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Contents

Comparison Of The Effectiveness of rbcL and matK in Amplifying The Genome of Diospyros

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ABSTRACT

Ebenaceae is a large angiosperm family that includes many endemic species; one of the genera is Diospyros. The aim of identified chloroplast genome marker in the Diospyros family is to enable precise species identification by analyzing a unique DNA sequence of a target gene. The current study was carried out to create a marker for different species of Diospyros growing in South Sulawesi. Three putative species of Diospyros (each represented by specimens collected from two districts in South Sulawesi) were evaluated using two regions in the chloroplast genome (matK and rbcL) in order to discriminate them at the species level. Results showed that matK yielded 891 bp after alignment. However, there was no precise identification to species level. The combined rbcL gene showed 100 percent amplification in three species, but combined MatK gene just 50 percent showed amplification. Considering the overall performance of these loci, we suggest the rbcL gene for amplification and matK + rbcL combination may amplify to determine Diospyros growing in South Sulawesi to the species level as distinguished on morphological grounds. These findings show the necessity of finding other candidate genes or markers that can potentially help delineate the various species of Diospyros and other related Diospyros genera. This study is very needed, especially for endemic.

Keywords: Chloroplast, Diospyros, Genome, matK, rbcL

Introduction

Diospyros celebica is one of the genera having the highest species number in Family Ebenaceae and also becomes one of the largest Genus in Angiospermae. The Genus has more than 500 species (1). Indonesia is the endemic region of Genus Diospyros. Bakh is an endemic species to Sulawesi (2). This species has been widely found in South Sulawesi, Central Sulawesi, and West Sulawesi. There are also ten other Diospyros species distributed in Central and Eastern Indonesia, i.e., Sulawesi, Bali, Nusa Tenggara, and Maluku.

The previous exploration by (3)showed eight Diospyros species found in Tangkoko nature preservation (D. minahassae, D. pilosanthera, D. cauliflora, D. maritima, D. hebecarpa, D. malabarica, D. korthalsiana, and D. ebenum). There are also D. buxifolia and D. javanica that thrive in North Sulawesi. Information from the Natural Resource Conservation Center of South Sulawesi (Sulawesi BKSDA) through direct communication stated that exploration by the Indonesian Institute of Science (LIPI) observed two species of Diospyros in Lohe Island, D. nigra and D. foliosa (Rich. Ex A. Gary) Bakh.

Phylogenetic and barcoding analyses of endemic Diospyros in Indonesia have never been studied efore. Phylogenic analysis of species in the same Genus can be performed using a DNA barcode. The DNA barcode can provide information on nucleotide sequences from a specific region that can be used for identifying an individual (4).

DNA barcoding is an effective method for identifying and documenting the species in the axonomy group (5). It also has been applied for analyzing Genus Diospyros in New Caledonian, which is located in Southwest Pacific (6). There are two main genes used by the DNA barcode consortium in plant identification, i.e., matK dan rbcL (7). Both genes are chosen due to their ability to distinguish individuals. They are from the chloroplast genome and located in the coding region (8).

Universal primers used in DNA barcoding (matK and rbcL) need to be screen in order to determine the suitable primers for phylogenetic analysis between species in Genus diospyros that thrive in a tropical area, such as Indonesia. The objective of the study was to evaluate and compare the success rate of matK and rbcL in amplifying DNA for barcoding and Phylogenetic analysis of Indonesia's endemic Diospyros.

II. MATERIALS AND METHODS

A. Genetic Material and DNA extraction

The samples of Genus Diospyros used in the study were collected from different areas. D. malabarica was from the orchard collection area of the Regional Tree Seed/Seedling Office (Sulawesi BPTH), D. buxifolia was collected from Kalaena natural preservation, D. macrophyllawas from Ponda-Ponda natural preservation, and D. celebica was from Poso, Central Sulawesi. Leaf sample collection of D. buxifolia and D. macrophylla in natural preservation areas were conducted after obtaining the permit from Sulawesi BKSDA. The leaf samples were then extracted using DNeasy Plant Mini Kit DNA extraction protocol (Qiagen) (9) with little modification by adding RNase solution into the DNA at the last step of extraction.

B. Primer Selection and DNA Amplification

Two chloroplast primers used in the study were rbcL and matK (four rbcL, four primers,and seven matK primers). From these primers, we obtained four rbcL primer and 12 matK primer combinations. Sixteen of the total rbcL and matK combinations were used in the study. Primer names and sequences are presented in Table 1 based on information from (10). Primer screening was performed to obtain information on whether primer pairs can amplify the DNA samples and determine the suitable annealing temperatures (11).

The DNA was amplified using PCR (Sensoquest Thermocycler) machine. PCR amplifications were conducted using the following steps: one cycle of pre-amplification at 95°C for 3 minutes, 35 cycles of amplification steps at 95 °C for 30 seconds (template denaturation), annealing temperature for 50 seconds (primer annealing), and 72 °C for 1 minute (primer extension), and one cycle of final extension at 72 °C for 5 minutes and then stored at 4°C. During the primer screening process, gradient temperature was performed using ±5°C from the given melting temperature in order to obtain the suitable annealing temperature (Ta).

The amplification products were then separated using horizontal electrophoresis on 2% agarose and TBE (1x) at 100V for 80 minutes. Agarose dyeing was done by adding GelRed (biotium) right after agarose dissolve completely. The visualization was performed by Geldoc (Biostep Gel Documentation System). To measure the amplified products, we used a DNA ladder of 50bp (bioline).

III. RESULTS AND DISCUSSION

Three out of four rbcL primer (75%) combinations could amplify DNA of Genus Diospyros and then used in the sequencing process, whilst only three out of 12 matK primer combinations (25%) were able to generate clear bands; thus, a total number of primer pairs used in this study was six primer pairs. The primer pair that could amplify and generate clear bands are described in Table 2.

There were one species out of four evaluated Diospyros species that showed unsuccess amplification using matK4. RbcL primers were able to amplify all evaluated samples (Figure 1). It indicated that rbcL has a higher success rate of amplification on Genus Diospyros than matK primer.

This study presents similar results to previous studies that analyze plants from different tropical regions. A previous study on 2052 samples, representing 655 species, 259 genera, and 76 families from a tropical rain forest area in Xishuangbanna, China, reported that rbcL managed to amplify up to 98% of the all evaluated samples, whilst matK amplified only 90% (12). It was similar to the study by (13).

They observed that the rbcL sequences only confirmed five seagrass species out of seven morphologically identified species, and the sequences generated from this study cannot discriminate Halophila ovalis and H. minor.

A study by (14) used five DNA barcodes (rbcL, matK, trnH-psbA, ITS, ITS2) were evaluated for species identification ability across 669 samples representing 314 species and 100 genera in the Arecaceae. Among the four analyses used, the barcode combination ITS2 + matK + rbcL gave the highest resolution among all single barcodes and their combinations, followed by ITS2 + matK. Among 669 palm samples analyzed, 110 samples (16.3%) were found to be misidentified. Those studies indicated that rbcL has a higher success rate than matK in amplifying the DNA. It is not only observed on Genus Diospyros in the tropical area but also on different Genus in other tropical forests and arid areas. The rbcL showed the best performances: the greatest amplification success, the best sequencing performance both in terms of the number of sequences obtained and in terms of quality of the sequences obtained (15)

The sequence of rbcL is more universal so that it is easier to amplify the evaluated DNA samples and has a higher success rate of the amplification process. Moreover, this primer is also easier to sequence yet has a lower ability to distinguish the individuals (16). On the other hand, matK has a lower success rate on amplification but a higher accuracy level in distinguishing the individuals at the species level. Study in the Genus Amentotaxus, consisting of five or six species, is confined to South China, Northeast India, Laos, and Vietnam, the species discrimination rate increased for all two-barcode combinations, except for matK+trnH-psbA (17).

DNA barcoding studies in plants use one or more plastid regions, such as rbcL and matK, as well as the non-coding spacer trnH-psbA and ITS (Internal Transcribed Spacer) nuclear ribosomal DNA (18–22). Figure 2 presents that matK4 was unable to amplify the D. macrophylla species. A similar result also observed in a previous study by (23) matK used in that study was failed to amplify one of the evaluated species, Viscum articulatum. The unsuccessful amplification of D. macrophylla samples using matK is due to the different sequences of primers with DNA templates. MatK has large sequence variation so that half of the binding site sequence from this species are different, which eventually lead to amplification failures (19).

The development of DNA barcodes is very needed, especially for endemic species. Research (24) results show for the first study to report a strategy for developing specific DNA barcodes of Orchidaceae plants, laying the foundation for the conservation, evaluation, innovative utilization, and

protection of Orchidaceae germplasm resource. Their work highlighted the potential of the barcoding approach for the rapid identification of plant species in order to solve taxonomic disputes and support the commercial traceability of floreal products. In the study of rice report, The six rice-specific chloroplast barcodes revealed that 17% of the 53 seed accessions from rice seed banks or field collections were mislabeled. These findings are expected to improve rice biodiversity by clarifying the concept of rice species, assisting in the identification and use of rice germplasms, and promoting rice biodiversity(25).

IV. CONCLUSION

This study showed universal primer of rbcL and matK genes could be used for amplifying Genus Diospyros's DNA. The success rate of amplification of rbcL was higher than matK. As many as 75% of rbcL were able to amplify all DNA samples, whereas matK only amplified 25% of the total samples. The primers that were successfully amplifying the samples showed sharp bands and suitable for gene sequencing.

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TABLES

Table 1. rbcL and matK primers name and sequence for primer selection of Diospyros

Gene	Primer name	Sequence
rbcL	rbcL724R	5'-TCG CAT GTA CCT GCA GTA GC-3'
	rbcL1F	5'-ATG TCA CCA CAA ACA GAA AC-3'
	rbcL1460R	5'-TCC TTT TAG TAA AAG ATT GGG CCG AG-3'
	rbcL636Fn	5'-TAT GCG TTG GAG AGA CCG TTT C-3'
matK	matK1300R	5'-CGA AGT ATA TAY TTY ATT CGA TAC A-3'
	matK800F	5'-CAT GCA TTA TGT TAG GTA TCA AGG-3'
	matK1710R	5'-GCT TGC ATT TTT CAT TGC ACA CG-3'
	matK1070F	5'-CCA TAG TTC CAA TTA TTC CTC TG-3'
	matK1900R	5'-ATT CGA GTA ATT AAA CGT TTT ACA A-3'
	matK55F	5'-CCC CCA YAT ATT TGA TAC CTT CTC-3'
	matK880R	5'-CCA GAA ATT GAC AAG GTA ATA TTT CC-3'

Source : Duangjai et al. 2009

Table 2. Primer and band quality generated by each selected primer

No	Primer Combination	Ta (°C)	Band
	Primer rbcL		
1	R1 rbcL724R rbcL1F	55,9	Clear
2	R3 rbcL1460R rbcL1F	60	Clear
3	R4 rbcL1460R rbcL636Fn	55,9	Clear
	Primer matK		
4	M1 matK300R matK800F	55	Clear
	M4	55,9	Clear
5	matK1710R matK800F		
	M5	54,2	Clear
6	matK1710R matK1070F		

FIGURES

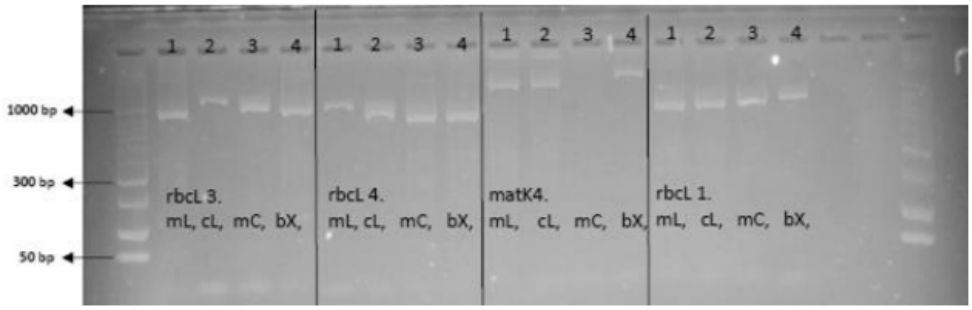

Figure 1. Presents that Genom chloroplast markers amplify the Diospyros genus.

