficacy in wastewater treatment.

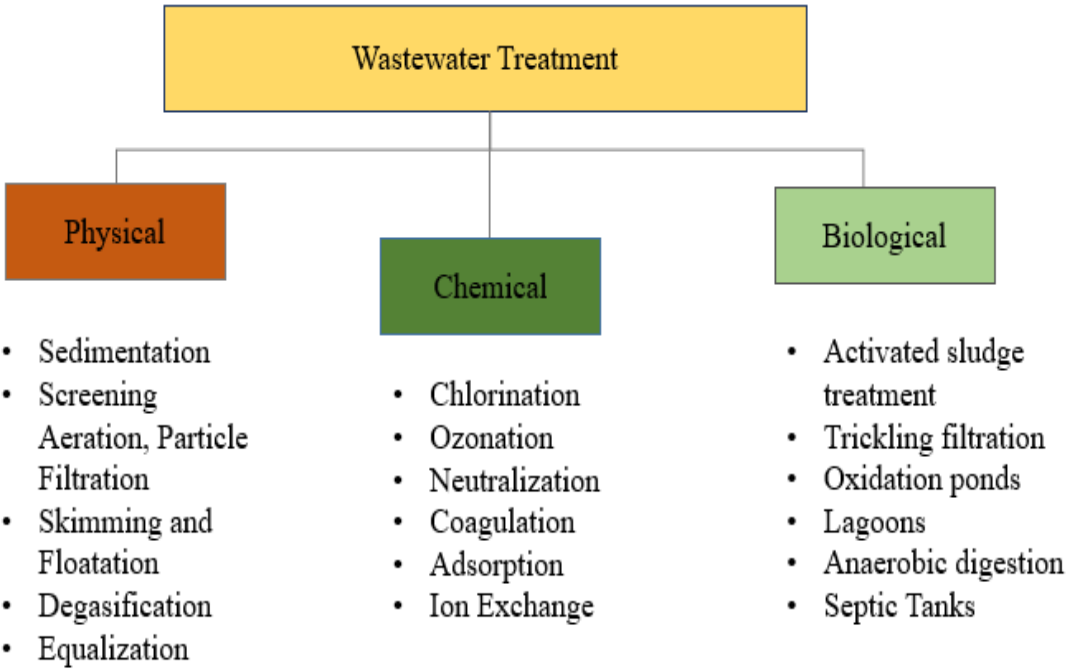

Figure 11: Illustrating the classification of wastewater treatment methods; i) "Physical method", ii) "Chemical Method", and iii) "Biological Method".

This study aims to provide an intensive review of existing literature on the novel approaches that have been developed by researchers to treat wastewater. The first section of this study discusses the background of the topic of wastewater and its generation with the limitations of conventionally available treatment methods. The second section reviews the novel approaches that have developed in recent years and have been published in peer-reviewed journals. The third section provides a critical discussion of the limitation of the previously employed methods with the steps that are employed in current wastewater management and treatment. The discussion section also provides an acritical discussion on the need of advance treatment methods which is a growing need as the population is growing rapidly. The last section provides a concluding remark.

2. LITERATURE REVIEW

Eukaryotic cyanobacteria and algae are two types of microalgae that are an environmentally sound and long-lasting substitute for the currently utilized, energy-intensive, traditional biological treatment methods. Numerous studies have assessed the research into the ecological

elimination of nitrogenous, phosphorus, and carbonaceous, material in wastewater effluents by microalgae.

Moondra et al. investigated the ideal concentration of "microalgal-bacterial" conglomerates for substantial cuts in physical-chemical parameters of raw wastewater from domestic sources with a variety of concentrations (20 %, 30 %, and 40 %). They found that the maximum removal efficiency of "biological oxygen demand", "chemical oxygen demand", "ammonia", and "phosphate", demonstrated the potential of micro-algal-bacterial consortium in effectively treating the wastewater [12].

Luis D. et al. conducted another investigation to assess a new treatment method that includes "thermal hydrolysis (TH)" pretreatment at various periods, accompanied by "anaerobic digestion" of the solid matrix and "photo-fermentation" of the liquid part. The combined use of anaerobic digestion and TH reduced the waste volume that needed to be discarded by 59–61%, which is 5–11% greater than what was achieved with the process of anaerobic digestion [13].

P is typically the rate-limiting element for phytoplankton development in ecosystems, hence lowering P inputs to recipient systems is thought to be essential for lowering eutrophication. Becker & Kruse created a cutting-edge method for recovering phosphate from sewage sludge that specifically used hydrothermal carbonization. Because the maximum overall recovery rate of phosphate from sludge was determined to be 82.5 weight %, the results showed the potential performance of "hydrothermal carbonization (HTC)" as a substitute sewage sludge treatment [14].

Nguyen et al. devised a novel BOD (Biological Oxygen demand)/pH device that relies on the "respirometric principle" that can concurrently and unceasingly measure both the pH and BOD of wastewater for approximately 20 days. In the laboratory setting, the suggested device's performance is compared to that of commercial devices (HACH, BOD Trak II,). The results of their comparison indicated that the suggested device can detect variations in BOD and pH throughout time. In addition to that, it is also cheap cost and has great potential for a wide range of applications [15].

Researchers are paying more and more attention to white-rot fungus as biological techniques for wastewater treatment gain popularity. By using grain sorghum as a carrier and the only source of carbon and nutrients for white rot fungus (WRF).

Zahmatkesh et al. investigated a novel method to simplify the deployment of WRF in non-sterile circumstances. Immobilized *Trametes versicolor* on sorghum was used to test the wrf's effectiveness in removing "humic acid (HA)" from both simulated and actual wastewater from the industry. The results of their experiment revealed that using immobilized WRF on sorghum under sterile conditions resulted in % color removal for both real wastewater and synthetic without the inclusion of any additional carbon or nutrition components. During non-sterile conditions, immobilized fungi removed 80% of the pigment and achieved a maximum laccase activity of 40 U/L, showing the WRF's capability in wastewater treatment [16].

Another study, conducted by Ning-Jie Li and Han-Qing Yu, explored soluble microbial products (SMP) from WRF "*Phanerochaete chrysosporium"* as a bioflocculant for the treatment process of municipal wastewater. The findings demonstrated the high flocculation behavior of SMP-P in kaolin suspension at a dosage spectrum of 0.6-0.8 mg/L with the assistance of Ca+, similar to

that achieved by market polyacrylamide, exhibiting the viability of using fugal SMP as a flocculant and providing advice for their practical implementation [17].

By sonicating ZIF-67 at room temperature, Saghir and Xiao formed "Novel CoCu-LDH nanosheets". The importance of the produced nanosheets in obtaining strong adsorption properties for methyl orange was shown to be significant (MO). The results of a thorough adsorption kinetics analysis show that the "CoCu-LDH" is an efficient and cost-effective adsorbent material for wastewater of anionic industrial effluents. The findings were consistent with a kinetic model of "pseudo-second order (PSO)" with "good correlation coefficient (R2) values" [18].

The above studies have investigated and developed novel approaches and methods one at a time and further efficacy of the developed methods was analyzed. However, the present study reviews as many studies as possible that have been carried out for the development of novel approaches which can further provide a one-stop paper for future researchers to ponder on the developed approaches and their associated limitations for effectively implementing them.

3. DISCUSSION

To address various treatment scenarios, chemical and biological treatment approaches have been developed. However, these applications are frequently hampered by the high cost of treatment, the constant addition of harmful chemicals, the large amount of area required for implementation and installation, the adverse effects of secondary pollution, and so on. As a result, numerous more unique techniques for the successful treatment of wastewater from various sources are presently being explored.

Water pollution may come from several places, such as homes, businesses, mines, and irrigation systems, but one of the biggest contributors is the industry's extensive usage of water. In general, four types of water are distinguished:

- Rainfall (runoff from impermeable surfaces)
- Agricultural water
- Domestic wastewater
- Wastewater from industry

Process water or manufacturing (biodegradable and/or potentially toxic), washing effluent (varying composition), cooling water, and the final category may all be further separated into these subcategories. The most troublesome substances are often processed fluids, commonly referred to as wastewaters or events. The source of the wastewater generation is illustrated in Figure 3 below.

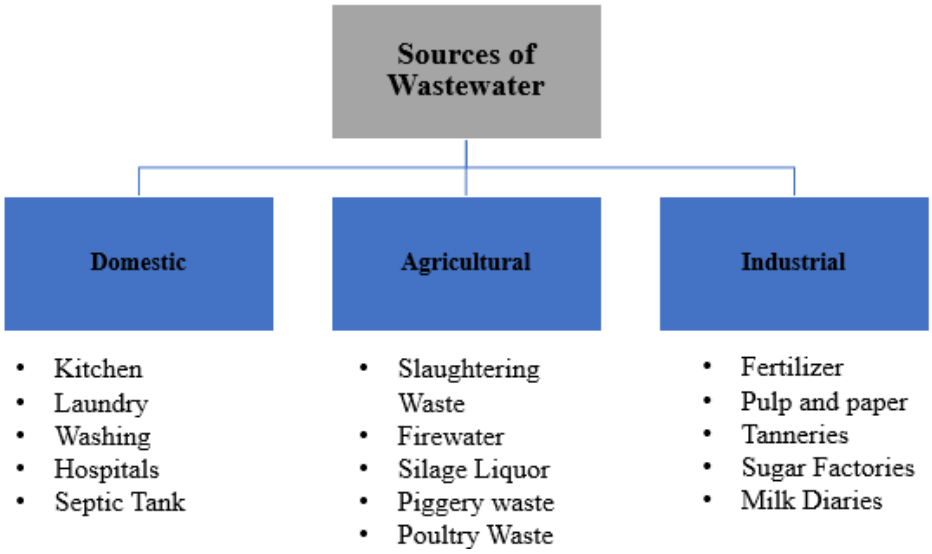

Figure 12: Illustrates the different sources of wastewater; (i) Domestic, (ii) Agricultural and (iii) Industrial.

