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Mozambique Civil War and Farida Karodia's *A Shattering of Silence*

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ABSTRACT

Mozambique civil war was fought between Mozambique's ruling Marxist 'Front for the Liberation of Mozambique' (FRELIMO) and anti-communist insurgent forces of the 'Mozambican National Resistance' (RENAMO). Through the story of Faith, the novel reveals the reality of hundreds of thousands of children all over the world who are the victims of war, hunger, and political corruption. Being the sufferer of apartheid system, Farida Karodia has extensively written about the war, segregation during the apartheid, social and political situations in South Africa. 'A Shattering of Silence' deals with the history of colonialism and its brutal effects on the people of Africa.

***Keywords*-** war, apartheid, segregation, colonialism.

A Shattering of Silence is written by South African writer Farida Karodia. Being the sufferer of apartheid system, she has extensively written about her past, war, segregation during the apartheid, social and political situations in South Africa. *A Shattering of Silence* deals with the history of colonialism and its brutal effects on the people of Africa. Colonialism comes through the violence which is one of the prominent features of apartheid system. Many African novelists wrote in the apartheid and post-apartheid period use violence as a weapon to express the effects of ongoing war and cultural awareness of that country.

In the present text, Farida Karodia talks about Mozambique civil war that took place between 1977 and 1992. Mozambique got independence in 1975. After independence, the civil war began. The war was fought between Mozambique's ruling Marxist 'Front for the Liberation of Mozambique' (FRELIMO) and anti-communist insurgent forces of the 'Mozambican National Resistance' (RENAMO). FRELIMO were trying to establish a single party socialist state. However, RENAMO strongly opposed the FRELIMO attempt. RENAMO were supported by anti-communist governments in Rhodesia and South Africa. In the civil war between FRELIMO and RENAMO around one million Mozambicans were killed and starved due to shortage of food supply. The civil war ended in 1992 and the government was established by FRELIMO. The war broke the daily lives of the people and physical infrastructure of the rural area of Mozambique. It includes hospitals, rail lines, roads, and schools. In the present text, Farida Karodia talks about the violence of colonialism that emerged due to the Mozambique civil war.

A Shattering of Silence is divided into five books and has twenty chapters. This novel has Prologue and Epilogue in which the novelist tells about the narrator of the story, Faith. The prologue reveals that Faith's story is representative of the reality of hundreds of thousands of children all over the world who are the victims of war, hunger, and political corruption. The novel deals with the suffering experienced by children of war.

The novel begins with Faith's journey to Mozambique, after 30 years of exile. Along with her there is an American woman on the journey known as 'Sue'. Faith is the narrator and she explains to her friend how issues of the African continent are misinterpreted by western governments. According to her, the real life of Africa and the concept of Africa that westerns associate with their social, political, and cultural structures are different one. She has spent many years of her adulthood

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in Mozambique. She has been the victim of the Mozambique civil war. So in 1972, with the help of Portuguese government forces and colonial secret police, she escaped from Mozambique. Now after 30 years of exile, she returns to visit her past in her home country. In this journey, she recalls her past, childhood memories, her family, and friends. She realizes her return brings a flood of memories that she is unable to control. She tries to search various images from her childhood but she becomes aware that the war of colonization has changed many things. Even though, she has a different pride about Mozambique and it can be sensed in her explanation to her friend:

> "Although Mozambique is on the African continent, it has its own social, tribal, cultural, linguistic and economic structures that distinguish it from other countries or the continent"(Karodia ix).

With all these memories, Faith arrives at Mozambique and her past drags her in the memories of her childhood, when she is six years old. She lives in the remote village of Mozambique. Her parents, Rebecca and Alex Smith are the Protestant missionaries who have left a cosy life in Manitoba, Canada to settle in Mozambique. After living in the remote village, Faith's parents notice that the life of inhabitants is more difficult and suffering one in Mozambique, due to the control of colonial society. The war of liberation created the sense of inferiority among indigenous. The land and fields of villagers are owned by Raul Morais - one of the largest landowners in the region. The Portuguese colonial estate owners in her remote village are very powerful and they oppose the government forces. Joseph Coelho is one of the laborers, who oppose the procedure of landowner, Raul Morais. This causes a frightful massacre in the village of Mozambique.