Notes :(1) mL : Diospyros malabarica

(2) cL : Diospyros celebica

(3) mC : Diospyros macrophilla

(4) bX : Diospyros buxifolia

A review on the localization of drugs by utilizing monoclonal antibodies

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ABSTRACT

Monoclonal antibodies are developed from the cell which may be a single cell or a complete cell line. They were firstly discovered by a scientist named Earlich in the time period of 20thcentury. At that time these antibodies are known as the bullets of magic hunting the toxicsubstance throughout the body as these magic bullets can circulate in the blood freely. This technology continues to develop from time to time and being used in many other fields of medical sciences like oncology, molecular biology, immunology, cytology, now a day monoclonal anti-bodies are being widely used in the targeted drug administration in the cancer treatments. Most of explicit mono clonal anti- bodies responding to human neoplasms being created by inoculating mouse with arrangements of cancerous cell lines hybridoma exuding mono clonal anti-bodies are produced by combining B lymphatic cells from such vaccinated mouse with cancerous plasma cell called myeloma the defined hybridoma when isolated from uncombined B lymphatic cells and cancerous cells filtered to distinguished singular hybridoma duplicates which discharge a cancerous responsive mono clonal anti-bodies perceiving a cancerous cell antigen. Mono-clonal anti-bodies offer the open door for particular conveyance of a scope of against neoplatics operators to cancer. In creature models investigation noteworthy anti-cancerous impacts have been exhibited utilizing mono-clonal anti-bodies to specifically covey hostile to cancer medications. Poisons radio nuclides and proteins pro drugmixes. Anti-bodies may likewise offers the chance of specifically conveying different specialist of possible use in malignancy treatment radio nuclide and protein and pro drug.

INTRODUCTION.

Monoclonal antibodies are developed from the cell which may be a single cell or a complete cell line. They were firstly discovered by a scientist named Earlich in the time period of 20th century. At that time these antibodies are known as the bullets of magic hunting the toxic substance throughout the body as these magic bullets can circulate in the blood freely.

This technology continues to develop from time to time and being used in many other fields of medical sciences like oncology, molecular biology, immunology, cytology, now a day mono clonal anti bodies are being widely used in the targeted drug administration in the cancer treatments. The Foundational organization of the low sub- atomic mass of anti-cancer medications for the patients having persistent tumors resultantly particular appropriation by themedication within the typical tissue relatively then the tumor as many persistent tumors are inadequately vascularized contrasted with majority of typical tissue.

The selectiveness of many anti neoplastic medications within medical utilized consequently depends at the way that they destroy the multiplying cells. In any case two significant downsidein utilizing them as a reason for refinement right off the typical tissues having multiplying cells community and the decimation of the prompts portion constraining toxicants having the mostanti cancerous medications in medical uses. Furthermore the portion of multiplying cells whenever in most persistent tumors is regularlyexceedingly low and in this way such factor justdestroys a little level ofthe cancerous cells rehashed organization of these factors are in this way needed to execute a noteworthy amount of cancerous cells.

The point of medication focusing on is to restrict specifically neo plastic factors present on neoplasm position and along these lines saves typical tissues from damage. Like specific localization ought to permits the utilization of factors, that destroy the couple of multiplying and calm populace in the neoplasm.

After developments in this filed the concept of heptophore is define by the scientists as this is section of the toxin or a molecule, which has ability to tie to a cell or to additional attachment station which can be a receptor, presently everywhere now again recorded regarding the hypothesis. Heptophore which conveyed a toxophor, specifically to a cyst introductory endeavors to incorporate this idea of utilizing an assortment of focusing the factors as the heptophore in particular regarding the poly clonal antisera whereas showings the achievability of this idea in trial model having minimal medical effect (1).

The approach of hybridoma innovation during the 1970's has brought about the age of countless mono clonal anti bodies coordinated against cell surface antigens specifically displayed on cancerous cell. This promoted a rise in enthusiasm of drug focusing on utilizing monoclonal anti bodies. We will discusssome ofthe strategies oftargeting the mono clonal antibodies regarding to this benefits and restrictions by different approaches (2).

Tumor specified monoclonal antibodies:

Most of explicit mono clonal anti bodies responding to human neoplasms being created by inoculating mouse with arrangements of cancerous cell lines hybridoma exuding mono clonal anti bodies are produced by combining B lymphatic cells from such vaccinated mouse withcancerous plasma cell called myeloma the defined hybridoma when isolated from uncombinedB lymphatic cells and cancerous cells filtered to distinguished singular hybridoma duplicates which discharge a cancerous responsive mono clonal anti bodies perceiving a cancerous cell antigen. For recognizing the tumor explicit mono clonal anti bodies the resulting hybridoma discharging cancerous responsive for screening of mono clonal anti bodies opposing typical tissue of human. Numerous hybridoma from a solitary splenic combinations are prone to be cancerous receptive however scarcely any will be sarcoma specific these only react when antigen is available at tumor as well as typical tissue (3).

Monoclonal anti bodies receptive with every one of antigen that can cross respond with typical tissue the ordinary tissue communicates a similar antigen or a basically related atom. Theabsolute finest described and broadly utilized cancer specific anti bodies at the point whencarcinoma particular mono clonal anti bodies are connected to strong cytotoxic factors as the result crossreactivity as for as poisonousness to typical tissue that can be significant it along these lines stays a significant objective in the focusing on the field to distinguish the recent tumours specific mono clonal anti bodies with insignificant or no ordinary tissue cross reactivity. Mono clonal anti bodies are responsive with every one of these antigens that cross-respond with ordinary tissue since the typical tissue communicates with a similar antigenor a fundamentally related particle the absolute best portrayed and most generally utilized cancer particular antibodies . At the point when neoplasm specific mono clonal anti bodies are connected to powerful cytotoxic specialists the results of this crossreactivity regarding poisonousness to typical tissue can be significant it in this manner stays a significant objective in the focusing on domain to recognize new tumor specific mono clonal anti bodies with negligible or no typical tissue crossreactivity. Majority of anti-bodies perceive antigen which are communicated variously in the cancer cell community. Subsequently an extant of cells won't tie the counter acting factor. On the off chance that the remedial methodology requires direct authoritative of the neutralizer to the objective cell this could speak to a significant issue.Potential approaches to beat this issue are to utilize a mixed shot of mono clonal anti bodies responsive with various diverse tumor associated antigen or by consolidating the treatment other remedial approaches which doesn't depended on the antigen articulation for its helpful impact (4)

Immunotoxins

Immunotoxins comprises mono-clonal anti-bodies conjugated to strong poisons of bacterial or plant inception. There are many overlays about 1000- 10000 more intense than conjugates utilizing customary of antineoplastic cyto-toxic medications. These are finest broadly utilized in this methodology are the bacterial poisons diphtheria pseudomonas exo-toxins. Every of these three poisons contain restricting locales which empowers to outside of most human cell. When bound, the poison is disguised enters in cytosol and chemically inactivates protein union. Immune- toxin arranged with flawless poisons for most part uses a non-divisible linkage to join the counter acting agent to the restricting subunit. Following disguise ofthe coupled into the cell the inhibitory subunits is discharging by decreased or then again peptide cleavage. (5)Conversely Immuno-toxins arranged with the A chain of ricin or RIP commonly use a divisible disulphide linker in the light of the fact that arrival of the free chain A or tear in cytosol seems basic for activity of the activity of the immuno-toxin. New frustrated disulphide jointers have been produced for connection of chain A once the immuno toxins have been disguised. Not at all like medication conjugates just 2- 3 molecules of poison are appended per counter acting factors. The toxins of immune have been appeared to repress cancer development in a wide scope of tumor design. An immuno toxin created for the treatment of Hodgkin's syndrome gave total reduction in mouse bearing set up Hodgkin's tumor xeno-graft. In the facility of ongoing outcomes with chain A and synthetically blocked immuno toxins coordinated against B cells leukaemia and lymphoma have come about in roughly a half reaction rate in stage one preliminaries. Medical preliminaries utilizing immuno toxins to treat nonlymphoid malignant growths have been progressively restricted and have created less promising results (6).

Cytotoxic drug conjugates

A broad scope of low sub atomic mass against neo plastic cyto toxic medications involving methotrexate vinblastine daunomycin and melphalan Methotrexate, vinblastine, daunomycinand melphalan joined the mono clonal anti bodies trying to convey specifically to neoplasm and therefore beat the harmfulness issue related to these medications. Various diverse conjugating procedures being researched at the point of which are present the most extreme numbers of medication particles as per anti bodies in status where immunizer restricting reactivity andmedication actions are held. In most cases destroying a cell with most cyto toxic medication conjugates including the beginning connection of conjugates to the cell surface antigen intervened disguise and arrival of free medications in the lyso-somal chambers of the human cell. Whereas utilizing of a high atomic mass bearer resulting in the best number of medication particles per counter acting agent particle the subsequent conjugate is frequent and incredibly huge further more in this manner cancer get to issue is exacerbated. (7) Moreover thepharmacokinetics of these conjugates in vivo might be undermined quick ensnarement of immune

response albumin of human sera methotrexate conjugates being accounted for in the liver of a mouse. In spite of the issue portrayed above immune response sedate conjugates have been found to apply predominant enemy of tumor impact in a few creature model frameworks when contrasted with free medication. While trying to diminish the quantity of medication atoms that should be conveyed to a tumor increasingly strong cyto toxic medications are being investigated which in their free structure have minimal medical utility because of serious unfavourable harmfulness (8).

Radionuclides

Radio nuclides iodine131 and yttrium90 emanate huge what's more middle of the road vitality betaparticles separately and half existence of 2.7 and 8 days individually which makes them appropriate for cancer focusing on. The betaparticles have way lengths that can traversenumerous cell widths. Thusly counter acting agent conjugate arranged with these radio nuclidesdon't should be disguised to slaughter the human cell. All the more significantly they can execute cell that compasses the directed cell permitting the annihilation of contiguous tumor cell which either do not have the objective antigen or territories of helpless vascularizationwhere the counter acting agent conjugate can't infiltrate. Conceivably radionuclide conjugate can beat the both issues of antigenic heterogeneity and cancer get to lamentably since the beta particles can reach over numerous cell distance across these conjugatescause radiation that can harm to ordinary tissue while they are coursing in the circulatory system and constrains the sum that can be regulated. Mono clonal neutralizers conjugates of both radio nuclides have been appeared to cause relapses of neoplasm in creatures in various examinations. (9) True to form portion restricting harmfulness was deep down marrow as a result of vague illumination by circling conjugates, there have been or are in progress, more than thirty clinical preliminaries with counter acting agent radio nuclide conjugate. Albeit complete and fractional reduction has been accounted for in an extent of the patients rewarded these have been limited mostly in lymphoid malignancies. Poisonous to ordinary tissue is limited since the chelated radio nuclide is quickly cleared from the blood circulatory system. The bio specific immune response at that point traps the chelated radionuclide at the cancerous site (10).