The best purification method should be used when the water has to be cleaned up after pollution, to achieve the desired decontamination. A purification process typically entails five steps that follow each other with four main steps illustrated in Figure 4 below:

(1) "Preliminary treatment"
(2) "Primary treatment"
(3) "Secondary treatment"
(4) "Tertiary or final treatment"
(5) "Treatment of the sludge produced"

According to the circumstance, the first two processes are typically referred to as pre-treatment or preparatory steps. Overall steps are illustrated in the Figure below that are used in conventional wastewater treatment.

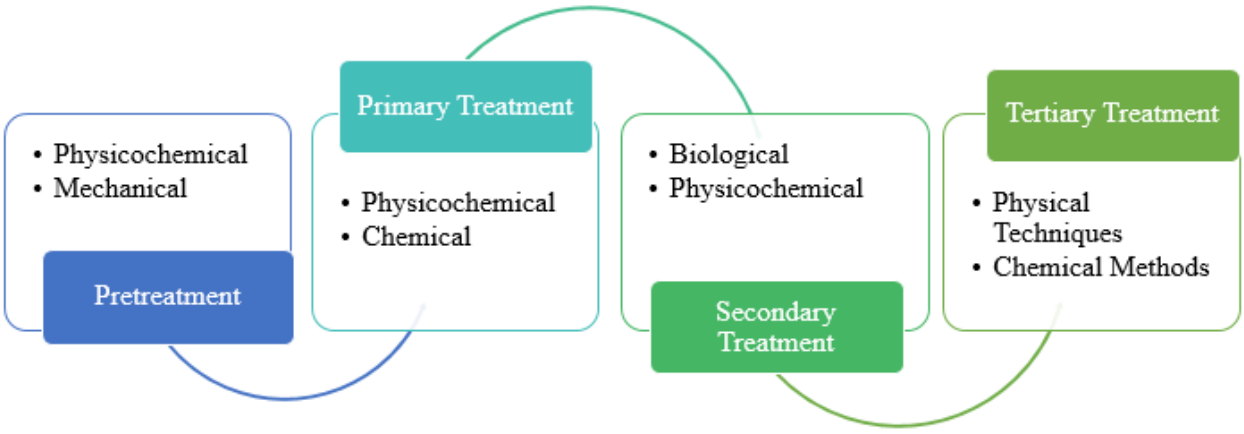

Figure 13: Illustrating the traditional method used for the treatment of Wastewater.

The regulations and high fines that are drawn when wastewater disposal does not match the stipulated discharge limitations are one of the key causes that have encouraged the genesis of new or better wastewater treatment technologies. This influence on manufacturing and industry financial well-being has fuelled the development of new or enhanced treatment solutions because of environmental issues. One of the biggest environmental issues is the chemical contamination of rivers and streams. Significant damage is caused by waterborne chemical contamination that enters rivers and streams. To prevent pollution of the environment, it is crucial to treat wastewater before it is released. It's feasible and practical to use wastewater for irrigation when disposing of garbage (as in slow-rate land treatment). To use municipal wastewater for industrial or agricultural applications, it must often first undergo some kind of treatment.

Any amount of treatment that is wanted may be accomplished with advanced wastewater treatment. In certain treatment systems, further treatment is required to remove nutrients from wastewater. Modern wastewater treatment facilities make use of cutting-edge procedures and tools. They require a fair amount of maintenance, and effluent quality and operating costs are related to how effectively they are run. To preserve the aquatic body that receives the discharge, wastewater treatment operations must be carefully managed. Operators of treatment plants who have undergone training and certification measures keep an eye on the final effluent as well as the treatment process.A combination of microalga and several bacterial strains has been used for research. By using resources for development and reducing a range of organic and physicochemical pollutants, microalgal-bacterial consortia may effectively clean wastewater. Algae, a water purifier, and pollution indicators are better alternatives to bioremediation. The treatment of household wastewater by consortia of microalgae and bacteria is efficient and rapid. In addition to being a possible alternative to conventional biological wastewater treatment methods, microalgae may also be a tempting addition.

Another research similarly found that the acid leaching, HTC, and struvite precipitation process combination performed well, recovering around 80% of the phosphate that was originally present in the native sludge. The limiting element, which was remedied by the use of nitric acid, turned out to be the process ammonium concentration of the liquid. As mineral acids are frequently introduced to HTC as proton donors for the catalysis of reaction, nitric acid can be utilized to generate ammonium and facilitate the production of struvite in the following phases.Along with emerging methods for treating wastewater, fresh tools are also being developed for characterizing it. A unique BOD/pH device proficient in long-term monitoring of “pH” and “BOD” was created successfully. It is based on the respirometric principle. The real-time applicability, however, is absent. Effective, cost-effective, and environmentally responsible wastewater management should be the ultimate objective of wastewater treatment.

4. CONCLUSION

There is now continuing research being carried out on the development of less costly, more efficient, and distinctive purification processes, as demonstrated by the multiple research that is published every year. Everyone is now extremely worried about the environment, specifically the problem of contamination of water, including members of the business, general public, researchers, decision-makers, and scientists, on a variety of levels, whether global or national. Due to the need of the public for pollutant-free waste discharge to receiving rivers, decontamination of industrial effluents has become a major problem. This is a difficult and

difficult undertaking, nevertheless. Additionally, a general technique that could be applied to remove all contaminants from wastewater is difficult to define.

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CHAPTER 20

A COMPREHENSIVE STUDY ON IMPLEMENTATION OF ULTRASOUND TECHNOLOGY FOR SAFE AND HIGH-QUALITY MEAT

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ABSTRACT:

Traditional food processing methods including cutting, degassing, freezing, filtering, meat tenderization, and thawing have been efficiently replaced by ultrasound. The ultrasonic method contributes to the improvement of already-established processes, as well as the analysis and modification of food and food products, and also their implementation for industrial scale production. Ultrasound has been effectively implemented in various areas of food technology as a substitute for existing techniques and as an assistant to traditional techniques. Despite this, additional study demand is suggested in terms of optimizing process conditions (scaling up of ultrasound equipment) and the improvement of existing processes' possibility for effective use in industrial food and product analysis and adaptation. This study provides a summary of the outcomes of studies that relies on ultrasound as a "green," nonchemical technology that might be utilized in the meat industry to enhance both the safety and quality of meat. Due to these possible benefits of ultrasonography, summaries of its notion and longstanding implementation in food business meat technology are evaluated. Ultrasonic processing is an inexpensive non-thermal method. Future ultrasonic applications would combine with non-thermal methods for better outcomes.

KEYWORDS:

Decontamination, Food Processing, Meat processing, Ultrasound Technology (US), Waves.

1. INTRODUCTION

A shift in consumer preferences coupled with the need of producing nutritious, high-quality meals drive the evolution of food processing. It seems that using emerging technology would be the most effective means of achieving the aforementioned attributes. To save money, time, and energy,Utilizing high pressure, pulsed electrical currents, microfiltration, and ultra-sonication are all examples of such technologies. The "Green Food Processing" process uses ultrasonography to assure that the food is safe and of excellent quality [1].In recent years, customers have demanded foods that have undergone a minimum amount of processing, which, in conjunction with shifting legislation around food, has spurred the development of several innovative technologies that have significantutility in the food industry. The food sector makes use of a variety of environmentally friendly and safe technologies, including ultrasound technology, which has a wide range of uses.As an alternative to or complement traditional processing techniques, this unique non-thermal approach has garnered significant attention. As a result of this innovation, food may be cooked without the use of fire [2].

Food businesses are always in search of breakthrough procedures that may not only create microbiologically safe and higher quality food items, but also manufacture such goods with attractive organoleptic features at the lowest possible cost and energy consumption.The production of safe food items has historically relied on the use of thermal processing methods such as pasteurization, sterilization, and canning, amongst others. However, high-temperature treatments affect the levels of vitamins, antioxidants, and polyphenols, in addition to sensory qualities [3].Therefore, the most significant hurdles lie in the development of cutting-edge methodsin a way that may provide top quality, security, and storage stability all at once. As a result, in the last several years, the food sector has been testing the exploitation of many newlyhigh-pressure processing and electric pulse fields are two examples of emerging technology, ultraviolet light, light pulses, ultrasonication, and irradiation. Ultrasonic technology is now being used in the food processing industry for cleaning and disinfecting manufacturing surfaces; however, many of the applications of this technology are still in the research stage [4].

Ultrasonic sound waves are used in this method; their frequencies are too high to be heard by the human ear. Ultrasonic technology typically operates within a frequency range of 20 Kilohertz (kHz) to 500 Megahertz (MHz).Freezing, crystallization, extraction, drying, sterilization, degumming, filtering, defoaming, emulsification, and preservation are just some of the many uses of cryogenics in the industry of food processing. High-intensity ultrasound, or power ultrasound, works between 20 and 100 kHz, whereas low-intensity ultrasound, or high-frequency ultrasound, operates between 2 and 10 MHz. Both of these categories utilize ultrasound waves [5].Sound is the movement of atoms and molecules in an elastic medium. When a vibratory action causes a wave to travel, the particles are displaced from their resting positions and the path of the wave. In this case, the source of sound is a specific item, which conveys its movement to the surrounding particles via the medium's mechanical characteristics. An elementary vibratory motion mechanically spreads to become a wave of sound when the particles communicate their motions to one other and other particles, resulting in a local fluctuation in pressure (or acoustic wave)[6].

An elastic medium is necessary for the propagation of ultrasonic waves (US) [7].Both sounds and ultrasound scans have distinct frequencies that range from 16 Hz to 16–20 kHz for sounds and from 10 MHz to the so-called hypersonic zone of 10 MHz for ultrasounds, which would be the maximum limit of human hearing for both.Mechanical waves, which are what ultrasound is made up of,transport energy rather than particles through a medium; particles, on the other hand, merely oscillate around their balance position in terms of the energy transfer from one particle to another. Ultrasound is made up of mechanical waves. Because of the oscillation's ability to travel through the medium in several different directions, we can differentiate between longitudinal waves and transverse waves.