Faith recalls the day, when she witnesses the violence that takes place in her village. The people of Senhor Morais arrive to find a labor organizer- Joseph Coelho. As village people refuse to tell the information about him, they start to shoot everyone with gunfire. Faith's father asks all family members to run and hide in the forest but they deny. Within a moment, the people of Senhor Morais destroy entire rural communities. Lodiya is Faith's nanny's daughter who pulls Faith with her and they go in a direction of forest.

Faith's carefree childhood is demolished with the arrival of people of paramilitary forces who massacre the entire village with their brutal act. Though Lodiya and Faith are hidden in the forest, they clearly view the massacre that takes place in their village. After demolishing every living being in that village the paramilitary forces return, then Lodiya and Faith come outside. They walk to the way of their home and they see the dead bodies of all villagers. As they reach home they find all family members dead. This is a great shock for Faith and she becomes traumatic by this massacre. This frightful violence leaves Faith in a stunning experience that she loses her voice and memory. Further, Faith and Lodiya go catholic mission at Sao Lucas. When Lodiya leaves the mission, Faith becomes alone and is converted to Catholicism and she shifts to Sao Thomas i.e. another mission church. In this mission church, Faith finds the children who have come there are half starved and ragged. It is a difficult life but children learn to survive. Then, a woman known as Mama Ria comes to church and she adopts Faith. At Mama Ria's home there are many children, one of them is Rita and she becomes her best friend.

After Mama Ria's death, again Faith shifts to Sao Thomas and is adopted by Dona Maria. She is sent to convent school at Santa Teresa. In this school Faith learns sign language because she is unable to utter the word. At Dona Maria's home Faith learns many things regarding Portugal war and politics. As she finishes her education at Convent, Faith is recruited in the clinic and she teaches sign language to deaf children. Later Faith is engaged with David but instead of marriage they prefer to live together. Further Faith gets attracted to Juan Guerra, who is a doctor working in the same hospital. Initially they come close to each other through their work and gradually both become attracted to each other. Meanwhile in Mozambique, the issue of 'disappearance of children' becomes a serious one. Faith becomes much worried about this and with the help of Helia de Souza and Rhonica, she tries to find the kidnapped children. Later Faith hears that the people from hospital staff provide medical assistance to the rebels. Rita is one of the members of this staff and she is arrested by the force and later beaten violently. As a result, she loses consciousness. Juan and Faith try their best to save Rita. Finally they arrive at Dona Maria's home for a safer place, as military police are searching for them. Dona Maria helps them to escape from this and she makes one plan for their escapement. With the help of this plan Dona Maria successfully relieved them out of the country. She makes arrangements for Faith and Juan to get to London and Rita is shifted to the convent at Santa Teresa. In the Epilogue of the novel, Faith tells that London is beginning as the new chapter of her life. She finishes minor surgery on her vocal cords and she gets her voice back that is lost due to the traumatic experience.

In *A Shattering of Silence*, Karodia tries to explore the effects of colonial war where a protagonist's identity and voice are lost. She focuses on the sense of loss of colonized people is very deep and distressed that creates the psychological, social, and political problem in the life of an individual. In the novel, she examines the plight of children who are brutalized by war, hunger and political corruption. The colonial conditions of the Mozambicans were pathetic and worst under Portuguese rule. The protagonist of the present text, Faith is a victim of this oppressive colonial conditions and she struggles to overcome her problem of lost identity. The suffering experienced by children of war in the present novel

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explores the predicament of Mozambican child victims of war. It is not limited to the Mozambique only but it extends to the children of 'color' in the apartheid South Africa who were also the victims of political and social abuse.

The present analysis of Karodia's *A Shattering of Silence* seeks to understand the violence experienced by Faith due to colonialism. Her formation as a voiceless character can render the problem of psychological as well as linguistic development of the person in Africa. It can be said that Faith's character is affected by unfavourable social and political conditions of colonial war. She has lost everything in her life due to this colonial violence, even her own voice and memory. After shifting at various mission churches she finds, there are thousands of children who suffer from difficult life to survive. This leads her towards the resistance and she tries to overcome her inability to speak.