Enzyme

Another counter acting agent based focusing on methodology that has as of late been created is immune response coordinated compound pro drug treatment. A chemical fit for changing over a nonpoisonous pro drug into a powerful cytotoxic medication is covalently joined to tumor particular monoclonal immune response. Following localization of the counter acting agent covalently joined to a cancer particular mono clonal immune response Covalently joined to a tumor particular monoclonal immune response. Following localization of the counter acting agent catalyst conjugate at the tumor site and

and freedom of remaining conjugates from the circulatory system the pro drug is directed which is changed over by the catalyst into a strong cyto toxic medication at the cancer site, so limiting vague poisonousness. (11) This methodology has various possible points of interest over the other focusing on methodologies. Cancer particular mono-clonal anti-bodies which are definitely not disguised and along these lines won't make intense medication conjugates or on the other hand immuno toxins can be utilized in this methodology. Constraints in sedate intensity are defeat since a solitary catalyst appended to a counter acting agent can create an enormous number of cytotoxic medicationparticles from pro drug atoms at the cancer site. Since the low sub atomic weight cytotoxic medication is produced outside the cancerous cell it can diffuse quickly to neighboring tumors cells which either come up short on the objective antigen or are in zone of helpless vascularization. In this manner the ADEPT like radionuclide focusing on can possibly beat these issues of antigenic hetero geneity and cancer get to. Not all like radio nuclide conjugate be that as it may both the counter acting agent protein conjugate and the pro drug ought to be generally non harmfully and subsequently that ought not result in poisonousness while flowing in the circulation system. Be that as it may vague poisonousness will happen if lingering counter acting agent compound conjugate stays in the circulatory system or ordinary tissue at the hour of pro drug organization. If endogenousis protein exists which can separate the pro drug if the counter acting agent coveys the catalyst to ordinary tissues if dynamic medication created at tumor escapes once again into general flow (12).

Conclusion

Mono-clonal anti-bodies offer the open door for particular conveyance of a scope of against neoplatics operators to cancer. In creature models investigation noteworthy anti cancerous impacts have been exhibited utilizing mono-clonal anti-bodies to specifically covey hostile to cancer medications. Poisons radio nuclides and proteins pro drug mixes. Anti-bodies maylikewise offer the chance of specifically conveying different specialist of possible use in malignancy treatment radio nuclide and protein and pro drug. Anti-bodies may likewise offer of specifically conveying different specialist of possible different specialists of possible utilization in malignancy treatment

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Isolation and Molecular Characterization of Exo-Polysaccharide Producing Weissella Confusa from Buffalo Ruminal Gut

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A B S T R A C T

Lactobacilli are the most important colonizers of the ruminant's intestinal tract and are an important source of probiotics. Among Lactic acid bacteria, Exopolysaccharide producing Weissella confusa has gained considerable interest due to its importance in the food industry and several health attributes. The present analysis was carried out to isolate and characterize exopolysaccharide producing W. confusa from buffalo ruminal gut. The initial characterization of the isolate was done morphologically and biochemically. Molecular detection of the isolate was done by PCR based amplification of 16s rRNA gene. Temperature, pH, incubation-time and media conditions were optimized for the enzyme assay. A total of 35 LAB isolates were cultured for exopolysaccharide production on MRS agar having ruthenium red dye. The pure isolates were also screened for proteolytic, amylase, lipolytic, and antibiotic susceptibility testing. Two Catalase and Oxidase negative strains were selected which shows their probiotic potential.. The two strains identified as W.confusa (by GenBank accession number MK128505, Mk212944), which was based on 99% nucleotide homology and phylogenetic analysis. Isolates produced amylase, protease, lipase and also displayed a high resistance range against selected antibiotics. These isolates also showed antimicrobial activity against pathogens Listeria monocytogenes as well as against Staphylococcus aureus. The isolates also produce EPS when grown on ruthenium red MRS agar. The temperature30°C-37°C and pH 4 were found optimum for enzyme assay. This study unravelled the capability and safety of EPS producing W. confusa for industrial use, and other potential applications.

Key words: Isolation; Molecular identification; Exopolysaccharides; Weissella confusa

Introduction

Buffalos, whose scientific name is "Bubalus bublis," are the biggest nutritionally and commercially valuable animals in the Bovidae family. The overall buffalo population in the world is estimated to be around 150 million. They are employed not just for flesh but also for dairy production due to their high mass, huge physique, and ease of growth. Kundi, Nili Ravi and Azakheli buffalo are the most common buffalo species in Pakistan (1). Franzolin and Wright (2016) suggested that the rumen may contain 300–400 different species of bacteria, while contemporary techniques such as 16s r RNA gene sequence analysis and real-time polymerase chain reaction (PCR) revealed that up to 2000 species may exist. While some microorganisms are pathogens that cause infection, other microbes, known as “Probiotics”, are beneficial to the host and help it fight pathogens (2, 3). Escherichia, bifidobacterium, Propionibacterium, Enterococcus, Weissella Spp and Bacillus are examples of bacterial probiotics, however the most frequent and effective probiotics are Lactic acid bacteria (LAB) (4). Different microorganisms have the ability to produce exopolysaccharides (EPS).exopolysaccharides is made of long polysaccharides chain (5). They may be papered within the cell or in secretary form (6). More research is being done on EPS, which have a role in the flavor, texture, perception and mouth feel of fermented foods (7). Weissella genus has a distinctivephenotypic trait that allows it to produce dextran. As a result, certain strains of Weissella, such as W. confusa and W. cibaria, have attracted researchers' interest due to their great potential for producing dextran, as well as heteropolysaccharides, new non-digestible oligosaccharides, and fractions (8). These are the exopolysaccharides that are the most significant. It also receives attention because of its prebiotics potentials, which include the ability to improve metabolism and bowel function, decrease the risk of illness and diarrhoea, and promote the unique gut microbiota, which includes theBifidobacteria (9). Prebiotic oligosaccharides are mostly utilized at the industrial level in the cosmetics, food, and feed sectors, as well as in the clinical setting and as dietary fibre (10, 11). Dextran and other similar oligosaccharides produced by W. confusa and W. cibaria are utilised in therapeutic settings, such as animal therapies, to enhance and activate the gut microbial flora and decrease illness risk (11, 12).

Galle and Arendt reported in 2014 that EPS may be utilized as a replacement for polysaccharides derived from plants, which are often employed in the food sector as gelling, stabilizing, thickening and texturizing agents (5). Food-related functions of EPS are important, although they are also concerned with general health issues, such as the prevention of cancer and ulcers, activation of the immune system, and reduction of cholesterol (13). EPS is mostly made by LAB, which provides a possible method for replacing hydrocolloid additives (14). The present study was piloted to isolate and characterize exopolysaccharide producing W. confusa from buffalo ruminal gut.

Materials and methods

The present research was carried out at the National Agriculture Research Center (NARC) in Islamabad, Pakistan. From three distinct sites in ChataBakhtwar, Bharakhao, and Rawalpindi, rumen samples were obtained from 35 different species of younger and healthier Nili Ravi, Kundi and Azakheli buffalos. Standard microbiological procedures were followed to transfer the samples using well-labeled, clean, and sterilized Polythene bags or sterilized flasks. Before processing, the samples were transported to the NIGAB Laboratory and kept in the refrigerator to avoid external contaminants (15). The bacteria were isolated using the De Man, Rogosa, and Sharpe agar (MRS) medium. Gram staining and standard biochemical assays were used to confirm the identification of bacterial isolates. The molecular characterisation of isolated bacterial strains was conducted out using 16S rRNA gene sequencing. To do this, the isolates were first cultivated on MRS solid medium plates and then incubated at 37°C for 24 hours. A new colony was chosen with the aid of a sterilised toothpick. Colonies were selected and immersed in a 20L TE buffer solution before being stirred. In a PCR strip tube, a 0.2(ml) suspension was produced. The PCR tube strips were put in a water bath at 95°C for 10 minutes to extract the DNA from the isolates. After that, the supernatant was obtained by centrifugation at 13000 rpm for 2-3 minutes. The DNA of the isolated bacterial templates was found in the supernatant. The pallet was dumped after centrifugation. The 16s rRNA gene was amplified using the template DNA that was acquired. The supernatant, which included 1µl of bacterial cell template DNA, was used to achieve the required Gene amplification.

Forward primer: (5'AGAGTTTGATCMTGGCTCAG-3').

Reverse Primer: (5'ACCTTGTTACGACTT3').

After that, the template DNA (1µl) and master mix (49 µl) were put in PCR tube and the reaction volume reached to 50 µl (16). For 1-2 minutes, the sample was centrifuged lightly. The PCR strips were maintained in the thermocycler for 16s rRNA Gene amplification before the reaction was begunand condition were set according to previous study (17). The amplified DNA was run on gel electrophoresis and the bands were observed through the Gel Doc System (17). PCR products from isolated isolates were sent to Macrogen (Korea) for 16S rRNA gene sequencing. With the use of the online NCBI BLAST, the acquired sequence was compared to the nucleotide database of 16S rRNA gene sequences. The top BLAST hits' phylogenetic tree was then chosen to create a phylogenetic tree. The MEGA7 bioinformatics program was used to measure evolutionary relationships and build a phylogenetic tree. The EPS production ability of isolated strains was tested similar to previous study (18). All the data was analyzed statistically by using SPSS version 23. A p value of less than 0.05 was taken as significant.

Results

In this research, 35 buffalo rumen samples were obtained from various areas of Islamabad, namely Bharakhao, Chatabakhtwar and Rawalpindi. In the microbial biotechnology lab, the specimens were

processed and analyzed. MRS (De Man, Rogosa, and Sharpe agar) agar was used to culture all of the samples. Pure growth was shown on this media by 24 isolates. For identification, the isolates were further examined by gram staining and biochemical tests (Table 1)

Table 1: Gram staining and biochemical characteristics of isolates

Strain ID	Gram staining	Morphology	Oxidase test	Catalase test
NMCC-M2	Gram positive	Rods	-ve	-ve
NMCC-M3	Gram positive	Rods	-ve	-ve
NMCC-M4	Gram positive	Cocci	-ve	-ve
NMCC-M5	Gram positive	Rods	-ve	-ve
NMCC-M7	Gram positive	Cocci	-ve	-ve
NMCC-M8	Gram positive	Rods	-ve	-ve
fM-13	Gram positive	Rods	-ve	-ve
fM-14	Gram positive	Rods	-ve	-ve
fM-20	Gram positive	Short rods	-ve	-ve
MF-9	Gram positive	cocci	-ve	-ve
FI-10	Gram positive	Rods	-ve	-ve

The DNA was extracted using a kit that was readily available (FavorPrep cat No-FAVNK001-2). Using the primers described in the previous section, the 16s rRNA gene was amplified. Figure 1 shows the Gel Doc system used to analyse the amplified DNA sequence. A band size of 1465-1522bp was used to confirm 16s RNA.