In the past, meat and poultry were considered to be the source of several cases of food-borne diseases in people. It has been reported by the aforementioned sources that, the bulk of food-borne dangers to human health is caused by microbiological hazards that are mostly carried by healthy animals. Potential microbiological threats include, but are not limited to: "*Salmonella enteritidis, "Campylobacter jejuni", "Escherichia coli", Shigella, Cryptosporidium, Clostridium perfringens, "Yersinia enterocolitica", and "Listeria monocytogenes"*[8]. Even though there are many rules concerning food safety and efficient monitoring systems in place for the food sector, one of the most difficult tasks that authorities face is the prevention of illnesses that are transmitted via food. The continued high rate of foodborne illness outbreaks linked to the

consumption ofthe safety of meat and poultry items is a major cause of anxiety for consumers.The persistence of meat-borne illnesses that are important to the public's health has also been clearly shown by research on humans using monitoring methods.

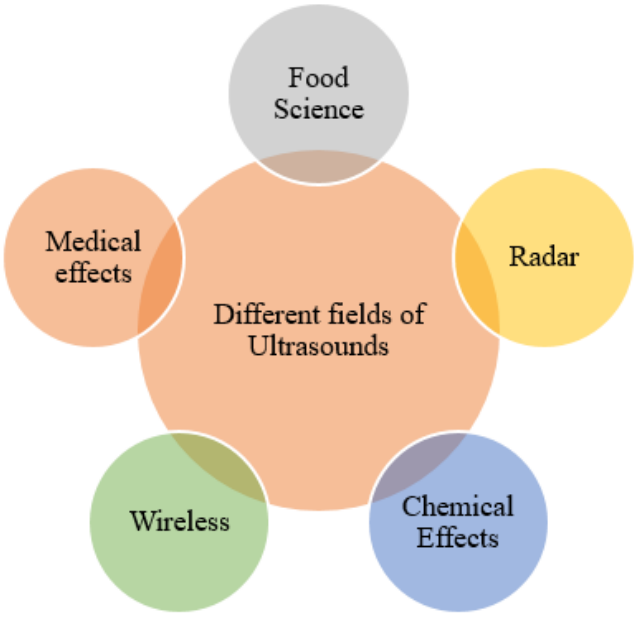

Figure 1: Domains Where Ultrasonography (US) Has Been Successfully Implemented.

The use of Ultrasonography in various domains \where the use of US (US) has been a success as seen in Figure 1. The benefits of sourcing food from the United States are many in the food industry. Using the US, Phyto complex extracts' organoleptic qualities were preserved while their functional principles were studied.Since any solvent can be used as an extraction solvent and also the process is carried out at room temperature, it is possible to save a lot of time, equipment, energy, and labor by using this method. In addition, the antibacterial activity of the US ensures that the finished product has a lower bacterial concentration. Finally, the extraction method used in the United States has been biologically certified for usage in the food and cosmetics sectors [9].As an alternate and novel way to existing decontamination applications, ultrasonic technology will be summarized in this study.Using simulations of the present body of research, we were able to evaluate the antibacterial effect of this technology, as well as its potential usage in ultrasonic applications in the wash water, for cleaning and decontaminating meats. It would be described how in the meat industry, ultrasonography is utilized to better track quality indicators including tenderness, marination, and cooking yields.

2. LITERATURE REVIEW

Monika Kordowska-Wiater and Dariusz Stasiak stated in their study that the research looked at the removal of bacteria such as skin bacteria such as "*Salmonella enterica" "ssp*. enterica "SV. Anatum", "*Escherichia coli", Proteus spf., and "Pseudomonas fluorescens"* were killed off by ultrasonication in water and a 1% aqueous lactic acid solution. A 40 kHz, 2.5 W/cm 2 ultrasound was applied to infected samples for 3 or 6 minutes. The bacteria *E. coli* were particularly vulnerable.After 3 minutes of sonication in a lactic acid aqueous solution, bacteria at

concentrations more than 1 log colony forming unit (CFU/cm^2), (up to 4.0 log CFU/cm 2) were destroyed. Pseudomonas became the most sonication-sensitive bacterium in lactic acid. Ultrasound coupled with lactic acid can disinfect fowl skin [10].

Dariusz Stasiak *et al.* studied using ultrasound for the cell survival and Salmonella on the epidermis of chicken broilers were studied using 40 kHz, 2 W/cm^2, 6 min, 20 °C, distilled water, and 1% lactic acid solution. Furthermore, adding lactic acid increased the sonication's impact. After six minutes of sonication, the number of living organisms and the presence of Salmonella on broiler skin were found to have fallen by over 1.8 and 3.6 log in a solution of 1 percent lactic acid, correspondingly [11].

In their study, Smith investigated the effects of static and ultrasonic marination on the decreased levels of *E. coli* and *Salmonella* in beef. Each broiler breast had one fillet static-soaked in a marinade for two hours and another fillet marinated in an ultrasonic bath for twenty minutes (Branson ultrasonic cleaners; ultrasonic settings were not mentioned). Ultrasonic and static marination did not significantly affect Salmonella and E. coli log counts ($P > 0.05$). The authors concluded that combining high-intensity ultrasound with antimicrobials may reduce pathogens [12].

L. M. Carrillo-Lopez *et al.* conducted a study that describes the process, operation, and current prospective uses of ultrasound in food systems, as also the physiochemical impacts of ultrasonic therapies on food conservation and modification. Acoustic energy is a promising food business technique. Acoustic cavitation, which alters food's physical, chemical, or functional characteristics, may enhance and generate new procedures. Ultrasonic energy combined with a sanitizer may enhance food quality by reducing microorganisms. Ultrasound may have applications outside of the food sector, but this has to be explored more [13].

3. DISCUSSION

Bacterial contamination of meat is unavoidable in industrial processing because of the high perishability of the product and the resulting risk of contamination. Water that hasn't been properly cleaned, excrement from animals or humans, and dirty processing surfaces are all common causes of contamination. It's not uncommon for the process of raising livestock to be a never-ending battle to maintain desired quality attributes while also trying to keep harmful bacteria at bay. Healthy and sanitary meat processing do not stop at the slaughterhouse or meatpacking factory; it also includes measures to avoid microbial adherence, remove pathogens physically, and destroy any remaining germs on meat [14].Several effective preserving food approaches have been studied because hurdle technology is still emerging as a food refurbishment strategy in the food industry.When it comes to preserving and processing food, there is a distinction to be made between thermal and non-thermal approaches, such as ultrasound and hermetic processes (such as pasteurization and sterilization), because of the variables required to produce more beneficial foods for human consumption, and also the drawbacks of thermal processing of foods [15].

As a result, ultrasonic technology is highly regarded for its outstanding capabilities as a processing and preservation tool. Although several alternative strategies have been investigated in the last decade, high-intensity ultrasound has garnered the most attention for food storage applications because of its ability to preserve food at high temperatures [16].In addition, ultrasound adaptation is an effective method that increases the flux in ultrafiltration or membrane

filtration methodologies and improves thecleansing of clogged membranes. Membrane technology is a tried-and-true advancement in the food and dairy industries, and it is also used to cleanse liquid sewage treatment streams and purify water [17].

3.1.A Review Of The Ultrasound Technology And Generating Process:

As seen in Figure 2, the ultrasonic wave generating system is made up of three different components: the Generator, the Transducer (which may be fluid-driven, magnetostrictive, or piezoelectric), and the Application System. The generator is responsible for the production of mechanical or electrical energy, which is then transformed into sound energy in the form of ultrasonic frequency by the transducer.The creation of ultrasonic waves was accomplished most often by the use of the electrostrictive transformer concept. This method was based on the deformations of ferroelectric materials themselves when they were subjected to an electrical field with a higher frequency. This elastic modulus was brought about by the interaction that existed between both the polarizations of the molecules in the field.

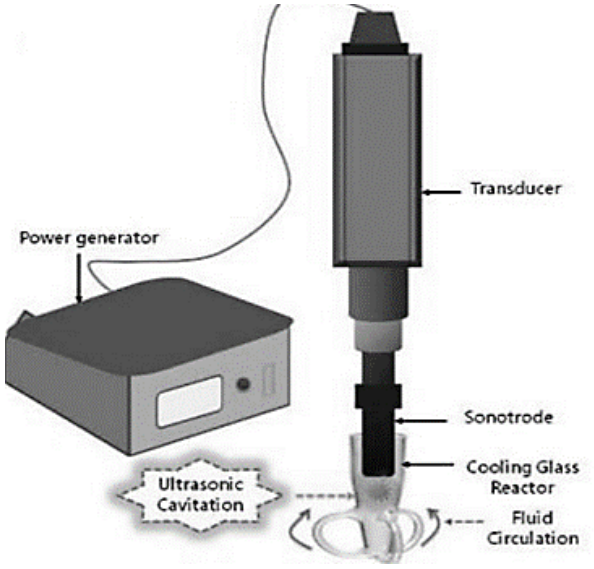

Figure 2: Ultrasonic Monitoring System [18]**.**

One sort of ultrasound system employs a horn as the sound emitter, while the other use a bath as the sound emitter. Both types of ultrasound systems are utilized in the food sector. Because of its convenient availability and accessibility, the bath has always played a role in the food processing industry.

3.2.Ultrasound's Chemical and Micromechanical Antibacterial Mechanisms:

For more than a century, scientists have known that ultrasound has an antibacterial impact on plants and other living organisms. Heat-sensitive nutritional, sensory, and functional properties of food are said to benefit from ultrasonic waves' ability to promote microbiological safety.Remember that ultrasonography is concerned with pressure waves of at least 20 kilohertz in frequency when talking about it. Power Ultrasound, shorthand forthe low-frequency, high-intensity ultrasound (20 to 100 kilohertz (kHz)), is capable of creating physical (micromechanical) as well as chemical antimicrobial effects. The creation of intracellular cavitation, which could induce cell membrane weakening, heating, and also the release of free

radicals, is thought to be the primary method of microbial inhibition [19].The fact that ultrasonic vibrations are harmless and non-toxic has contributed to the widespread acceptance of ultrasound as a viable alternative to conventional antibacterial treatments. The use of physical-biological approaches in conjunction with ultrasound helps to contribute to the increase in the inactivation and eradication of microorganisms. It is generally known that the impact that ultrasound has on various microorganisms variesmicroorganisms' physiological states, bacterial shape, size, and cellular type all have a role. Furthermore, from the perspective of industrial needs for foods, it is necessary to examine and identify the combined antibacterial impact of ultrasound or other "green" activities at the plant scale.