Karodia in the beginning of the novel through the narrator of 'Faith' tries to show the effect of the civil war and how people in Mozambique suffer its drought. On the way back to Mozambique Faith notices the scenario of African life. She realizes the issues of poverty and unemployment are still the biggest problems. She observes people either live in shacks or they are homeless, and their children are begging. It shows how Mozambique suffered by colonization as the number of migrants increased rapidly at various lands of Africa. Faith's village is also dominated by the estate owners who behave like masters of their land. It is evident when Faith says:

"The estate owners had always controlled every aspect of village life, determining everything from what we villagers ate to what they could grow and where they could sell their cash crops. Many of these estate owners were a law unto themselves, their dictatorial actions either condoned or ignored by the colonial government" (Karodia 7).

The villagers are always considered as a slave. With recalling these colonial memories, Faith recalls her childhood. She realizes the sense of despair as she tries to reveal her past and the violence she has seen. As village people refuse to tell the people of Senhor Morais about Joseph Coelho, they start killing everyone including children, infants, cattle, men and women. The massacre is going on till midday. The highest peak of their brutality can be traced in the following incident:

"One of the men pointed his rifle at a woman known as Firipa, who was wearing a bright yellow headscarf. In her arms she held a six-month-old son, Xavier. She got to her feet and shuffled forward, her son perched on her hip. She stood before her executioner as he leveled his rifle. A single shot rang out and she fell at his feet. Her son disengaged himself from her lifeless arm and crawled close to her body, wailing loudly" (Karodia 15).

After this, the attackers kill the children and infants heartlessly. They try to smash their skulls against the hard wall; they open the bellies of the three pregnant women, and destroy their foetuses. Finally, they rape the young girls and they remain silenced when there is not a single figure alive except Faith and Lodiya (Karodia 15).

Faith and Lodiya notice that their parents are the victims of these attackers and but they are helpless. When Faith and Lodiya see their parents as dead, they find them in the worst condition. It is evident in the description of Faith:

"The bullet hole my mother's forehead had turned black. My mother's head had ultimately come to rest on my father's chest. Her braids had come undone and were drawn across his chest like two pale ropes. One of my father's legs was curled under his body. His glasses were broken, the frames askew" (Karodia16).

Faith witnesses this brutal violence. She feels that all emotions and feelings have disappeared in her mind. Faith undergoes a drastic change in her personality. She becomes very frightened as the act of the estate owner is so brutal that she views it clearly. Looking at the dead bodies of her parents, Faith becomes unconscious. She is not able to cry and utter a single word as she loses her ability of speaking. It is the worst impact of violence on her mind.

To conclude, the novel clearly shows the impact of Mozambique civil war on the people. Their life is physically and mentally shattered. Korodia has broken the silence of the victims and showed to the world the brutality of colonialism which is the sole reason for the war. Though the violence has made Faith unspeakable, after 30 years she bounces back and shatters forced silence of colonialism and Mozambique civil war. She tells in a very clear and loud voice the injustice done to the native people by the people who fought for the power.

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Rapid and High yielding One Pot Efficient Protocol for the Synthesis of Pyrazoline Derivative

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Abstract:

A facile route was developed for the synthesis of 1,3,5-trisubstituted pyrazoline derivatives from Aromatic aldehyde, aromatic ketone and phenyl hydrazine in an aqueous media by using $CoFe_2(C_4H_4O_6)_3.6H_2O$ as a catalyst at reflux condition. The reaction protocol generate 1,3,5-trisubstituted-2-pyrazolines in good to excellent yields via a one-pot addition–cyclocondensation between aromatic aldehyde, acetophenone and aryl hydrazines. The catalyst can be reused without much loss in the catalytic activity. The structures of the synthesized compounds were confirmed by analytical techniques.

Keyword: Pyrazoline, reflux, $CoFe_2(C_4H_4O_6)_3.6H_2O$, aromatic aldehyde.