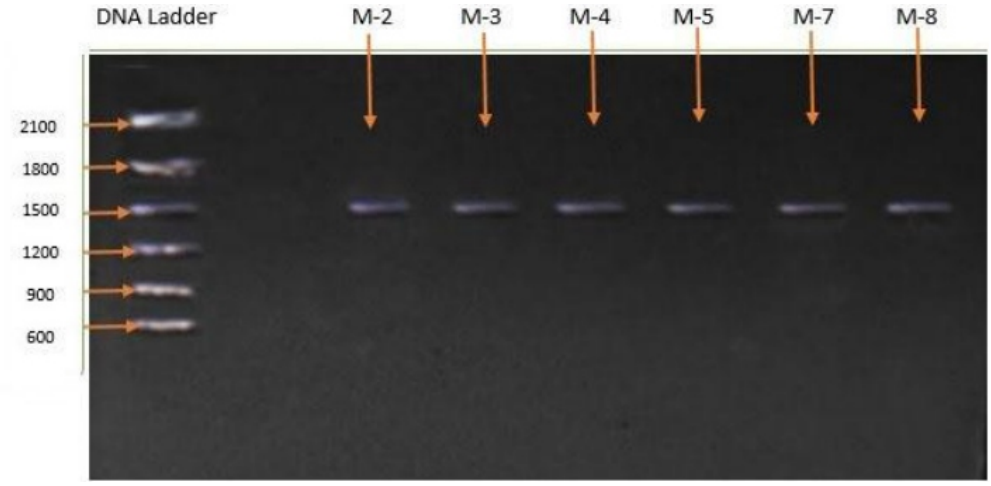

Figure 1: Agarose gel and 16s rRNA amplified bands of isolated strains:

The image above illustrates the amplified sequence of the 16s rRNA gene from isolated strains on an agarose gel. The 16s rRNA gene length in W. confusa strains with accession number (Mk128505) is 1465 base pairs (bp) in M3, whereas the 16s rRNA gene length in strains with accession number (Mk212944) is 1470 base pairs (bp). The M line depicts a DNA ladder with various sizes ranging from 600 to 2100 base pairs. The size of the isolated strains corresponded to 1500bp DNA bands. In the provided image M3 indicate lab ID (NMCC-M3) whereas M8 indicates lab ID (NMCC-M8) (NMCC-M8).

The data was analysed for phylogeny once it was sequenced and trimmed. Only NMCC-M3 and NMMC-M8 (BLAST, NCBI) showed 99 percent identity with the available sequence of W. confusa JCM strain accession number NR113258.1 in a sequence similarity search for all identified strains.ClustalW of MEGA7 was used to do the phylogenetic analysis, and maximum-likelihood was used as the statistical metric. The fact that both strains have a bootstrap value more than 50 indicates that they are fully accurate and very similar to the W. confusa JCM strain. Figure 2 shows a phylogenetic study of W. confusa in relation to other LAB using the maximum-likelihood technique.

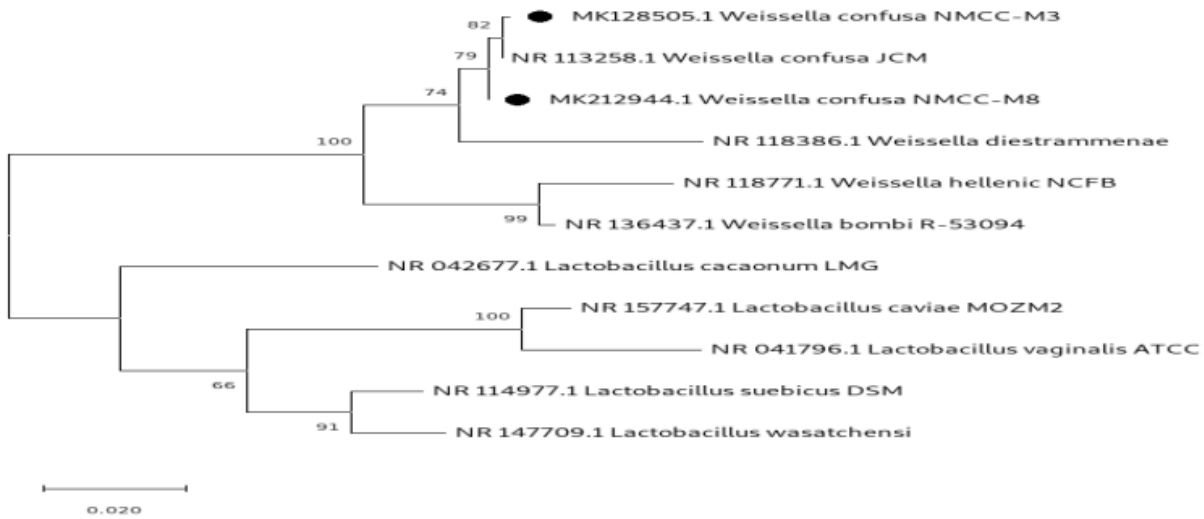

Figure 2: Phylogenetic tree : Based on examination of the 16S rRNA sequence, a maximum likelihood tree with 50 bootstrap values in MEGA7 shows the phylogenetic relationship of W. confusa. The tree was constructed through MEGA-7 by using neighbour-joining method (16).

EPS Producing Activity

On the MRS medium with a particular indication, the suspicious LAB isolates were qualitatively tested (ruthenium red dye). Ruthenium red dye is cationic, meaning it attaches to ionic sites in the bacterial cell membrane, giving it a red colour. EPS-producing bacteria, on the other hand, do not interact with ruthenium red dye, which results in a white colony on screening medium. In the case of both isolates chosen, white colonies of isolates were found, indicating favourable findings as shown in figure 3.

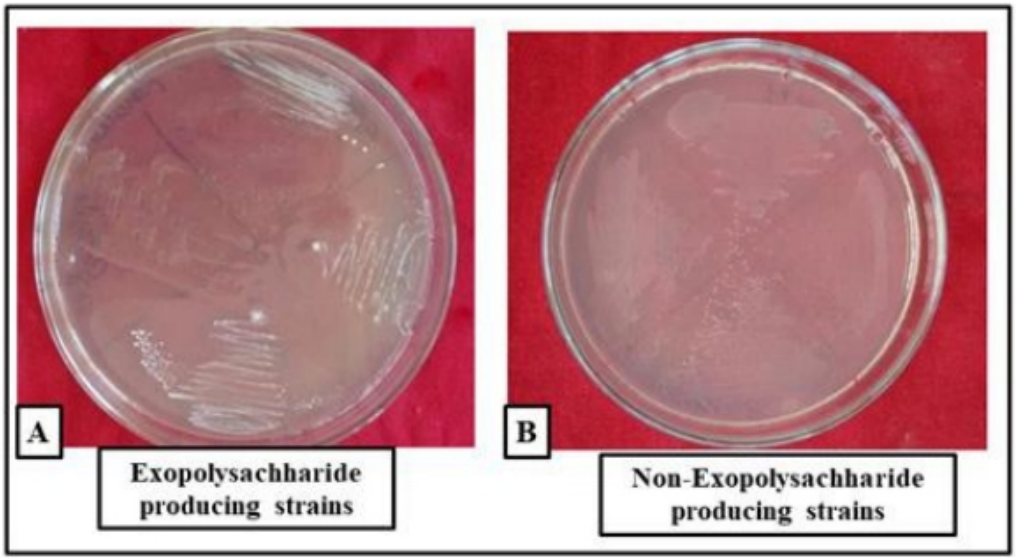

Figure 0: EPS production by the selected isolates

Discussion

Because of its probiotic potential and many industrial uses, LAB strains capable of generating EPS have been intensively researched. These EPS-producing LABS may be found in milk, dairy products, and the rumen gut of ruminants. The present research isolated and identified W. confusa that produces EPS from the ruminal gut of buffalo in order to evaluate their potential of exopolysaccharide production. Thirty five samples of buffalo faeces were collected from various locations throughout Islamabad, Pakistan. MRS medium was used to isolate lactic acid bacteria anaerobically. Colony morphology was creamy white and silky. The isolates (NMCC-M3 and NMCC-M8) were classified as round or punctiform. A previous study reported similar results (19).Gram staining was used to identify the LAB isolates morphologically. They showed purple rods under a light microscope following Gram staining, which is a hallmark of Gram-positive bacilli. Only 19 isolates were recognised as Gram-

positive rods in our research. In comparison to our study same results were reported in another study done by Jose, Bunt (20). The isolates were chosen for further investigation based on biochemical analyses. Only 14 Gram-positive isolates were catalase negative, with only 11 showing oxidase negativity among the 19 Gram-positive isolates. Barakat et al., for instance, found similar findings (21). Different settings were adjusted for the experiment in order to ensure that LAB's activity was as efficient as possible. At 30°C and 37°C, the LAB strains grew the fastest. At 45°C, though, no growth was seen. Same findings were reported by another previous study done by Lee, Park (22). LAB's probiotic capacity to survive within the gastrointestinal system is shown by their growth at 37°C. The strains were also unable to generate bacteriocins, among other things. W. confusa strains had previously been shown to be largely resistant to high salt concentrations, according to the authors. The strain's unique feature of acid tolerance and survival at low pH allows it to demonstrate its probiotic potential as well as its capacity to live in unfavourable circumstances (23). The strains in our research exhibited the best survivability at pH 2 for 2-3 hours. Montoro, Benomar (24) reported that W. confusa was shown to have similar survival behaviour at pH 2 without decreasing viability. H+-ATPase activity determines a strain's capacity to withstand high acid concentrations. Only two isolates were chosen for this study and were tested for EPS activity. On the MRS medium with ruthenium red dye as an indicator, these two isolates were then tested qualitatively for EPS production. Osmotic stress, biofilm and hazardous chemicals are all inhibited by exopolysaccharide. They are also suitable for usage in the textile, culinary, pharmaceuticals, and chemical industries. Both isolates formed white colonies on the medium, indicating that the EPS test was positive (25). The identification of LAB species is based on their physiological and biochemical properties, according to the literature. Furthermore, since 16s rRNA is the most conserved segment across various LAB strains, it is used for phylogenetic analysis. Exopolysaccharide-producing LAB was isolated from the ruminal gut of buffalo and genotypically identified using PCR-based amplification of the 16s rRNA fragment. Sequencing was used to improve the strain's characterisation. After comparing the sequence to other sequences in the Genbank database, it was determined that it was 99 percent identical to the W. confusa JCM strain accession number NR113258.1. Other researchers have also reported similar findings (26).

Conclusion

Our study concludes that isolates of W. confusa from the ruminal gut of buffaloes showed unique exopolysaccharide production activities. Coming scientific research is attempt on probiotics bacteria to explore novel and particular bacterial strains for the welfare of the different host such as animals and human. So, the future improvement and scientific research tendency will be.

More features of W. confusa will need to be determined before such strains may be used in the industrial zone. Apart from that, the strains' safety must also be assessed.

Further processed the isolated strains for whole genome sequencing.