3.3.Meat Industry Use of Ultrasound for Product Quality:

As an alternative to standard food processing operations, ultrasonic technology was used for crystallization,defoaming, and enzymatic activity, alterations to the functional qualities andmodifying the food's storage life or quality, killing harmful microorganisms, freezing, tenderizing, marinated, defrosting, freeze drying, and concentrating.Despite this technology's practicality, greater uniformity, and considerable energy savings, additional study is required and efficient ultrasonic systems must be developed to enable large-scale operations that can be applied to varied food systems.Meat includes the skeletal muscle and its linked tissues, as well as edible offal (organs and non-skeletal muscle tissues) from mammals, birds, reptiles, amphibians, and fish. A well-balanced diet necessitates the consumption of meat, which has been in high demand since ancient times owing to its nutritious value [20].

The use of real-time ultrasonography has seen widespread application in the estimate of chemical transcription in both the body and the carcass of animal products. If evaluated by, intra-muscular fat percentage, (sheep) carcass features, and reflection prototypes of tissues like muscle, fat, and vital organs in live animals were shown to be useful in genetic transformation via the application of low-intensity ultrasonography in meat technologies.Ultrasound has a dual effect on meat tenderness: first, it weakens the muscle structure directly, making the flesh tenderer; second, it indirectly stimulates proteolysis by causing the release of cathepsins from lysosomes or Ca++ ions, both of which break down proteins, which permits calpains to be triggered.

A few studies, however, have claimed that ultrasonography does not change the meat's texture in any significant way.Because marination is so highly respected in the meat processing industry, ultrasonography was able to efficiently cut down on the amount of time spent salting the meat, which improved the meat's softness, flavor, and shelf life. However, the ultrasound's intensity and repetition rate are the most crucial considerations to consider when trying to marinate meat successfully [21].Many organizations are hesitant to use ultra-sonication technology because of the weight of old food processing procedures and the unfortunate recognition of new process disciplines (ultrasound) by food specialists. Assessment of ultrasound energy transfer to medium effect during application for various test treatments and intensities of ultrasounds additionally, precise ultrasound frequency and treatment duration are required for a regulated and optimum ultrasound application.

Research that has been approved for publication suggests that ultrasound may contribute to improved performance from several meat quality criteria, including tenderness, although the results of various studies cannot be compared due to a large number of ultrasonic equipment and efficacy, relative intensity, frequency, or time length of treatments, as well as therapeutic applications, muscle type, animal age, and other factors.

3.4.Meats may be sterilized from microorganisms by the use of ultrasound processing:

The non-thermal method of ultrasound further increases microbial safety and increases the food's shelf life, particularly food that is heat-sensitive in terms of its nutritional, sensory, or functional properties.In ideal circumstances, the average number of microorganisms on a corpse may be reduced by one to three log colonies forming units per square centimeter by the employment of detoxification techniques. Foods like meat, poultry, fruits, and vegetables all benefit greatly from the washing decontamination approach being used early on in the commercial production process. Some of these technical solutions are already in widespread use in industry; for example, water spray washing, steam-vacuuming, chlorinated, organic acid, or trisodium phosphate formulations, heated water and pressured steamwhile others are still in the washing decontamination methodology. This is because washing eliminates potentially infectious germs [22].

The antibacterial impact of ultrasound may be improved when combined with the other decontamination/preservation treatments, like hypochlorite, moderate temperature, compression, steaming, or organic acid.. In general, there seems to be no research that specifically examines the impact that high-powered ultrasound has on the bacteria that are present during the spoilage of fresh meats.Equipment hygienic management is a key strategy for preventing the transfer of potentially dangerous bacteria to carcasses, as it may impact the microbiological state of delivery crates for live birds and processing equipment in plants. This is why the use of modern technologies like ultrasound on equipment may also be highly beneficial to reduce the microbial contamination of carcasses from such sources. Some cases include using a stainless steel ultrasonic generator (4 kW) in a tank of boiling water (approximately 60 °C), then submerging the processing facilities in the water for 10 minutes to eliminate gas [23].

3.5.The Positives and Negatives of Utilizing Ultrasound Technology:

According to Chemat *et al.* [24], the following are the primary benefits of using ultrasonic technologies in the food processing industry:

1. The efficiency of items being mixed and micro mixed,
2. The ability to move mass and energy at a much faster rate,
3. During processing, keep the temperature low and make no adjustments,
4. Extraction of certain components with precision,
5. The miniature size of equipment.

According to Alexandre *et al.* [25], the following are some of the potential drawbacks:

1. Largely dependent on the composition of the sample matrix,
2. Strong dependence on the existence of a dispersion phase, may result in a reduction in the efficiency of the technique.

4. CONCLUSION

Ultrasound technology provides a novel approach to problems that have long plagued the food processing and manufacturing sectors. One of the many advantages of this technology is that it has several potential applications within the food processing sector, a few of which could be integrated with the usage of other technology. Ultrasonic technology's ability to inactivate germs

that cause foodborne disease and spoilage while maintaining the food's organoleptic, sensory, nutritious, and compositional qualities is today unmatched.When the US is used in conjunction with heat and high pressure, it activates bacteria and enzymes to preserve or disinfect food. It's becoming increasingly commonplace for manufacturing operations to use ultrasonic (US) as a tool for everything from material mixing and agglomeration to dust precipitation and filtration improvement, as well as to extract liquids and solids from plants and foods, and also bioactive substances. Traditional sanitization procedures may be replaced with ultrasonic sanitization, and the organoleptic qualities of food are unaffected.Both low- and high-intensity ultrasound have potential uses in the meat processing industry, and ultrasound is a green technology. Furthermore, there are unique challenges related to the development of ultrasonic industrial equipment and control systems due to process variations; nevertheless, once these challenges have been met, the technology should be able to broaden its scope of use.

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CHAPTER 21

AN EXPLORATORY STUDY ON MOLECULAR DIAGNOSIS OF *LEISHMANIA DONOVANI* USING NASBA AND QRT-PCR ASSAY

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ABSTRACT:

Leishmania is a parasitic protozoan that belongs to the genus trypanosomes. The parasite causes a zoonotic disease in humans called leishmaniasis mostly in Tropical countries such as Africa, Somalia, Kenya, India, and Yemen. The disease is caused by a sandfly bite. The disease affects several people all over the world and could turn out to be fatal if not treated at the right time. Kala-azar is a disease that affects homeless people in India, the only responsible parasite is which causes this particular disease is "Leishmania donovani". The symptoms of the disease include extreme loss in weight, weakness, high fever, and lack of blood in the patient's body. The detection along with the right diagnosis is highly important for controlling the progression of the disease. This research paper reports the use of two laboratory-based methods "nucleic acid sequence-based amplification (NASBA)" and Real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) assay for the detection and diagnosis of the parasite.

KEYWORDS:

Kala-azar, Leishmania, Nucleic acid sequence-based amplification (NASBA), Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR), Sandfly.

1. INTRODUCTION

The protozoan parasite leishmania taxonomy is still in the process of evolution and is unclear. The conventional system of categorization uses demographical, clinical, and geographical characteristics for underlying the structure of classification. The parasite falls under the category of eukaryotes specifically in the Trypanomastidae family. Figure 1 shows the leishmanial parasite. The family members of Trypanomastidae under the class of invertebrates or vertebrates are parasites and are susceptible to morphological changes during the transformation in various stages of the life cycle. The trypanomastidae family comprises diverse species out of which "Leishmania L. (Leihmania) and L. (viannia)" are well known.

Overall 14 species of leishmania are considered to be a threat to animals, a total of 9 are known for causing infections in humans. The parasite exists in two kinds: an oval and a round stage which lives and reproduces inside the host vertebrate. Leptomonad is an organism with a long, motile flagellated structure found inside the digestive tract of the parasite. Apart from this, the organism has 3 variants that are similar in appearance but instigate 3 various types of diseases in humans. Leishmaniasis. *L. donovani* targets the human spleen, liver, and bone marrow causing kala-azar in countries like Europe, Asia, and Africa. The symptoms of its invasion include blisters on the skin of the hand, legs, face, and feet. Some of these variants cause deep painful blisters in the oral cavity and mucous membrane of the nasal area.

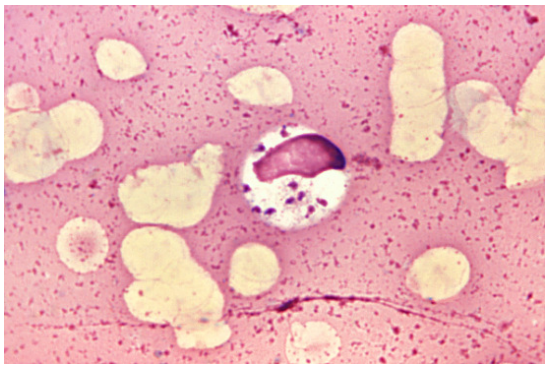

Figure 1: Illustrating the Diagrammatic Representation of Leishmanial Parasite (Circle) in Bone Marrow Cells [1]**.**

1.1.Lifecycle of Leishmania

The parasite causes the disease called leishmaniasis which is spread by the bite of the sandfly. The disease is distributed in 3 subtypes i.e. visceral, mucocutaneous, and cutaneous. Their host target involves animals such as rodents, vertebrates, and canines. The disease transmission starts with the sandfly bite which has a parasitical mode of infecting mechanism. It belongs to the genus lutzomyia. It comprises 2 major life stages during its lifetime one of its kind involves inhabiting the digestive tract of the sandfly it is in a long-elongated form with flagella that is known as "promastigote or leptimonad". Another kind known as amastigote is a shape oval and nonmotile prevalent in vertebrate macrophages. The disease expands by the infestation of sandflies on a target vertebrate that is already infected. Figure 2 explains the lifecycle of Leishmania [2].