Introduction:

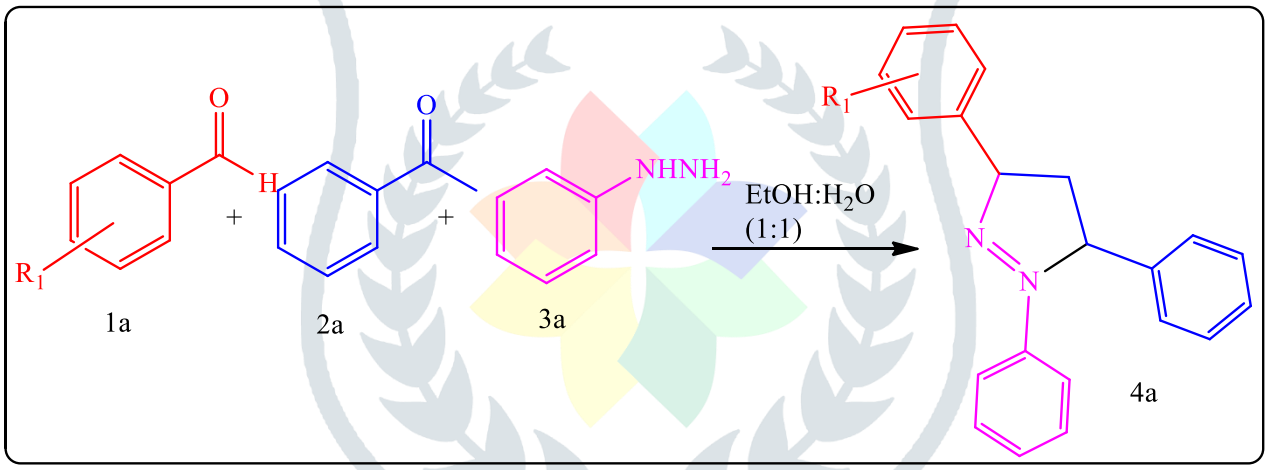
In Recent era Considerable interest has been concentrated on the pyrazole structure, which has been recognized to possess a wide range of biological activities such as tranquillizing, psycho analeptic, anticonvulsant and antihypotensive activities [1-2]. Pyrazoline derivatives are attracting many researchers, not only in biological concern of their bioactivity but also several pyrazoline derivatives possess important pharmacological activities and therefore they find wide application in drugs synthesis. Pyrazolines are biologically active moiety with a diversified biological activities like and anti-HIV [3], antimicrobial [4], antitubercular [5], antiinflammatory [6], antitumor [7], anticancer [8], anticonvulsant [9], Some of the pyrazoline derivatives are also reported to possess anti-inflammatory [10], antidiabetic [11] and antibacterial properties [12].

Experimental:

Chemicals were procured from sigma aldrich and were used without further purification. Melting points of compounds were determined by in an open capillary method and are uncorrected. The product confirmation was done by TLC plate method. IR spectra were recorded (in KBr pallets) on Bruker spectrophotometer. ^{1}H NMR spectra were recorded (in DMSO-d6) on Avance-300 MHz spectrometer using TMS as an internal standard.

General Procedure for the synthesis of pyrazoline:

Mixture of aromatic acetophenone (2 mmol), aromatic benzaldehyde (2 mmol), potassium hydroxide (20%, 5 mL) and phenyl hydrazine (4mmol) in Ethanol: Water as a solvent in 1:1 ratio (15 mL), a catalytic amount $CoFe_2(C_4H_4O_6)_3.6H_2O$ of catalyst also added and were allowed to react in round bottom flask under reflux condition. Reaction progress was monitored by using TLC plates after regular interval of timetime. After the completion of reaction, the mixture was poured to crushed ice to yield solid product. The solid compound was filtered and washed and then recrystalized using methanol to yield pyrazoline derivatives.



Catalytic Activity:

Synthesis of 1,3,5 triaryl pyrazoline is carried out by the reaction of aromatic aldehyde, aromatic ketone and phenyl hydrazine under optimized reaction condition. In order to select appropriate solvent the synthesis we carried different trial which were listed in table 1.

As seen from the table no polar benzene media yields 58 % of desired product with elevated time period of 6 hr., the formed was not satisfactory so several other trials were taken in which Acetonitrile, ethyl alcohol and water were used which deliver 66%, 74% and 68 % of product yield with time taken for the reaction 5,5,6 hr respectively. In last attempt and experiment was made by taking EtOH: H_2O as a solvent in 1:1 ratio and surprisingly product yield was raised to 82% with short of reaction time.

Table 1: Optimization of solvent for reaction condition.