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Microbiological Analysis of Drinking Water of Gravity Flow Water Scheme Abbottabad, Pakistan

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A B S T R A C T

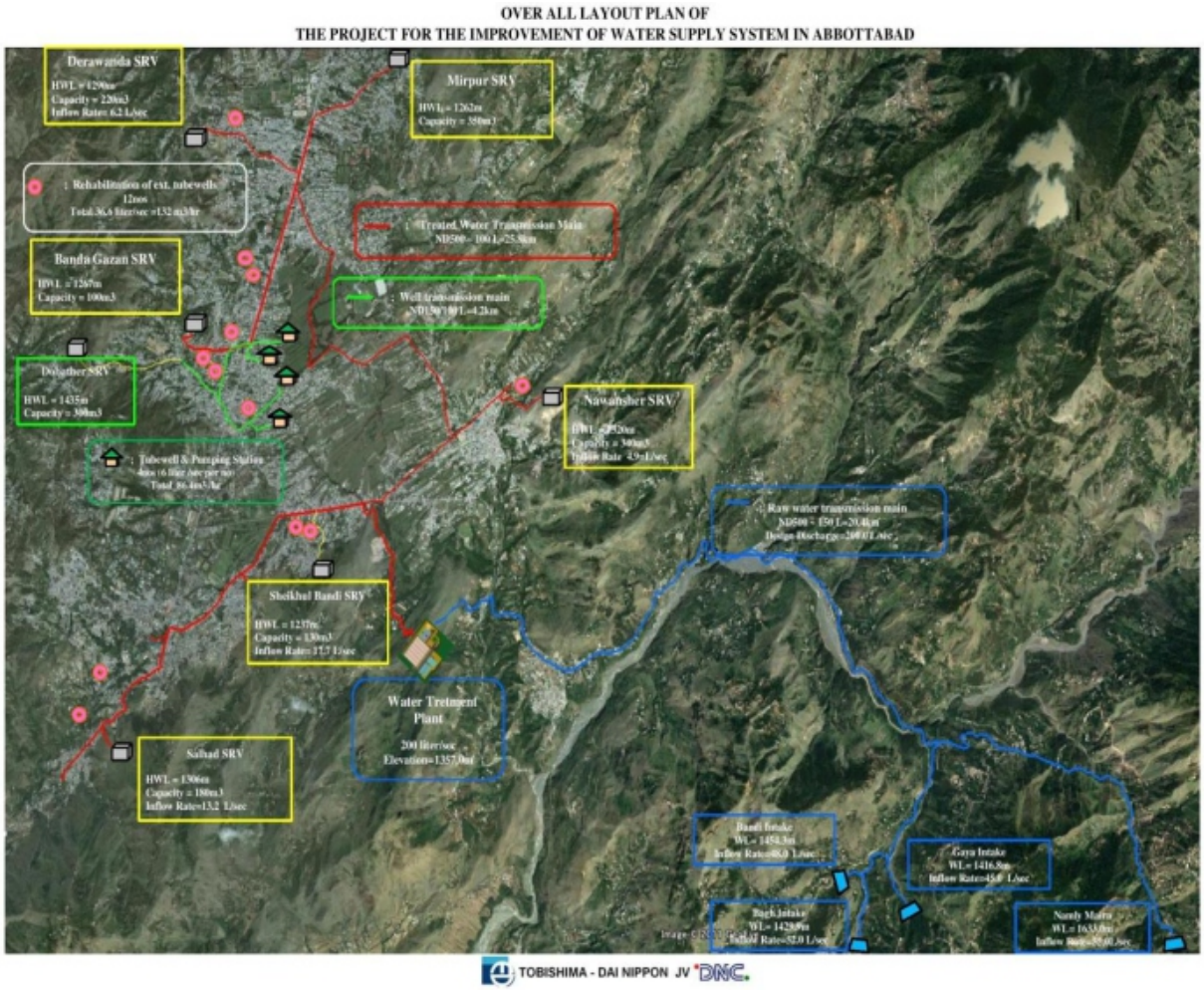
Since pure drinking water is good for health, it must be assessed for bacterial contamination. This research was conducted on the gravity flow water scheme of District Abbottabad, Pakistan. Pakistan. Twenty water samples were collected in different areas of city. Physiochemical analyses including pH, temperature, and chlorine residue showed that all water samples were according to prescribedvalues by the World Health Organization. This research work aims to analyze the total viable count (TVC), Total Coliform Bacteria (TCB), Total fecal Coliform Bacteria (TFCB) present abundantly in drinking water. The results showed that highest TVC values 5.6020 log cfu /100ml and lowest were 3.7781 log cfu/100ml, TCB were found in the range 250>2.0MPN/100ml, TFCB were found in the range 25>1.1MPN and E.coli were found in all water samples except sample-11. The water samples analysis data indicated that 100% water samples were unfit due to high TVC values, 85% samples were unfit due to higher (TCB) and 45% TFCB than permissible limits and 75 % samples were found to be contaminated with E. coli. Escherichia coli, Klebsiella oxytoca and Pseudononas aeruginosa were identified based on morphology, gram staining and conventional biochemical testsand the identification was further verified by API 20 E kit.

Key word: API 20E kit, Microbiological Analysis; Drinking Water; Gravity Flow Water Scheme

Introduction

The project of gravity flow water scheme is located in the district Abbottabad, Pakistan. The project initiated on 15th April, 2011 and was completed in September of 2013. The total expenditure to complete this project was 4.28 billion rupees out of which 3.7 billion rupees were provided by the Japanese government, and the rest was provided by the KPK provincial government [1].The plan provided the city of Abbottabad with drinking water and benefited 216,353 people. Among them, 65% of the population lives in urban areas and 35% of the population lives in rural areas. To store water, four different pools were built in Namly Maira, Bandi and Bagh [1].

To purify water, a treatment plant was built in Chuuna Kala Pul Abbottabad to purify 38,000 gallons of water (200L/S) per day. Drinking water is supplied by this plant in different areas of Abbottabad, including Sheikul bandi, Salhad, Newansher, Mirpul, Derawanda and Danda. In addition to the camp tube well pumping station is used to provide, drinking water in the Dobass region.



More than 3.4 million people are affected each year due to contaminated water as reported by the WHO. The chief culprits of causing major diseases and deaths around the world are water-borne pathogens [2]. The feces of humans or animals and chickens are major causes of water contamination. The, fungi, viruses, protozoa, and worms are generally secreted by feces.The intestinal diseases and some others infectious diseases are mainly the result of using contaminated water [2]. According to the latest report of the Pakistan Water Research Council, the water quality of most water supply projects in Pakistan exceeds the drinking water quality standards set by the World Health Organization [2].

Materials and Methods

Site description

Abbottabad is a city of KPK provience of pakistan. It covers an area of nearly 1969 square kilometers;

the city of Abbottabad is surrounded by Serbian mountains on all sides.

Sample collection

20 water samples at different sampling points in Abbottabad were collected. They were put into 150ml sterile polyethylene bottles. The pH and temperature of the sample was noticed at the sample collection site, and kept them in an ice box and transported them to the Quaid-i-Azam University Soil Microbiology Laboratory for further processing

Enumeration of total viable count

1 ml water sample was taken from each collected sample and added to a serial dilution of 9 ml normal saline. Then inoculated 0.1 ml of the dilution on the nutrient agar plate and the three un inoculated plates, and incubated at 37°C for 24 hours. After incubation, visible colonies formed on each plate, but no visible colonies appeared on the un-inoculated plates. Then colony counter was used to count colonies on plates that contain 30 to 300 colonies, and the results were presented as cfu/100 ml.

Multiple Tube method

Multi-tube method was used for determining the most probable number (MPN) of coliforms and fecal coliforms and Escherichia coli. The test was carried out according to the standard protocol [3].Sterile 10ml and 1ml water samples were added to test tubes containing 10ml and 5ml double strength Mac Conkey broth respectively.In addition, 0.1ml of water sample was added to a test tube containing 5ml of MacConky Broth of a single concentration. All test tubes contain sterile inverted Durham test tubes and were incubated at 37°C for 48 hours.

Positive samples were sub-cultured into 10ml single tube of brilliant green bile broth with inverted Durham tubes and10ml Peptone Water to find out presence of fecal Coliform and E.coli and the tubes were incubated at 44◦C for 24 hours. The MPN of fecal Coliform and E.coli was determined according to the standard WHO guidelines.

Procedure for isolation of selected colonies

1ml of coliform and fecal coliform cultures on the MacConkey agar plate was inoculated. Sterile spreader was used to evenly distribute the cultures on the plate. The plate was kept in the inverted position in incubator and keeps growing at 37°C for 24 hours. After culturing for a period of time, colonies of different colors were formed, and some suspicious colonies were selected from every plate to precede further work.

Identification of microorganism

For confirmation of suspected colonies Gram staining technique, conventional biochemical test and API-20E kits was used [4].

Result

During November to December, the pH, temperature as well as residual chlorine content of drinkable water samples were measured. The maximum pH value of drinking water was 9.3, the minimum pH value was 7.43, and the average value was 9.36. The highest temperature of drinking water samples was 18 C°, the lowest temperature was 9.7 C° and their average value was 12.675 C°. The maximum value of residual chlorine was 1.7 ppm, the minimum value was 0.4 ppm and their average value was 1.025 ppm. (Table 1)

Physiochemical parameter

Table-1: Physiochemical character of water samples

NOV TO DEC	Parameter	MINMIUM	MAXIMUM	MEAN	STD DEVIATION
	pH	7.43	9.3	9.365	0.3969
	Temp(^{O}C)	9.7^{O}C	18^{O}C	12.675 ^{O}C	4.4364 ^{O}C
	Chlorine residual	0.4ppm	1.0ppm	1.025ppm	0.3339ppm

Total viable count

The total viable counts of individual drinking water samples were relatively high, which means that these water samples are not safe for drinking .The maximum number of total viable bacteria was 5.6 log cfu/100ml, and the minimum log was 3.8 log cfu/100ml. eighteen drinking samples have a medium value (Figure 1). Therefore, the result is not significant and does not meet the requirements of the WHO

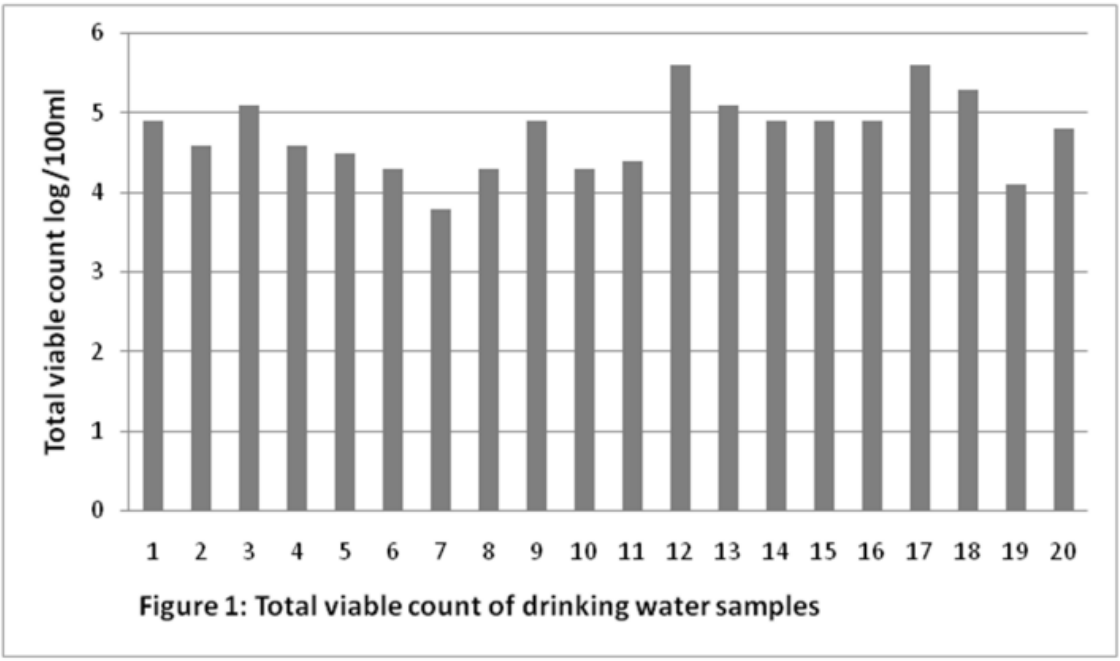


Figure 1: Total viable count of drinking water samples

Most probable number of drinking water samples

The MPN/100ml values of coliform, fecal coliform and E. coli were analyzed from water samples from various locations in Abbottabad, such as residences, schools, hotels and restaurants, and distribution lines. The MPN/100ml value of various drinking water samples is greater than the standard protocol set by WHO, so the water is not suitable for community use.