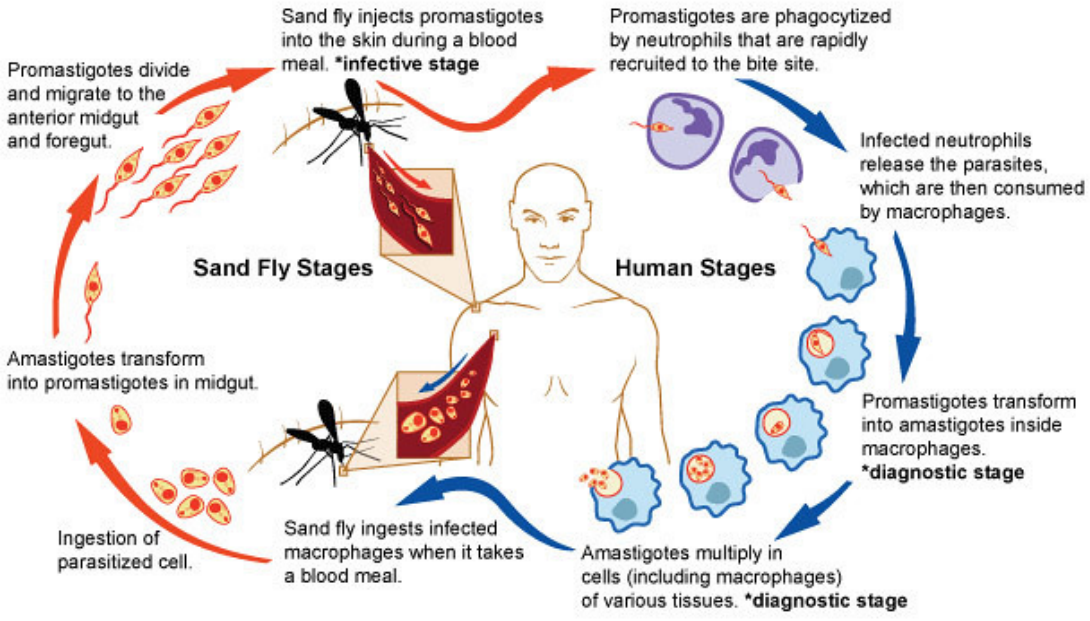

Figure 2: Pictorial Representation of Stages in the Life cycle of Leishmania causing leishmaniasis in Human beings.

1.2.Treatment & Detection

For the diagnosis of visceral leishmaniasis, a "combination of parasitological and clinical tests helps in the diagnosis of the disease". In the case of "mucocutaneous leishmaniasis, the blood tests have a restricted value and the laboratory tests confirm the disease diagnosis". The treatment is dependent on many aspects which also include disease type, species of the parasite, and geographical location. The disease is treatable and curable too with proper medication and a healthy diet [3].

1.3.Diagnosis via a laboratory-based method:

1.3.1. qRT-PCR Assay:

qRT-PCR stands for real-time quantitative reverse transcription polymerase chain reaction. The technique is similar to PCR but it enables to detection and measurement of the product generation in every PCR cycle. The analysis becomes successful post the addition of a probe oligonucleotide which is constructed for hybridization with the sequence target. The activity of 5' during the cleavage of the probe in a PCR cycle along with the Taq polymerase activity can also be used for the amplification detection of a product that is target specific. Figure 3 explains the process of qRT- PCR [4].

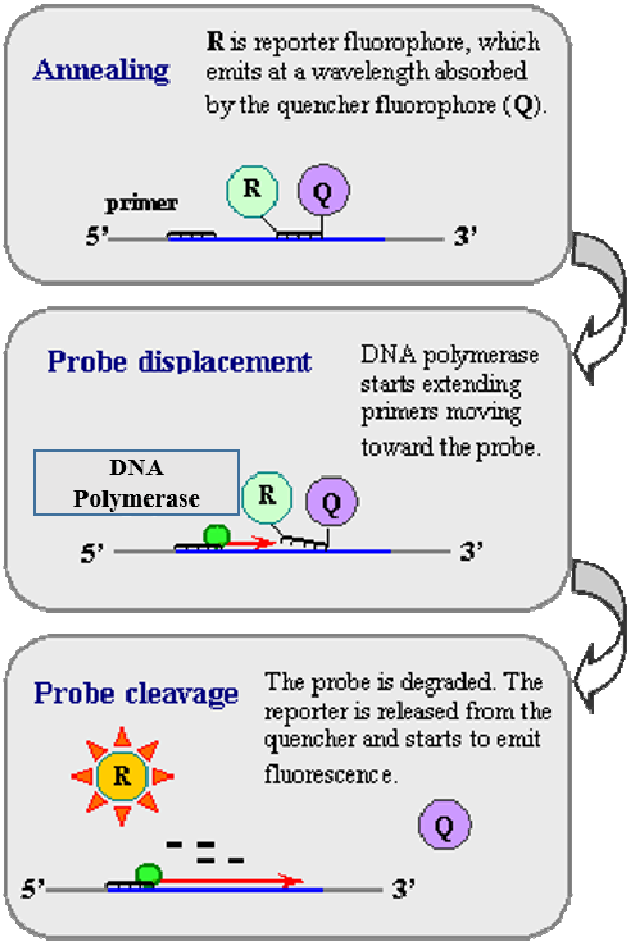

Figure 3: Diagrammatic Representation of the Stages Involved in the qRT- PCR Assay. Enzyme DNA Polymerase Helps in the Extension of Primers.

1.4.NASBA:

"Nucleic acid sequence-based amplification also known as NASBA" is a molecular biology application employed in the production of a single-stranded RNA with multiple copies. It is a two-step procedure that assists RNA molecule anneal especially in primers that are designed and use an enzyme cocktail for amplification. Figure 4 explains the process of NASBA in detail [5].

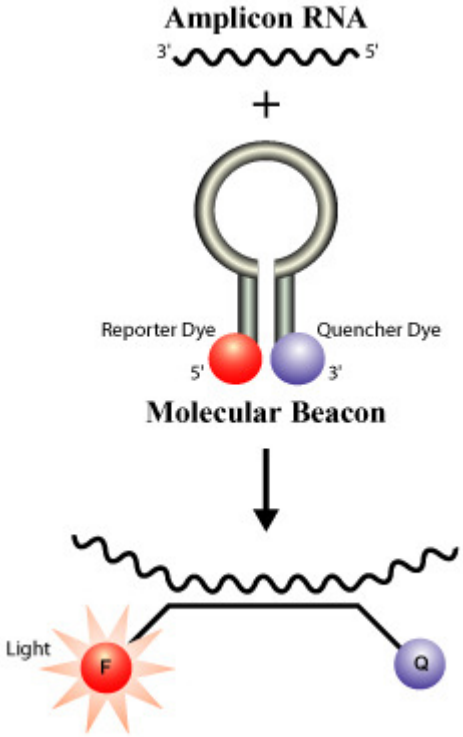

Figure 4: Pictorial Representation of the Nucleic Acid Sequence-based Amplification Assay in Detail.

1.5.Control and Prevention

Early or timely diagnosis of the disease can minimize the severity of the disease and provides prevention against physical and mental disabilities also it will help in reducing the transmission and assist in monitoring the spread of the infection. Another method for controlling the spread of the transmission is by limiting the population of sandflies. The use of insecticides and other insect killer sprays can help in managing the population of the parasite. Other management methods are effective monitoring of the growing number of cases and keeping a record to evaluate the disease spread and providing the required education or awareness for keeping one self-clean and safe.

2. LITERATURE REVIEW

Kid Kohl *et al.* described their views on polyphosphate relevance in the life cycle of Leishmania. The authors describe the parasitic protozoan and its biological makeup. The study deals with the confirmation of polymerase polyp vacuolar transporter chaperone for the production of polyp in Leishmania parasites. Furthermore, the study also discusses the characteristics of the bacteria leishmania [6]. Lima Tariqand and Kasim Sakran Abass expressed their research views in a review paper on the lifecycle and virulence of Leishmania. The authors discuss the

characteristics and biology of the parasite leishmania along with its life cycle. The paper also contains a brief discussion on the chemical and biological nature of the organism[7].

Paul A. Bates elaborated on research in a form of a review article on the life cycle of Leishmania. The author discusses the parasite leishmania in detail with a discussion of its sub-types along with an explanation of stages in the lifecycle, the role of the sandfly, and the development of diseases. The study also elaborates on the transmission mechanism of leishmaniasis and the symptoms it causes in a host body [8]. Andreas Damianou et al. explicated research on the importance of deubiquitination in the progression of the Leishmania life cycle. The authors demonstrate the role of deubiquitination in the cellular activities of leishmania about the different pathways it employs in the completion of the life cycle. The research study further elaborates on the proteasome and its association with the proliferation of parasites. The research study comprises bar sequencing and the use of CRISPR – Cas9 in creating null DUB mutants [9].

Santanu Sasidharan and Prakash Saudagar reviewed a topic on Leishmania and Leishmaniasis. The review article discusses the disease leishmaniasis and the human and animal skin infestation by the sand-fly, a parasite-infected fly that causes disease in the human body. Further, the study also elaborates on the past, present, and future conditions of the disease leishmaniasis along with the preventive measures that need to be taken into consideration for disease management [10]. Mohammad Akhoundi et al. proposed their research on the use of Molecular targets in the diagnosis of Leishmania infections. The authors proposed an updated version of the categorization of species of leishmania along with their visible symptoms. Moreover, the paper also provides a list of methods that are presently in use and explains their associated advantages and disadvantages. The authors also provided a genome map as a reference for the diagnosis of the disease [11].