Entry	Solvent	Time (hr.)	Yield (%)
1	C_6H_6	6	58
2	CH_3CN	5	66
3	EtOH	5	74
4	H_2O	6	68
5	EtOH:H_2O	4	82

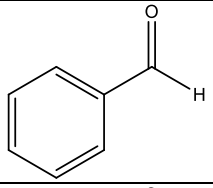
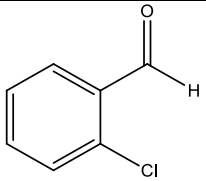
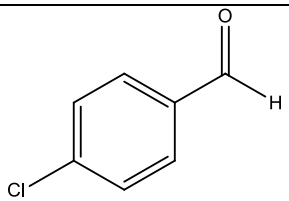
Optimization of Amount of catalyst was confirmed by carrying different set of separate reaction with different amount of catalyst and it was found that synthesis of 1,3,5 triaryl pyrazoline was found to be most feasible with 10 mol% of catalyst.

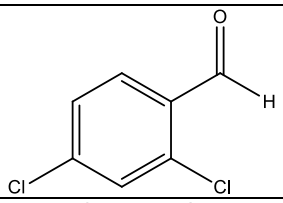
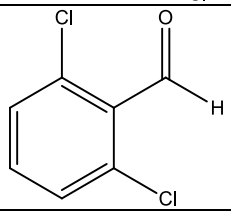
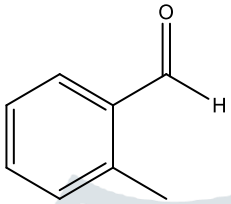
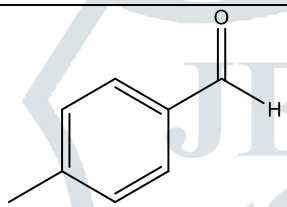
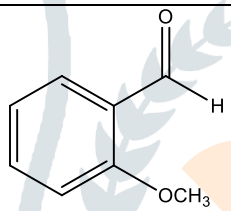
Table 2: Optimization of amount of catalyst for reaction condition.

Entry	Amount (mol%)	Time (hr.)	Yield (%)
1	5	4	78
2	10	4	82
3	15	3.5	86
4	20	3	86

Synthesis of 1,3,5 triaryl pyrazoline by one pot cyclocondensation of substituted benzaldehyde, aromatic ketone and phenyl hydrazine under the $CoFe_2(C_4H_4O_6)_3.6H_2O$ as a catalyst under reflux condition. The reaction was tested with differently substituted electron withdrawing and releasing group. The result was displayed in table 3. As seen from the Table 3 electron withdrawing group dominates the yield of pyrazoline product. Entry 4 clearly shows 90% of pyrazoline was obtained with very short of time 3hr. Table 3 indicates that synthesis of pyrazoline under these optimized redaction condition delivers good to Excellent yield.

Table 3: Optimization of Synthesis of 1,3,5 triaryl pyrazoline from differently substituted aromatic aldehyde

Entry	Aromatic Aldehyde	Product	Time Hr.	Yield[X]
1		**4a**	4	82
2		**4b**	3	88
3		**4c**	3.5	86

4		**4c**	3	90
5		**4d**	3.5	85
6		**4e**	5	71
7		**4f**	4.5	74
8		**4g**	6	68
X represent isolated product				

Conclusion:

Synthesis of 1,3,5 triaryl pyrazoline from aromatic aldehyde, aromatic ketone and phenyl hydrazine under reflux condition by using $CoFe_2(C_4H_4O_6)_3.6H_2O$ catalyst afford good to excellent yield. The major advantage of this protocol is short reaction time, excellent yield and easy recovery of catalyst at the end of reaction. reaction proceeds smoothly and the catalyst used three time for further synthesis.

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Effect of climate-smart agriculture practices on energy, greenhouse gas mitigation and resource use efficiency of rice-wheat cropping system in North West IGP: A review

PK Singh, RK Naresh, Rajan Bhatt, Himanshu Tiwari, Aashu Rajput, Lavudya Sampath, Smruti Ranjan Padhan and Goyal Akash M