The higher MPN/100ML value of total Coliform (250,35,50) were reported in Serbund Student Berger Restaurant, Storage Water Govt Girl High School Abbottabad and House No :261 and lower value (2,2,2) in House No:k494, House No:50/2 and Bandkhou Abbottabad as presented in fig.2A

Fecal coliform (35, 25, 25) in House No: 261, Govt Girl Hostel No: 1 and Serbund Student Berger Restaurant were higher MPN/100ML and lower value of fecal coliform were reported in seven localities in House No:k494, House No:204, House No:247, Storage Water Govt Girl High School Abbottabad, House No:50/2 and Bandkhou Abbottabad as presented in fig.2B

The highest value MPN/100ML of E.coli (25,9,9) of drinkable water samples were reported in House No: 261 Boland Hotel and Serbund Student Berger Restaurant and lowest value in House No:247 was shown as presented in fig.2C .

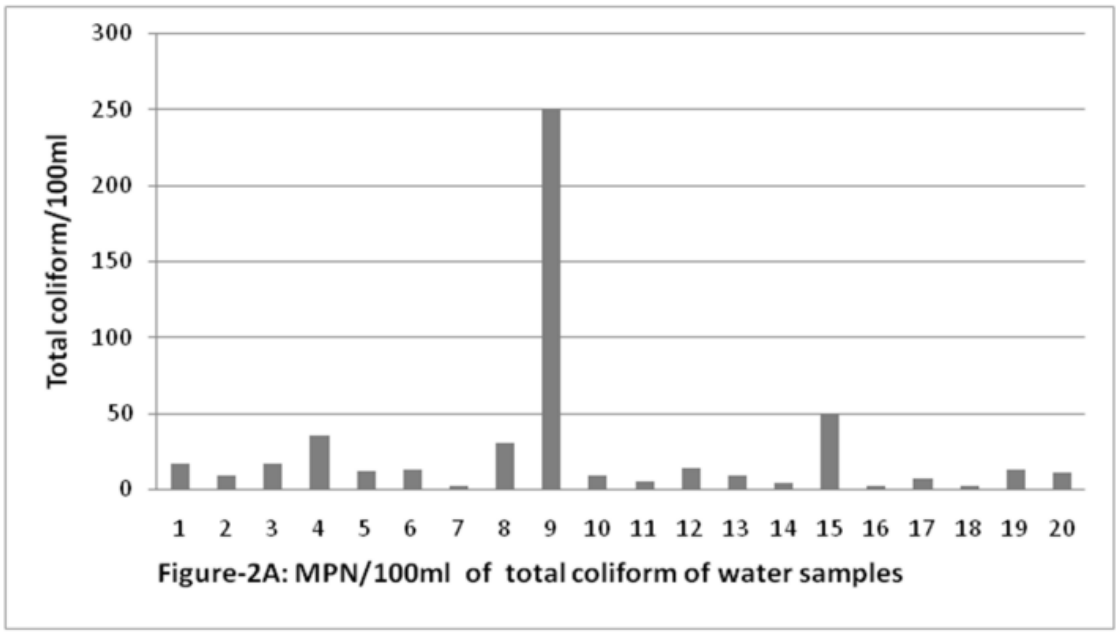


Figure-2A: MPN/100ml of total coliform of water samples

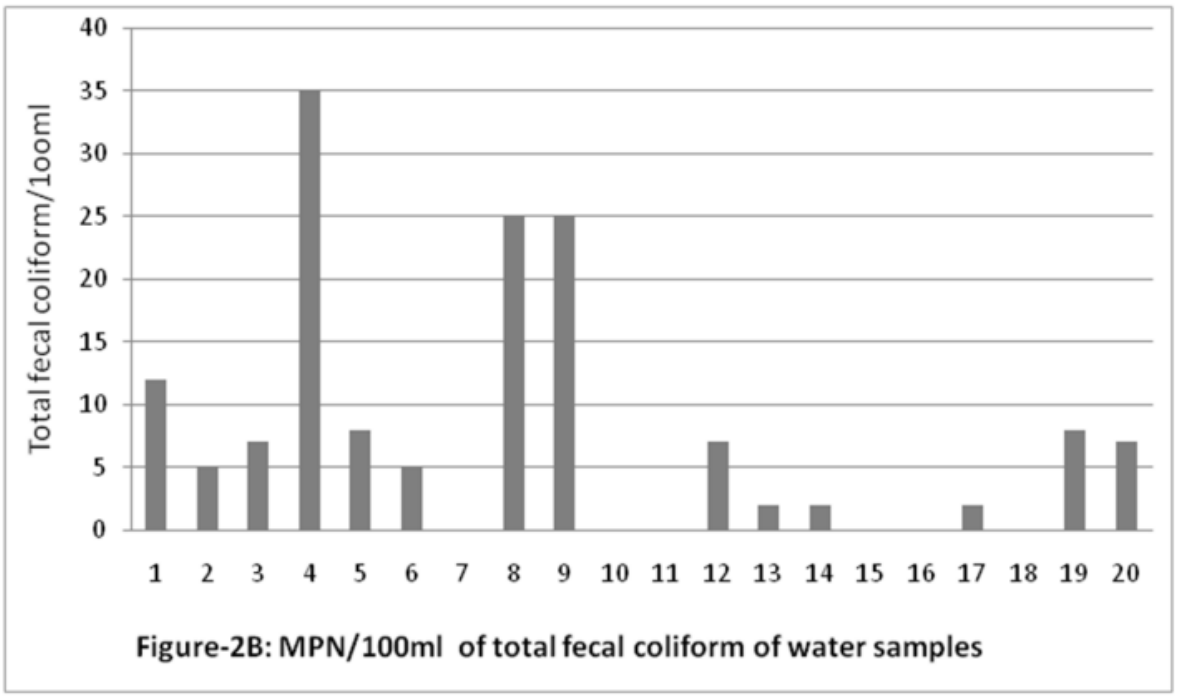


Figure-2B: MPN/100ml of total fecal coliform of water samples

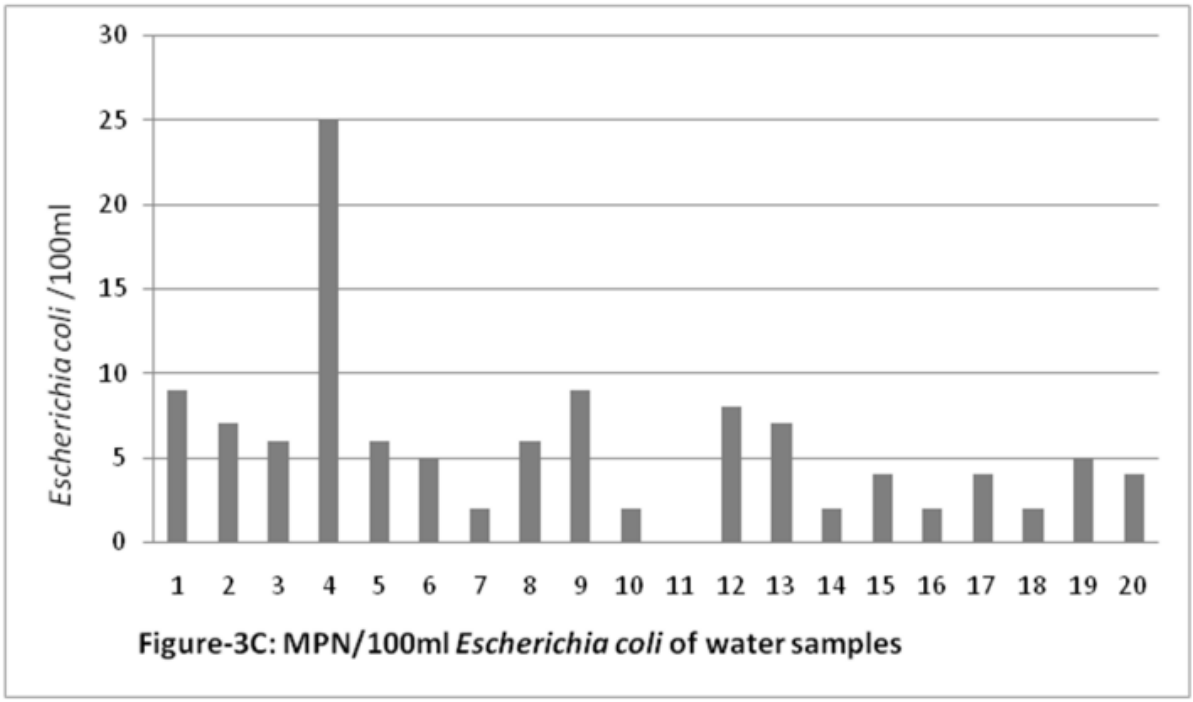


Figure-3C: MPN/100ml *Escherichia coli* of water samples

Isolation and identification of waterborne Pathogens

Eescherichia coli, Pseudomonas aeroginosa, Klebsiella oxytoca isolated from drinking water by using different microbial technique including Gram staining, Oxidase, Mobility and further bacteria verified by API-20E kit.

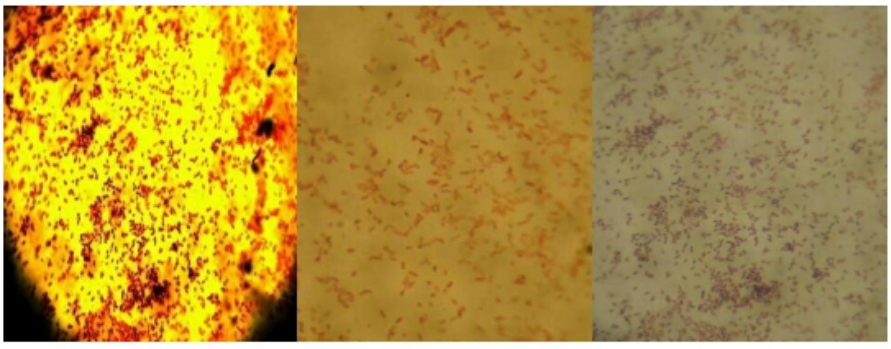
Table 2: Biochemical identification of pathogenic Bacteria

S/N 0	Full name of substrate	*Escherichia coli*	*Pseudomonas aeroginosa*	*Klebsiella oxytoca*
1	Gelatinase	+	+	–
2	Voges-Proskauser	–	–	+
3	Indole	–	–	+
4	L-tryptophane Deaminase	–	–	–
5	Urease	–	+	+
6	Hydrogen Sulphide	–	–	-–
7	Citrate	–	+	+
8	Decarboxylase Ormithine	–	–	–
9	Lysine Decarboxylase	–	–	+
10	Arginine Dihyrolase	–	+	–
11	Ortho-Nitro Phenyl-BD Glactopyranoside	–	–	+

12	L-Arabinose	_	_	+
13	Amygdaline	_	_	+
14	L-Melibiose	_	_	+
15	D-Saccharose	_	_	+
16	L-Rhamnose	+	_	+
17	D-Sorbitol	+	_	+
18	Inositol	_	_	+
19	Mannitol	_	_	+
20	Glucose	+	+	+
21	Gram staining	_	_	-
22	Oxidase test	_	+	_

Table 3: Morphological identification of pathogenic Bacteria

S/N0	Morphological character	*Escherichia coli*	*Pseudomonas aeroginosa*	*Klebsiella oxytoca*
1	shape	rod	rod	rod
2	Motility	motile	motile	non-motile
3	Color appearance	pink	colorless	creamy mucoid
4	Baceria gram staining	negative	negative	negative



Escherichia coli Pseudomonas aeruginosaKlebsiella Oxycota

Discussion

According to world health organization standard, total viable count and coliform should not be greater than 100 cfu/ml [5]. It has been observed that the total viable bacteria counts of water samples collected from different sampling points in Abbottabad are very high. 5.6020 log cfu/100ml compared with water tape and tube well 4.0791 log cfu/100ml of Peshawar drinking water [6, 7].The results of MPN/100ml drinking water samples included 17 out of 20 samples (85%) contaminated with total coliforms, 11 (45%) contaminated with fecal coliforms, and 15 (75%) contaminated with Escherichia coli Contamination (similar report by Abdul et al., 2009) All 120 (100%) samples in Sukkur City were contaminated with total coliforms, and 98 (82%) samples were contaminated with heat-resistant E. coli.The same research work was done to determine microbial contamination of individual drinkable water sources for example ground, well and river water reported E.coli and fecal coliform [8] are the main contaminants of 67% of total water sample. Comparable results were also reported by another study [9].