Haroun Zangger et al. expressed their research views on “Detection of RNA virus in Leishmania parasite”. The authors have explicated the patients suffering from “cutaneous leishmaniasis” and the associated symptoms such as the presence of blisters on the skin and more lesions. The study further explains the correlation between the Leishmania RNA viruses with other strains of Leishmania. The objective of the study is to understand the relationship between the leishmania and Leishmania RNA virus [12]. S.M. Wilson proposed their research views on the Detection of leishmania by DNA-based method for applications in fieldwork. The author explains the existing methods for detection via DNA starting with hybridization by employing DNA probes as they use chemiluminescent and are slightly sensitive in comparison to the amplification. Further, the study also discusses the various stages of “polymerase chain reaction” in the “detection of leishmania disease” and its application in the diagnosis [13].

3. METHODOLOGY

3.1.Design:

The primary identification of the leishmania disease was done by staining the sample with a Giemsa- stain smear of the skin. The skin samples were put under the influence of anesthesia followed by “QT- NASBA”, and “qRT- PCR”. The analysis of “Q-RNA” quantitation in vitro requires the addition of the sample before proceeding with the extraction and amplification process. The detection of the samples was done by electrochemiluminescence (ECL)

3.2.Sample and Instrumentation:

The samples were taken from 80 patients from a renowned institute situated in the southern part of India. The sample was taken from the age group between 18 to 60 years with a doubt of CL and a consent written application.

3.2.1. Analysis by QT- NASBA

The technique involves targeting the 18S rRNA 170 base pair region by the use of commercial kits for the process of amplification followed by the use of primers and probes5′-GATGCA AGG TCG CAT ATG AG C CAA AGT GTG GAG ATC GAA G-3′ as a forward primer and 5′-AAT TCT AAT ACG ACT CAC TAT AGG GAG AAG GGC CGG TAA AGG CCG AAT AG-3′ as the reverse primer along with the use of biotin probe("5′-Biotin-GAC CAT TGT AGT CCA CAC TG-3′)". The quantitative in-vitro analysis RNA (Q-RNA) is introduced before the extraction process to the sample as it acts as an RNA competitor for the QT-NASBA assay along with the internal control. Post the process of extraction followed by amplification, the detection of the samples is accomplished by the electrochemiluminescence (ECL) method.

3.2.2. Analysis by qRT- PCR

The procedure involves the use of probes and primers for 170 basepairs and "18s rRNA along with rDNA sequences" on basis of the above "QT- NASBA" analysis. "Same IC Q-RNA was utilized, and TET (5′-tetrachloro-fluorescein) and FAM (6-carboxyfluorescein) reporter dyes on two probes were designed for Q-RNA sequence and wild-type target.

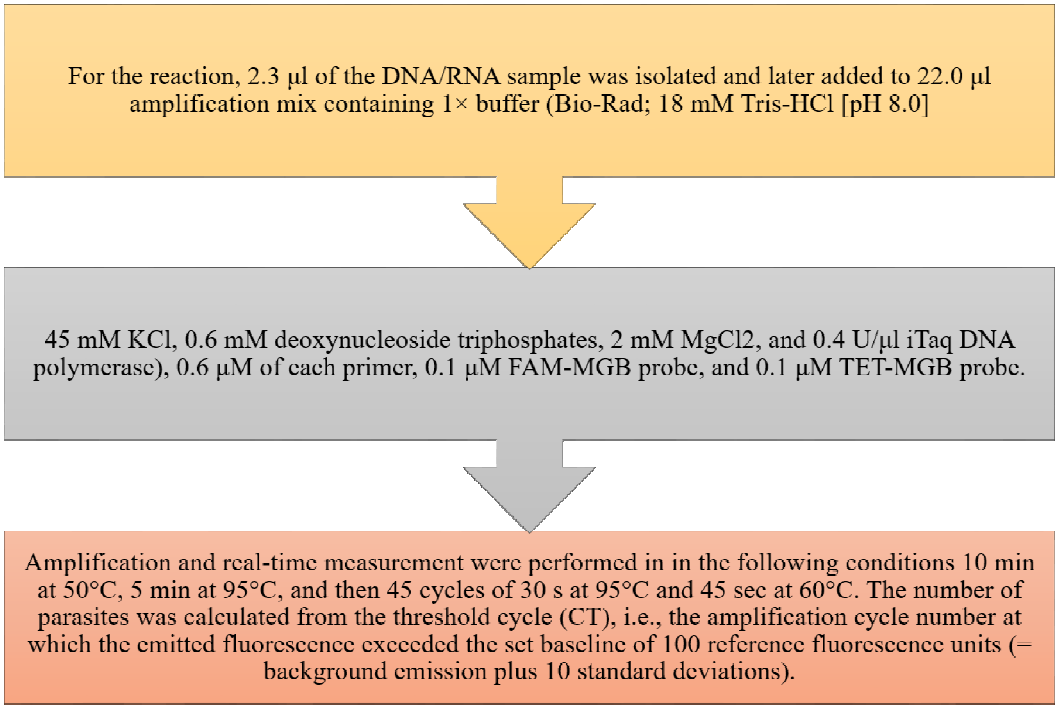

Figure 5: Representing the Stages Involved in the Procedure of qRT- PCR Assay.

The employment of 2 probes with various probes using varied dye reporter, "TET". 5′-C CAA AGT GTG GAG ATC GAA G-3′ was employed as a forward primer and "(5′-GGC CGG TAA AGG CCG AAT AG-3′)" as the reverse primer with the 5′-6FAM AC CAT TGT AGT CCA CAC TGC-NFQ-MGB probe. The reporter dye was constructed for the target which is a "Q-

RNA sequence plus a wild type". The probes were subjected to conjugation with a slight grove binding at the 5′ ends which ultimately leads to affinity with high binding for the target against the probe. Figure 5 describes the procedure of qRT- PCR".

3.3.Data Collection

The techniques qRT-PCR and QT-NASBA are 10-fold more sensitive and detected around 100 parasites per ml. among some samples the QT-NASBA also detected parasites with 10 parasites per ml concentration. Both the assays amplify the rRNA parasite whereas qRT-PCR was able to amplify rDNA as well. As the DNase step was not performed before proceeding with the amplification step, the rRNA parasite copy number was observed to be more than a hundredfold in abundance in comparison to the copy number of the gene. This gives us an idea about the nature of other assays such as qPCR that amplifies only the target DNA.

3.4. Data Analysis

All the 72 CL patients confirmed tested "positive" by the "QT-NASBA" and "qRT- PCR" technique earlier in the management with the count of 25,000 median parasites (ranging from 3 to 4, 650,000) and 5,120 biopsy/ parasites respectively. Observations show that 4 out of 5 patients infected by the parasite were not recognized by the microscopy. On an estimation 8 out of 9 patients with a non-confirmed status who earlier tested negative by the PCR and microscopy, they too recorded negative by "QT-NASBA" and "qRT –PCR". The results that were found to be negative could not be only because of the inhibition as altogether every IC was already positive. Out of them one of the patients with CL non-confirmed was found to be positive in both the assays against the target that is wild type. The patients were treated based on the test results accordingly. Both the techniques are "ten times more sensitive. Some of the samples showed the concentration of parasites to be 10 parasites per ml in QT-NASBA analysis.

4. RESULTS AND DISCUSSION

Few studies have been reported on the applications used for the recognition and diagnosis of the disease leishmania in skin biopsies of humans. Past research studies are required for the examination of the best-suited diagnostic assay to implement in regions that have tagged the disease as "endemic". The present research investigation comprises applications based on RNA ("QT-NASBA" and "qRT- PCR"). The following results were recorded. The concentration of each parasite for the standard curve and the standard deviation was calculated using the formula CV (SD/Avg. × 100%) against the single run. The variation in intra assay was comparatively low in every concentration for parasite with a range of (0.4% to 3.2%) for QT-NASBA. Whereas in qRT- the CV was reported (0.3% to 23.2%). The CV value for QT-NASBA was observed to be low as 6% whereas in the case of "qRT- PCR" was at 95 parasites per ml. the identification of the amplification completed in "QT-NASBA" was done by "ECL", the assay turns the procedure a little expensive. With the addition of molecular tools, such as the use of fluoresce labels in QT-NASBA assay.

5. CONCLUSION

The current study presented above put forth the detection and diagnosis of Leishmania in human skin samples. The method used for the diagnosis is an RNA-constructed method (qRT-PCR and NASBA). In conclusion, both the techniques were found to be reliable and the accuracy level too was found to be satisfactory. The use of ECL detection makes the QT-NASBA technique less

feasible for use. On the other hand, qRT-PCR is convenient in terms of application and easy to operate. Both techniques could be used for studying efficacy and vaccine trials.

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CHAPTER 22

A COMPREHENSIVE STUDY ON POTENTIALLY HEALTH HAZARDOUS EFFECTS OF FOOD ADDITIVES AND PRESERVATIVES FOR HUMAN HEALTH

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ABSTRACT:

During the manufacturing or processing of food, small quantities of food additives may be added to enhance the food's organoleptic properties. These attributes include color, flavor, aroma, appearance, taste, and texture. It is necessary to utilize additives to generate and maintain consistency and quality, to improve or maintain the nutritional content, to increase or maintain the wholesomeness and deliciousness, to leaven yeast, to regulate the pH, to enhance taste or color, and to impart color. It is common knowledge that some substances may have negative effects on the human body. If we eat an abnormally high level of food additives over an extended period, this might have a detrimental effect on our health. Ingestion of food additives is related to some possible bad health effects, some of which include allergic responses, cancer, damage to the brain, and hyperactivity. The purpose of this study is to investigate not just the many ways in which people both food manufacturers and consumers put chemicals to use, but also the myriad ways in which people are affected by these additions. Negative side effects, including allergic reactions, intolerances, cancer, hyperactivity, brain damage, nausea, and even heart disease, have been documented as a result of taking some medications.

KEYWORDS:

Allergy, Flavor, Food Additives, Food Preservatives, Nutrients, Organoleptic properties.

1. INTRODUCTION

Food may be broadly defined as any item or material that is consumed, either to provide the body with nutritional support or for enjoyment. Any material, usually derived from plants or animals, that is ingested and processed by an organism to supply energy, promote growth, and keep life continuing is considered a nutrient. Food chemistry is the scientific discipline that investigates the chemical processes and interactions of all the food's components, both living and nonliving [1].