Abstract
Rice–wheat cropping system in north-western Indo-Gangetic Plains performed a crucial role in the national food security. However, the widespread and intensive cultivation of this system has led to serious problems such as declining groundwater table with sharp increase in number of districts under over-exploitation category, higher greenhouse gases emission and herbicide resistance in weeds, causing stagnant crop productivity and lesser profitability. In this review article, an attempt has been made to discuss the major issues pertaining to intensive rice–wheat cultivation amidst climate vagaries and futuristic approach to address these challenges. Intensive tillage operations, indiscriminate use of irrigation water, chemical fertilizers, and pesticides and crop biomass burning have made the conventional rice–wheat (RW) system highly energy-intensive and inefficient. In the recent past, portfolios of climate-smart agricultural practices (CSAP) have been promoted as a potential alternative to improve the energy efficiency in conventional RW system. Therefore, to evaluate the energy input–output relation, energy flow and economic efficiency in various combinations of crop management options, a review study was conducted. The net energy, energy use efficiency and energy productivity were 11–18, 31–51 and 29–53% higher under CSAP in RW system than conventional tillage without residue, respectively. However, renewable and non-renewable energy inputs were 14 and 33% higher in conventional tillage without residue compared to CSAP, respectively, it showed that conventional tillage without residue practices mostly dependents on non-renewable energy sources whereas CSAP dependents on renewable energy sources. Similarly, the adoption of CSAP improved the biomass yield, net farm income and economic efficiency by 6–9, 18–23 and 42–58%, respectively compared to conventional tillage without residue. Greenhouse gas emissions were also ~63% higher in conventional practices compared to CSAP. The energy input of under traditional method was 85.4 GJ/ha, and the energy output was 59.7 GJ/ha. Among all energy input elements, mineral fertilizers accounted for the highest proportion of energy input, accounting for 48.31%. Under water-saving irrigation, the energy input and output are 72.3 GJ/ha and 62.3 GJ/ha; the highest energy input is also mineral fertilizer. The total input energy for rice-wheat cultivation as 63825 and 50799 $MJha^{-1}$ respectively. Main contributors are electricity, fertilizer and diesel for both crops; however irrigation water is also a significant contributor in rice. The yield per unit energy use is relatively low and warrants better crop management practices to reduce the environmental footprint of the rice-wheat cropping system. Overall, the adoption of CSAP could be a viable alternative for improving energy use efficiency, farm profitability and eco-efficiency in the RW system.

Keywords: Conservation tillage, greenhouse gases, soil quality, energy efficiency

Introduction
Agriculture is a major driver of climate change. According to 5th FAR (IPCC fifth assessment report), Agriculture and its allied sciences contribute 20-24% of human induced GHGs emission and IPCC estimates that agricultural contributes about 13.5% of GHGs emission. These emissions are largely from the results of synthetic fertilizers use; methane from large scale animal operation and some methane are released from rice paddies. It is projected that climate change affects around 49 million people at risk of hunger by 2020. RW system of the IGP, the energy is expensed in several forms such as labour, farm machines, fertilizers, insecticides, fungicides and herbicides, electricity for pumping irrigation water, manual transplanting of rice seedlings into the well-puddled soils etc. But presently, RW system are showing energy insecurity in the IGP`s region due to intensive energy used in various crop

production activities such as multiple tillage to get ready the field for rice and wheat planting (Kakraliya *et al*., 2018; Chaudhary *et al*., 2009) [15, 5]. Further, the use of more manual labour in transplanting of rice seedlings into well-puddled soil also consumes an enormous amount of energy. In PTR, puddling alone needs approximately 25–30% of the total irrigation water requirement of rice (Kakraliya *et al*., 2018) [15]. Higher water requirement in rice is also due to more water losses in the form of puddling, percolation and surface evaporation which ultimately leads to more consumption of electricity for groundwater pumping for puddling, nursery raising and frequent irrigation to keep the fields flooded throughout the growing season (Kakraliya *et al*., 2018) [15].

In upper and middle IGP, irrigation water is mostly driven by electricity pumps whereas in lower IGP diesel pumps are mainly used, and both consume a huge quantum of energy Kakraliya *et al*., 2018) [15]. Approximately 84% of wheat production costs incurred from these energy-intensive inputs (Saharawat *et al*., 2010; Naresh *et al*., 2018) [27, 20]. In South Asia and elsewhere, published outcomes from diverse research findings have highlighted that intensive tillage practices accounts~25% or more of the total production cost in RW system. This energy-intensive system has started suffering from other production fatigue owing to over mining of nutrients, declining factor productivity, increasing production cost, reducing farm profitability, deteriorating soil health and labour shortage causing concern about its sustainability (Kakraliya *et al*., 2018; Abbas *et al*., 2020) [15, 1]. Escalating the production and energy costs in the RW system is not only harmful to keeping productivity and farmers' farm incomes but are also a major challenge for global food and energy security (Abbas *et al*., 2020) [1].