The present study showed that drinking water samples were more contaminated. They consist of number of bacteria but the most abundant bacteria found in different drinking water samples were commonly Escherichia coli, klebsiella Oxytoca and Pseudomonas aeruginosa. Another study made a similar report [10]. The results showed that analysis of drinking water samples from the Kathmandu Valley and they identified 238 strains of intestinal bacterial isolates. Among them, Escherichia coliwas 26.4%, Pseudomonas aeruginosa was 6.3%, Klebsiella 5.4%, Shigella 4.0%, Vibrio cholerae1.0%, Salmonella was 3.0%.

Conclusion:

Microbiological analysis of drinking water samples revealed that all water samples were contaminated with microbes such as coliforms, fecal coliform and E.coli. It is responsible to cause diseases in community and kept extra burdon on the society as a result the area cannot progress properly. So government should have to pay attention towards these problems and revises the policy and do work for it

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Conflict of interest

The author declares that there is no conflict of interest in the publication of this paper.

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Impact of Rainfall on Indian Economy: With Reference to Crop Production

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A B S T R A C T

It is a well-known fact that agriculture is measured as the strength of India's economy. The world wide studies through different researchers have shown that the rainfall has great impact on the crop production which ultimately influences our economy. In India we could differentiate the different type of available crops broadly as Rabi, Kharif and Zaid where the growth of Kharif crop majorly depends on the rainfall. This growth of crop ultimately impacts the Indian economy as 22.5 percent of Rajasthan state's GDP (Gross Domestic Product) comes from agriculture alone. This paper is presented here to analyze the impact of rainfall on the productivity of different kind of crops and to evaluate the economic effect of Kharif crop production onto it which furtherinfluences the states GDP growth.

Keywords: Rainfall, Environment Change, Agricultural Impacts, Rabi and Kharif Crops.

1. Introduction

Agriculture is a major source of India'seconomic growth. It has a large contribution inIndian GDP byadding 16.5% GVA (GrossValue Added) in 2019-20. As we know in India we grow three main kinds ofcropsnamely Kharif, Rabi and Zaid, where the production of Kharif crops mainly dependsupon the rainfall. The Kharif Crops includes Bajra, pulses, Jowar, maize, gaur and groundnuts as the major cropelements. Kharif crops include rice, maize, sorghum, pearlmillet/bajra, finger millet/ragi (cereals), arhar(pulses), soyabean, groundnut(oilseeds),cotton etc. In India, the monsoon season startsin June and ends in October. The Kharif cropsare harvested at the end of the monsoon seasonOctober or November month. By end of July,2020, the total Kharif crops has been sown on882.18 lakh hectare (hectare) area against 774.38lakh hectare (ha) area during the corresponding periodof last year (2019). 266.60 lakhhectare areascovered under rice and about 111.91 lakh hectare areascovered under pulses. Coarse Cereals covered148.34 lakh hectare areas, about 175.34 lakh hectare area covered under oilseeds. Sugarcane covers51.78 lakh hectare area, Jute & Mesta covers about6.95 lakh hectare areas and Cotton covers 121.25 lakh hectare areas. So, it's well known fact that the rainfall plays a crucial role in the economy of our country and our farmers. Here, Weather works alike to a ―natural experiment‖ meanwhile it has peripheral causes [1]. There is a random control on economic events. Whereas we are capable to distinguish the statistical fundamental influence of weather on economic results [2].

So, here the paper is presented to evaluate the crop production against the data sets of the rainfall which is then compared with the economic growth of the country in the specific time stamp. In this way the paper concludes that there is an impact on our economic growth due to the rainfall. This further focuses on the reasons of protecting our environment not only for the human health but also for the productivity of the crops which is a major source of Indian economy as India is a land ofFarmers.This paper is structured as: The first sectionhaving introduction, the second section withliterature review on existing studies and theimpact of rainfall on economic conditions offarmers. The next section has dataset details and methodology to evaluate the proportionality of rainfall with the crop production and further the financial growth of the country. The results are discussed in 4th section and in the last section the conclusion is stated to focus on the need of increasing rainfall in the country with the evidences of different specific reasons.

2. Literature Review

Siriklao Sangkhapha and Yang Shu discovered the influence of rainfall on the progress of thegross provincial product (GPP) by economic sector. They used provincial-level panel datafrom the timeperiod of 1995 to 2015for analysis. In the regression model they used FGLS(feasible generalized least squares) estimator along withfixed influence. The results show that the chief effects of the weather are fallen with help of rainfall andreduced GDP growth. Experiments also demonstrate that the rainfall had a negative influence on the agriculture [1]. Marcelo Torresand et al., introduced a novel hydro-financial model with planning and forces of precipitation influence the profitability of an in part inundated horticultural framework in Brazil. The result recommended that rainfall timing was an essential economic parameter and models thatwere used to find outinfluences of water shortage on agricultural income predict underestimate values [2].

Mariana Camarin Gazonato and MariaAparecida Silva Oliveira analyzed the limit of Brazilian economysegments to move theirprofitability increases over the beneficial chain from 2000 to 2009. In this manner, it has beenutilized basic disintegration procedure and a philosophy that manages the variety of workefficiently. The after effects of this article yield in the period under audit are that theServices and not the Industry were answerable for transmitting these additions of efficiency.Be that as it may, the intensity of transmission of efficiency variety of the tertiary part comesto be moderately low when contrasted with the capacity of the Industry to transmit its profitable varieties [3]. Tara Iyer and Abhijit Sen Gupta built a toolbox to now cast, or produce timely gauges of GDP development in India. They utilized a dynamic factor model (DFM) to now cast GDP development in India on a quarterly premise from January 2000 to December 2018. The results shown that, the precipitation has high prescient substance for GDP development in India [4].

Shreekant Gupta et al. examined the impacts of precipitation and temperature on yields of paddy and millets (pearl millet and sorghum) in India for the period 1966–1999 [5]. Julie A. Silva and Corene J. Matyas have built a connection amongst precipitation and economic well of the monetary prosperity of rustic agriculturalists in Mozambique, who fundamentally depend on down pour took care of agribusiness for food and salary. Authors built up connection by isolating the examination locale into precipitation zones dependent on the level of ordinary precipitation got during the developing seasons and from two tropical typhoons at every one of 536 towns. The precipitation examination uncovered that four of the nine precipitation zonesexperienced outrageous wet or dry conditions, while high month to month and inter-annual fluctuation in precipitation happened all through the nation [6]. Abdul Rehman examined the relationship between rural GDP and the yields of major crops. These crops were sugarcane, wheat, maize, rice and cotton. They collected data of GDP and crops production fromdifferent publication over the time of 1950 to 2015 ofPakistan. They used ADF unit root test,common least square strategy and Johansen'sco-coordination test for analysis of data. The results

of analysis using regression representedthat the output of maize, wheat, cotton and rice have positive relationships GDP. The output ofthe sugarcane has a negative impact on GDP [7]. Khaled Ramadan Elbeydi experimentallyresearched the causal connections between GDP and agricultural production. Inthisexperiment the author used annual time series data of the Libya during the time period of1970 to 2012. Error correction, integration and Granger causality methods were implementedto regulate the long runequilibrium relationship. The integration test showed thepresence of long run equilibrium association amongagricultural production and the GDP. These results demonstrated that theagricultural section play a vital role in GDP ofLibya [8]. Abidoye et al.,examined the experimental connection between economic growth and climate variation in Africa. In theexperiment they used annual data of 34countries from 1961 to 2009 time period. Theyfind out negative influence of climate variationon economic growth of these countries. Theexperimental results show a 1°C increase intemperature decreases gross domestic product(GDP) growth by 0.67 percentage points. Theyalso find out that there was no impact of average long-run temperature changes affectlong-run economic growth, they average 5years temperature [9].

M. Gilmont et al., examined relationship between Indian state- level economic and rainfall data from 1961 to2012. In experimentaldataset, from 15Indian states, those populations exceeding 20 million as of 2000 and totaling 920 million people, about 12% of the global population. From experimental results, they found thatsome patterns of interdependence between rainfall inconsistency and economic growth. They found continuous correlation of rainfall and economic growth rates [10]. Berlemann et al., have analyzed short term and long term economic growth effected by rainfall variations. For experimental analysis, they collected 150 countries data from 1951 to2013. From experiment, they found strong and extremely robust empirical proof for long- lasting negative growth effects of rainfall more on poor and underdeveloped countries [11].

Lertamphainont et al., investigated the impact of rainfall variation, how rainfall effect human life in term of floods and droughts, how affect income, consumption, and coping responses of farming households in Thailand. They collected household-level data from repeated cross-sectional farm household surveys over the time of 2006-2010. From data analysis, they find out that crop income falls sharply as resultsof rainfallvariation. Also find out the emphasize wealth-differentiated effects of rainfall shocks as landless households seem more vulnerable to rainfall shocks than landholding households due to their limited ability to smooth income and consumption [12].

3. Dataand Methodology

3.1 Data source

In this paper the data for Rainfall and crop production is collected from Agriculture department, Government of Rajasthan. Here, we have chosen Rajasthan state average rainfalls in millimeter from

2010 to 2018 for our study and experimentation. The results of Crop data is also taken from 2010 to 2018 time period of total production of Rajasthan state of all three types of crops namely Rabi, Kharif and Zaid.

3.2 Methodology

In this analysis we select Rajasthan state total crop production on entire year and state average rainfall in that particular year of all districts. The Rabi crops are winter crops, seeded in October or November months and get product in March and April months. The Kharif crops are summer, seeded in June or July and get product in September or October. Zaid crops are summer season crops and they grow in time period from March to June. Here, the Linear Regression is used to show relationship between rainfall and crop production (Productivity per hectare Rabi and Kharif (kg/hectare), Productivity per hectare Rabi (kg/hectare), Productivity per hectare Kharif (kg/hectare), Total production Rabi and Kharif (Tonnes), Total production Rabi (Tonnes), and Total production Kharif (Tonnes). This methodology is used to evaluate the growth of crop in relation to the rainfall occurred so that a proper comparison could be made to note the impact of rainfall onto the crop production.

4. Results Analysis

The following table-1 show Statistics of state average rainfall, productivity and production with detail of minimum, maximum, mean, standard deviation (S.D.) and Coefficient of Variation (CV).