Additives are substances that are put into food for one of two reasons: to improve the flavor, consistency, coloring, or synthetic preservatives, taste, or appearance, or to serve as an assistant in the preparation of the food. The term "food additive" refers to non-nutritive compoundsthat are inserted on purpose into foods, often in very minute amounts, to enhance the meal's appearance, flavor, texture, or preservation characteristics [2].Additives are compounds that are purposely added to food by food producers in tiny quantities during the preparation or handling of food to improve its organoleptic properties [3].By ensuring that the food remains uniform, healthful, and fresh, they assist to extend its shelf life. By eliminating the need for

regular grocery shopping and cooking, they provide a wide variety of easy foods. All food additives must be introduced in controlled amounts and concentrations within Acceptable Daily Intakes (ADIs), otherwise, they may have detrimental impacts on the consumer's health and well-being.

In foodstuffs, preservatives and additives have been around for a long time.Barbecuing creates compounds with antioxidant and antibacterial characteristics, such as butyl gallate and butylated hydroxyl anisole (BHA).Salt has been employed for preservation for millennia. Salt increases the shelf life by reducing water activity (aw) and preventing bacterial development in meats and other foods. Microorganisms, such as fungi and bacteria, may thrive in food that is too wet. Acids like vinegar are used in pickling to reduce the pH of food to a level that inhibits bacterial development. Antioxidant-rich herbs and spices like cinnamon, chile, and curry, for example, may have bactericidal properties.

1.1.Classification of Food:

Agriculture, the practice of raising crops, raising livestock, and fishing for sustenance, has been practiced by humanity for thousands of years. In the food business, run by multinational companies and using intensive farming and industrial agricultural practices,the bulk of the food energy required by the world's population is currently supplied by crops. Plant and animal-based foods make up the vast majority of our diets. There is no other crop that supplies as much dietary energy as cereal grains do.87 percent of the world's grain output is comprised of three crops: corn, wheat, and rice. Many edible fungi, such as mushrooms, may be found in the wild and can be eaten:Fermented and pickled foods include leavened bread, alcoholic drinks, cheese, pickles, kombucha, and yogurt, as well as blue-green algae such as Spirulina. Inorganic compounds such as baking soda and cream of tartar may be employed to chemically alter a substance [4]. A wide variety of plants and plant components are used in culinary preparations. Food-growing plants number in the thousands, with many having dozens or even hundreds of different varieties. A valuable source of nourishment for animals, particularly humans, is the seed of a plant because it contains nutrients essential for the plant's early development, including numerous beneficial fats, such as omega fats. Seed-based foods make up the bulk of the food people eat daily. Cereals (like rice, corn, and wheat), and nuts are all edible seeds, and legumes (like beans, peas, and lentils). Rich oils such as sunflower, flaxseed, rapeseed (especially canola oil), or sesame are commonly produced by pressing oilseeds. Additives can only be allowed provided they don't deceive customers, have a clear technical purpose (such as improving food stability or preserving nutritional value), and don't compromise the food's quality.

2. LITERATURE REVIEW

B. Linke *et al.* stated in their study that some foods' inherent qualities are lost in the manufacturing process, thus food additives are employed to make them last longer on the shelf and/or improve their other properties. Modern living has led to an increase in the food industry's usage of food additives. Despite their widespread usage, these drugs are no different from any other medication in that they might have negative side effects. Sodium benzoate is a food ingredient that has beenassociated with several health issues, including cardiovascular illness and cancer. This chemical preservative is sodium salt, and it is found mostly in manufactured drinks/beverages, such as soda. The consequences of sodium benzoate on human health are still being debated, although major regulatory authorities deem it harmless [5].

Konstantinos Gerasimidis *et al.* conducted a study that the influence of dietary effects of additives, artificial sweeteners, or home cleaning agents on gut flora and fiber fermentation capacity. In their study, feces of Thirteen healthy participants were fermented in fermentation media using dietary additives and home hygiene products. Gas chromatography was used to determine the amount of short-chain fatty acids produced. The makeup of the genome was determined using 16S rRNA sequencing and quantitative polymerase chain reaction (PCR). Dishwashing detergent or sodium sulfate, on the other hand, had the opposite effect on the concentration of acetic acid. This work adds to the knowledge of how gut microbiota composition and fiber metabolic activities may be affected by additives, which have several impacts on human health [6].

Dr. Serap Kayisgolu and Dr. Fatma Coskun discussed in their study that this research surveyed consumers on food additive knowledge and safety. In June and July 2015, 300 residents in the heart of Tekirdag province were surveyed concerning food additives. Before the survey began, it was tested with ten persons for clarity and validity of questionnaire items. Data were analyzed using SPSS ver18 and Chi-square (2) test.Food additive knowledge and respondents' professions were correlated. Education level was also correlated with food additive knowledge. Gender and controlling food packaging labels during shopping had a significant correlation. 70% of people who read labels are controlled by food additives. Education and income boosted women's food safety awareness. According to study findings, consumer and government collaboration is needed for effective food additive use [7].

Chinaza Godswill Awuchi *et al.* stated in their study that the research looked at food additives and preservatives, their health advantages, kinds, and safety requirements, including flavor ants. Food additives protect or improve food's flavor, appearance, taste, or other properties. The systematic study analyzed food additive updates and developments in food businesses and residences.Current food additives include emulsifiers, colorants, flavorings, micronutrients, or preservatives. Food preservatives suppress yeast, bacterium, and mound development. Intentional or inadvertent food additives may be applied. Foods could include human growth hormones, pesticides, antibiotics, etc. utilized in animal or plant agriculture. The research gives extensive and simple data on the primary food additives used in households in the food business to preserve flavor or enhance appearance, taste, or other factors [8].

3. DISCUSSION

Natural foods are foods that have not been processed in any way and do not include any additives of any kind, including but not limited to preservatives, artificial colors, chemicals, fillers, or artificial flavors. The greatest and healthiest source of nutrients may be found in natural foods. Additives are substances that are put into natural foods to retain their flavor and extend their shelf life. These substances are referred to as food additives. It is necessary to use additives and preservatives in food that are going to be kept in storage for an extended length of time to keep the food's quality and flavor intact. Due to the presence of excess water in the meals, the chemicals and preservatives stop the development of harmful bacteria and fungi [9].Food additives can have immediate effects or long-term consequences depending on how often they are consumed.Acute repercussions may include headaches, effects on energy levels, and shifts in focus, behavior, and immune response. The long-term use of food additives has also been linked to increased cancer risk. Allura Red (E129), Sodium benzoate (E211), Tartrazine (E102), Quinoline Yellow, and Carmoisine are only some of the most common food additives.

3.1.Food Additives Are Classified According To Their Function:

There are a few categories that may be used to classify food additives, even if there is considerable overlap between these categories:

3.1.1. "Antimicrobial agents":

These prevent food from spoiling due to germs. In addition to both vinegar and salt, foodstuffs like baked goods, salad dressings, cheeses, kinds of margarine, and pickled foods contain components like calcium propionate and sorbet acid [10].

3.1.2. Antioxidants:

By preventing oxidation, anti-oxidantspreserve fat-based foods, improve their flavor, and even slow the aging process. An anti-oxidant mustn't impart any unpleasant flavors, aromas, or colors to the fat or food it's in.As food additives,antioxidants aid in the preservation of food. Because bacteria thrive in an oxygen-rich environment, antioxidants serve as oxygen scavengers. Unsaturated fats oxidase in the lack of antioxidant food additives, resulting in food with a bad odor and color [11].

3.1.3. Flavors:

Foods may be given a distinct flavor or aroma by adding flavorings. Spices, herbs, and other substances with a primarily sweet, sour, or salty flavor are examples of natural food flavorings. Additives that improve the flavor: Flavor enhancers are substances added to food to bring out more of the flavor already there. The chemical senses of flavor and smell are principally responsible for determining a meal or other substance's flavor [12].

3.1.4. Coloring Agents:

Color retainers, color stabilizers, color fixatives, and other similar substances fall within this category. They are made up of colors that are both synthetic and derived from natural sources. Even though the majority of colors do not contribute in any way to the nutritional content of meals, the majority of customers will not purchase or consume specific foods if they do not have particular colors. Therefore, colors are commonly added to replace the natural ones that are lost during the processing of foods or to provide a natural color to the preparation [13].Food additives may also be made from a variety of natural food colors that have been taken from seeds, flowers, insects, and even foods themselves.Bixin, a well-known and frequently used red pigment, is derived from the seeds of the lipstick pods plant, also referred to as Bixa Orellana. This plant is native to South America.There is no proof to substantiate the hypothesis that bixin is carcinogenic.

In addition, margarine uses a carotene obtained from carrots as a yellow colorant. Saffron has long been used as a culinary dye because of its flavor and color-giving qualities. Curries, various meat products, and salad dressings all benefit from the addition of turmeric to their color. As a food ingredient, cochineal (also known as carnum), the female insect's excrement, grape skin extract, or caramel, the brown color generated from burned sugar, are some of the natural colors [14].

3.1.5. Decolorizing agents (Bleaching):

In the process of bleaching foodstuffs like wheat flour as well as cheese, peroxides are used.When freshly milled flour is exposed to oxygen in the air, it begins to age naturally, although this may take up to two months. Commercial mills use bleaching chemicals and other chemical additions to make their flour white and ready to consume as soon as it is milled.

3.1.6. Chelating agents:

Antioxidants do not include chelating agents. These are scavengers of metals that aid in the oxidation of other materials. Antioxidant levels between about 100 and 200 ppm are usually suggested for citric acid use (0.1 and 0.3 percent). Chemical preservatives such as EDTA, a chelating agent used in the food sector, are allowed on the market. Calcium disodium has been approved for use by the Food and Drug Administration (FDA). Ethylene diamine tetraacetic acid (EDTA) and disodium EDTA as food additives. Tartaric acid, Citric acid, and Malic acid are instances of chelating compounds used to prevent food discoloration, flavor alteration, and rancidity throughout preparation [15].