Table-1: Statistics of state average rainfall, productivity and production

Indicator	State average rainfall (MM)	Productivity (kg/hectare)			Production (Tonnes)		
		Total	Rabi season	Kharif season	Total	Rabi season	Kharif season
Minimum	464.00	1202.00	1873.00	745.00	30667740.00	16886820.00	12342637.00
Maximum	719.90	1386.00	2271.00	1061.00	36155659.00	21906279.00	16842656.00
Mean	555.49	1298.44	2005.11	888.33	33222358.22	19014653.33	14207704.89
S.D.	94.34	60.01	152.35	84.77	1537730.06	1302759.203	1233483.904
Coefficient of Variation (CV)	0.17	0.05	0.076	0.096	0.046	0.068513434	0.086817956

The figure-1 represents year wise total crop productivity along with Rabi and Kharif season individually.

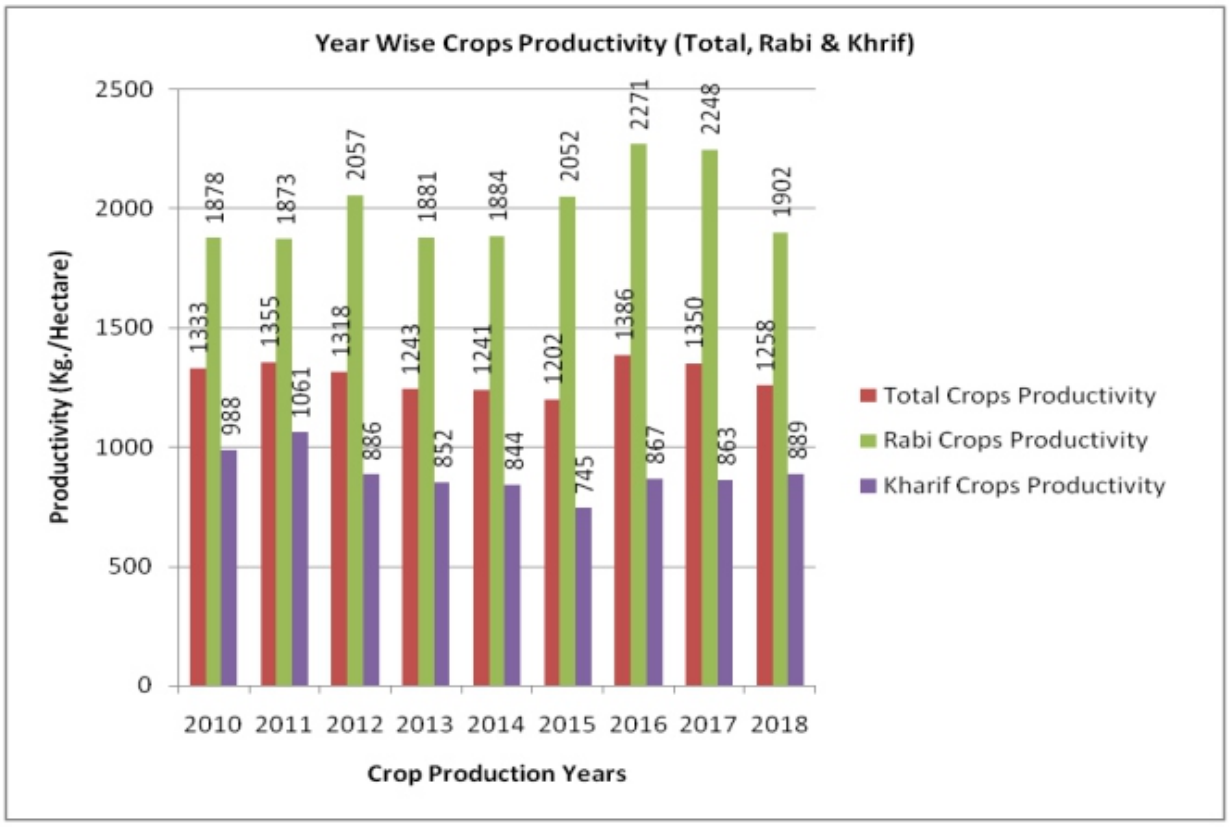

Figure-1: Yearwise total productivity,RabicropsproductivityandKharifcrops productivity

The figure-2 shows the correlation between rainfall and total productivity (Kg./Ha) of Rabi, Kharif and Zaid crops. From graph we can conclude that that rainfall has great impact on all types of crop production.

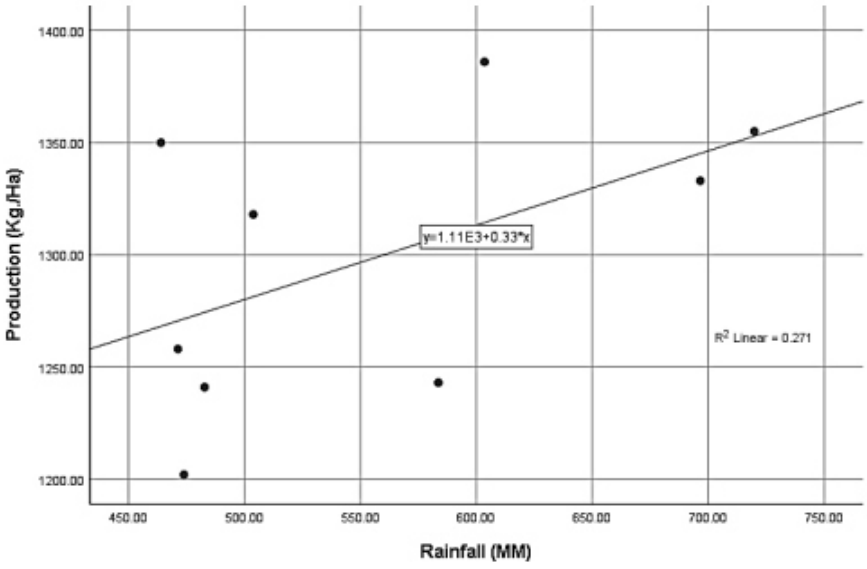

Figure-2: Rainfallandtotal productivity(Kg./Ha)

The figure-3 shows the correlation between rainfall and total productivity (Kg./Ha) of Rabi crops. In graph we can see that Rabi crops production have negative correlation with rainfall, because Rabi crops are fully depends on other irrigation System, with compared to rainfall.

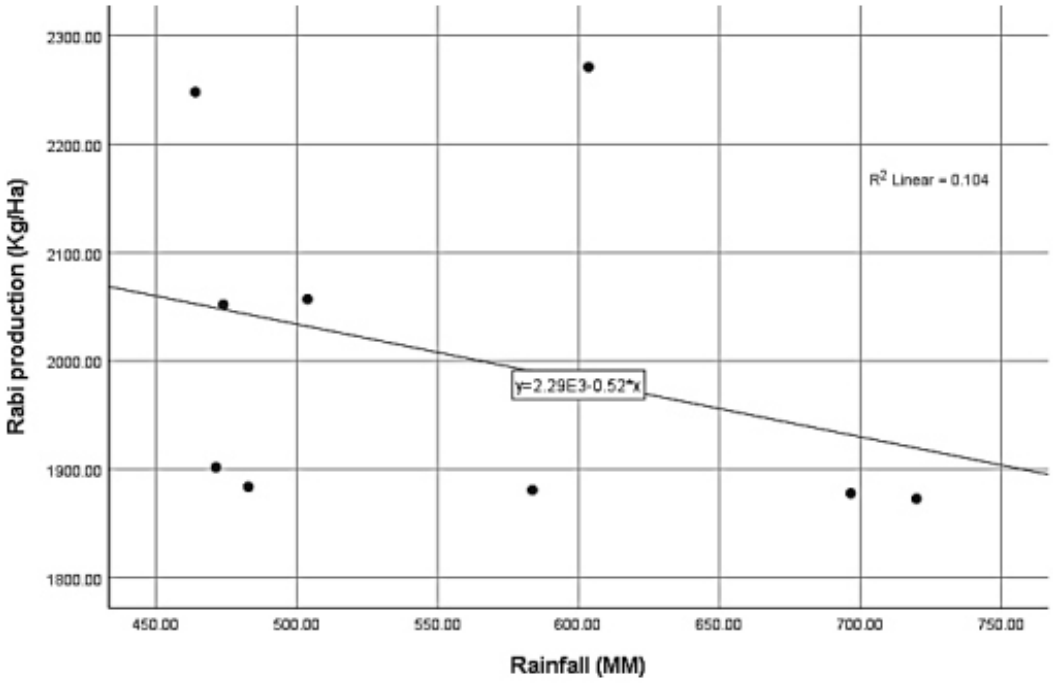

Figure-3: Rainfall and Rabi productivity (Kg./Ha)

The figure-4 shows the correlation between rainfall and total productivity (Kg./Ha) of Kharif crops. In graph we can see that Kharif crops production have high positive correlation with rainfall, because Kharif crops are fully dependent on rainfall.

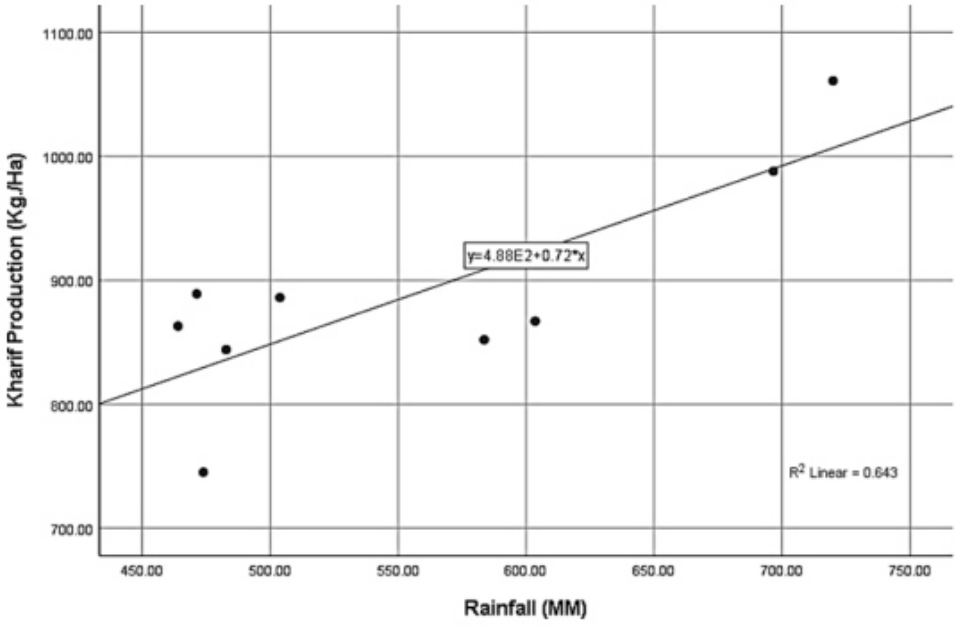

Figure-4: Rainfall and Kharif productivity (Kg./Ha)

5. Conclusion

Thepresent paper concludes that the agriculture is measured as the strength of India's economy as there is a large support from agriculture in Indian GDP (Gross Domestic Product), by adding 16.5% GVA (GrossValue Added) in 2019-20. In this paper we performed the analysis of the impact of rainfall on different season of crops and then calculated the productivity to find out the economic growth of the region due to the rainfall. For this, the Rainfall and crop production data is taken from the Agriculture department, Government of Rajasthan. Then, Rajasthan state average rainfalls in millimeter from 2010 to 2018 are takenfor experimentation. This data is taken from 2010 to 2018 time period of total production of Rajasthan state of all three seasons of crops as mentioned in the paper. The results showed that, there is a positive impact of state average rainfall onto the total Kharif crop production.

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