3.1.7. Supplements with nutrients:

Nutrient supplements replenish nutrients that have been depleted during production or storage, or they guarantee that the body receives more nutrients than it would have received from food alone. Some nutrients could be lost during the processing of food, and additives could be employed to make up for the lost nutrients. For example, to strip away the grain's vitamin and mineral-rich dark color, wheat is processed to make white flour. The flour is fortified with Calcium, thiamine, nicotinic acid, and iron. When citrus fruits are canned, they get an injection of vitamin C to make up for the nutrients lost during processing [16].

3.1.8. Stabilizers:

It is applied to the cuisine to give the dish a more defined body and to smooth out the consistency of the food that has already been prepared. Stabilizers are chemicals or substances that enable food components, which do not mix well, to stay in a homogeneous form after mixing. Food stabilizers are added in very modest amounts, which makes the impact of emulsifiers worse [17].

3.1.9. The thickeners and gellers:

Food preparations may benefit from the use of thickening agents, also known as thickeners, which are compounds that can enhance the viscosity of the food without affecting other qualities, such as flavor. If you add a food thickener or a thickening agent to a beverage, the thickening will absorb some of the liquid, which will cause the beverage to become more viscous [18].

3.2.Food additives may affect human health:

The rising demand for fresh food items that are already prepared to consume has presented food distributors with several issues in terms of maintaining the quality and safety of the meals they sell [19].Numerous forms of preservatives are used in the production of many of the foods that are sold in stores. These compounds have been linked to a variety of adverse health effects. The continually excellent quality of meals is maintained by the use of various additives.

Additives are known as food preservatives, and their primary function is to stop the development of microorganisms like yeast, mold, and bacteria in food.Some additives are sourced from natural sources such as corn, beets, and soybeans, whilst others are created artificially by people and hence classified as man-made additions.The ever-increasing demand for fresh food items that are ready to eat has presented food distributors with some issues concerning the security and quality of the meals they sell. Artificial preservatives provide a solution to some of these problems since they extend the amount of time that freshly may be maintained, but these preservatives also have the potential to have unintended consequences. Meats including lunch meats, hams, sausages, hot dogs, and bacon are preserved using sodium nitrite to prevent botulism [20].

1. Synthetic food dyes have been linked to several health problems, including asthma, hyperactivity, and even cancer.
2. Nitrosamines, which have the potential to cause cancer, may be formed in the body from nitrites and nitrates.
3. Sulfites, also known as sulphur dioxide, have been linked to asthmatic and allergic symptoms.
4. Obesity, tooth decay, diabetes, hypoglycemia, increased triglycerides (blood lipids), and Candida (yeast infection) have all been related to sugar and artificial sweeteners.
5. Saccharin and Aspartame are two examples of artificial sweeteners that have been linked to a variety of negative side effects, including hyperactivity, behavioral issues, and even the development of cancer. Children and women who are pregnant are especially discouraged by the authorities from using any kind of artificial sweetener.
6. Headaches, dizziness, or difficulty breathing are just some of the frequent allergy and behavioral problems that may be brought on by monosodium glutamate (MSG).
7. Some preservatives have been linked to allergic responses, hyperactivity, and even the development of cancer, while BHT has the potential to be harmful to both the nervous system and also the liver.
8. Artificial flavors have the potential to provoke allergy or behavioral responses both fluid retention and elevated blood pressure have been linked to salt consumption.

Food additives and preservatives are required for long-term preservation; yet, they have been linked to a variety of negative health effects. Some persons who are sensitive to certain chemicals may develop a variety of allergies as well as disorders like hyperactivity and attention deficit disorder as a result of being exposed to them.These responses might include rash, nausea, headaches, heart palpitation, warts, as well as a deterioration of eczema. Asthma and hay fever are two of the conditions that can be triggered by meals that contain chemicals [21].One should steer clear of foods that include additives and preservatives to reduce the likelihood of acquiring health issues as a result of their use of food additives and preservatives. Checking out the contents of the can of food is a necessary step before making the purchase. Users should consider purchasing organic foods since they do not include any man-made ingredients. Instead of eating meals that have been processed or canned, you should make an effort to consume as many freshly made foods as possible.

Food additives could have instant or long-term impacts, depending on the amount of consumption or accumulation one has. Headaches, a drop in energy, and changes in mental focus, behavior, and immunological response are all possible side effects.Because of the long-term impacts, a person's likelihood of developing cancer, cardiovascular disease, and other degenerative disorders may rise. It has recently come to light that several contemporary synthetic preservatives are linked to a variety of adverse health effects, particularly those affecting the respiratory system [22].

3.3.Additives in Food and the Risk of Malnutrition:

One of the most significant dangers presented by chemicals is the reduction in the nutritional content of the foods they are added to, which may lead to unhealthy diets and even a milder form of malnutrition. The widespread use of food additives may lead to malnutrition in the following ways; the majority of foods that include additives have a high level of salt, sugar, and fat; this is the common factor among all of these items [23].Pure sucrose, by principle, does not include any micronutrients at all and just contains calories; in contrast hand, fat contains relatively few nutrients and has a very high-calorie content. Furthermore, the majority of foods that include additives are processed foods; as a result of the processing technique, these foods have lost a significant amount of the nutritious content they once had.Some vitamins and minerals are occasionally given after processing, however, the ratio of vital elements to calories is frequently fairly insufficient, resulting in a high caloric consumption but poor nutritional content. As a consequence of its high caloric and poor nutritious content, this diet might result in subclinical or marginal malnourishment.

3.4.Side effects of several common food additives and preservatives:

Human and animal studies have demonstrated that certain food additives and preservatives may have negative impacts on health.

- *Tartrazine:*

In food, cosmetics, and pharmaceuticals, 7.5 mg/kg BW of azo pigment may be used as an acceptable daily intake (ADI) for its usage as a colorant. Tartrazine, on the other hand, may have some negative side effects if used for an extended period or in overabundance [24].For the first time, Mpountoukas *et al.* have shown that tartrazine may cause genotoxic damage in human lymphocytes [25].Because of the possibility of toxicity, it is of the utmost importance to exercise control over the amount of tartrazine that is included in food products. As a result, analytical methods must be developed that can evaluate the level of tartrazine exposure experienced by the general population. Chromatography, spectrophotometry, and electroanalytical procedures are only some of the methods that have been described so far as being able to identify tartrazine.These approaches are, however, costly, time-consuming, or difficult to use regularly, making them unsuitable for frequent broad monitoring of the chemical tartrazine. A more suitable option would be an enzyme-linked immunosorbent test (ELISA), which has the advantages of high sensitivity, speed, and low cost.

- *Nitrites and Nitrates:*

Meta-hemoglobin, a chemically-altered form of hemoglobin, is formed when nitrate attaches to hemoglobin (the component that delivers oxygen in the blood to tissues in the body), culminating in the blue hue of the skin. Increased exposure to nitrates and/or nitrogen dioxide (NO2) was

shown in some studies to be linked to an increased risk of malignancy in adults, as well as a probable increase in the chance of brain and tumors, as well as nasopharyngeal malignancy in children.After drinking water polluted with nitrates and nitrites, some people have developed methemoglobinemia, which is also known as the "blue baby syndrome." This condition is characterized by cyanotic symptoms, which indicate an oxygen deficiency, and it leads to decreased hemoglobin oxygenation, which in turn causes decreased oxygenation of the blood.

- *Artificial Sweeteners:*

Some food additives have side effects that are related to their sweetness, which makes them easy to employ by both food manufacturers and consumers alike. This review considers Saccharin, Aspartame, Sucralose, and Neotame as sugar substitutes.

- *Vinegar:*

There have been reports of esophageal damage caused by the use of a vinegar table, and since vinegar products supplied for medicinal reasons are not controlled or standardized, their volume, pH, and other characteristics vary greatly. One documented incidence of hypokalemia, hyperreninemia, and osteoporosis may have been caused by long-term excessive vinegar use.

- *Saccharin:*

After the warning was removed, the dangers of ingesting items containing saccharin remain. After NTP decided to remove saccharin from its list of probable carcinogens, the “Center for Science in the Public Interest (CSPI)” released a paper in 1997 stating that saccharin is still a potential carcinogen.Saccharin, a sweetener in certain newborn formulae, may also contribute to hyperactivity and muscular dysfunction in babies. Saccharin is still widely believed to be harmful to youngsters and pregnant women for these reasons. The FDA has not placed any restrictions because of the lack of data to back up these claims. Saccharin-induced bladder cancer was also discovered to be unique to rodents based on their physiology.

- *“Anti-caking agents”:*

Food additives known as "anti-caking agents" keep ingredients from clumping together and becoming unpleasant to the food product from becoming lumpy or clumped together after it has been packed. Anti-caking chemicals may either work as a moisture absorber or as a sealant, preventing water and oil from penetrating the substance. Anti-caking agents developed of metalbentonite, cellulose, antimycotic chemicals, mesh vegetable flour and bacterial cultures are all examples of ingredients, and they enhance the performance of cheese by reducing the adhesiveness of chunked, diced, or shredded cheese. Additionally, anti-caking agents reduce the likelihood of cheese becoming moldy. Anti-caking chemicals provide other benefits, including inhibiting the development of yeasts and molds.

4. CONCLUSION

Preservatives are added to foods to extend the amount of time that they can be stored without losing their quality and to extend the amount of time that they can be stored. Reactions between synthetic food additives and the biological components of the body may result in a wide variety of gastrointestinal problems (effects).Avoiding foods that contain food preservatives and other additives is the best way to reduce the chance of acquiring health issues as a result of these chemical preservatives. Investigate the ingredients of the canned food before buying it. To avoid

artificial additives and preservatives, stick to organic foods.They are added to these dishes to hide the low quality of the components that they contain. If consumers eat significant quantities of foods that contain these compounds, they run the risk of experiencing the harmful consequences of the additives. The immune system is exposed to a significant amount of danger as a direct result of the ever-increasing usage of a large number of food additives in the everyday life, which may result in a variety of ailments and diseases.Research on this complex and crucial component of the nutrition-immune system interplay will lead to the identification of numerous treatments to reverse these adverse effects as well as the development of future food additives that will include a diverse selection of healthful options.

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