Energy smart agriculture (ESA) practices namely laser land levelling, zero tillage (ZT), direct-seeded rice (DSR), site-specific nutrient management (SSNM) and precision irrigation management have been suggested as potentially sustainable alternatives to traditional energy-intensive practices. Non-requirement of intensive tillage operations in energy-smart agriculture translates into less diesel requirement, lesser working time and slower depreciation rates of equipments. These all are reducing energy inputs in various farm operations, particularly from land preparation, as well as from the agricultural machinery manufacturing processes. By adopting the ESA-based ZT system under the RW system, farmers could save 36 L diesel ha^{-1} which is equivalent to 2027 MJ ha^{-1}. In addition, energy-intensive agricultural practices have high carbon footprints especially; greenhouse gases (Yuan & Peng, 2017) [36] have enhanced the global energy budget by more than 10 times since the beginning of twentieth century (Pratibha *et al*., 2015) [16] and at the same time increased the cost of cultivation in crop production by approximately 4 times than ZT farming during the same period (Parihar *et al*., 2017; Naresh *et al*., 2021) [22, 21]. Therefore, energy requirements can be minimized by adopting of energy-efficient technologies. Furthermore, adequate availability of the accurate source of energy and its effective and proficient use are the prerequisites for the conventional RW system with the lowest energy inputs (Yuan & Peng, 2017) [36]. In energy budgeting, it is essential to identify or develop energy-efficient technologies, with less energy and environmental footprints. A number of climate smart agriculture (CSA) practices have been assessed in cereal systems as an alternative to energy-intensive traditional practices. So far, information on energy footprints of these practices together (as a portfolio) is scanty. Hence, there is an urgent need for a scientific assessment to use a holistic tactic of principles and procedures known to increase the energy-use efficiency (EUE) and decrease the input energy as well as associated carbon footprints in crop production.

Researchers expressed concerns on sustainability of rice cultivation associated with high energy demand, deterioration of the groundwater table and escalating cost of groundwater pumping from deeper depth as a result of puddling and ponding practices (Naresh *et al*., 2018; Chauhan *et al*. 2012) [20, 6]. It is estimated that out of total energy input (52.4 ±1.3 GJ ha^{-1}) required for rice cultivation, irrigation water uses about 40% of total energy followed by 17.7% for electricity in pumping out of groundwater (Singh *et al*., 2019) [33]. This is also accompanied by increase in associated carbon dioxide (CO_2) emissions emitted during the multiple wet tillage operations in puddling and water pumping in the cases where stationary diesel engines are used as power source. The practice of intensive puddling and continuous flooding in rice field also promotes methane (CH_4) emission as a result of methanogenesis (Sapkota *et al*. 2015) [30]. Grace *et al*. (2003) [11] reported that rice–wheat system emitted greenhouse gases with global warming potential of 13–26 Mg CO_2 ha^{-1} yr^{-1} in Indo-Gangetic Plains. The environmental threats of intensive rice cultivation are also encouraged by dominating chemical-based weed control strategies. Thus, climate smart (CSA) improves the EUE, decreases the carbon footprints, cost of production and efficient use of production inputs in the RW system without jeopardizing the productivity of the crops relative to those for the conventional management practice of the RW production system, and offers a hygienic and environmentally sustainable energy use efficient production technology for this IGP region of India. This review mainly focuses on the importance of the assessment and insight into: (1) to find out the energy conservation and energy-efficient agricultural practices for the RW system in western IGP of India; (2) to assess the key energy indicators and inputs for the RW system; and (3) reducing and/or removing GHGs, in the RW system.

Climate smart agriculture strives to sustainably increase productivity and profitability build resilience and adaptive capacity; where possible reduce greenhouse gas emissions (GHGs). CSA is an approach that helps to guide actions needed to transform and reorient agricultural systems to effectively support development and ensure food security with changing climate. Development of appropriate adaptation, mitigation of GHGs and food security strategy under rice-wheat production condition is important to cope with the progressive climate change and variability.

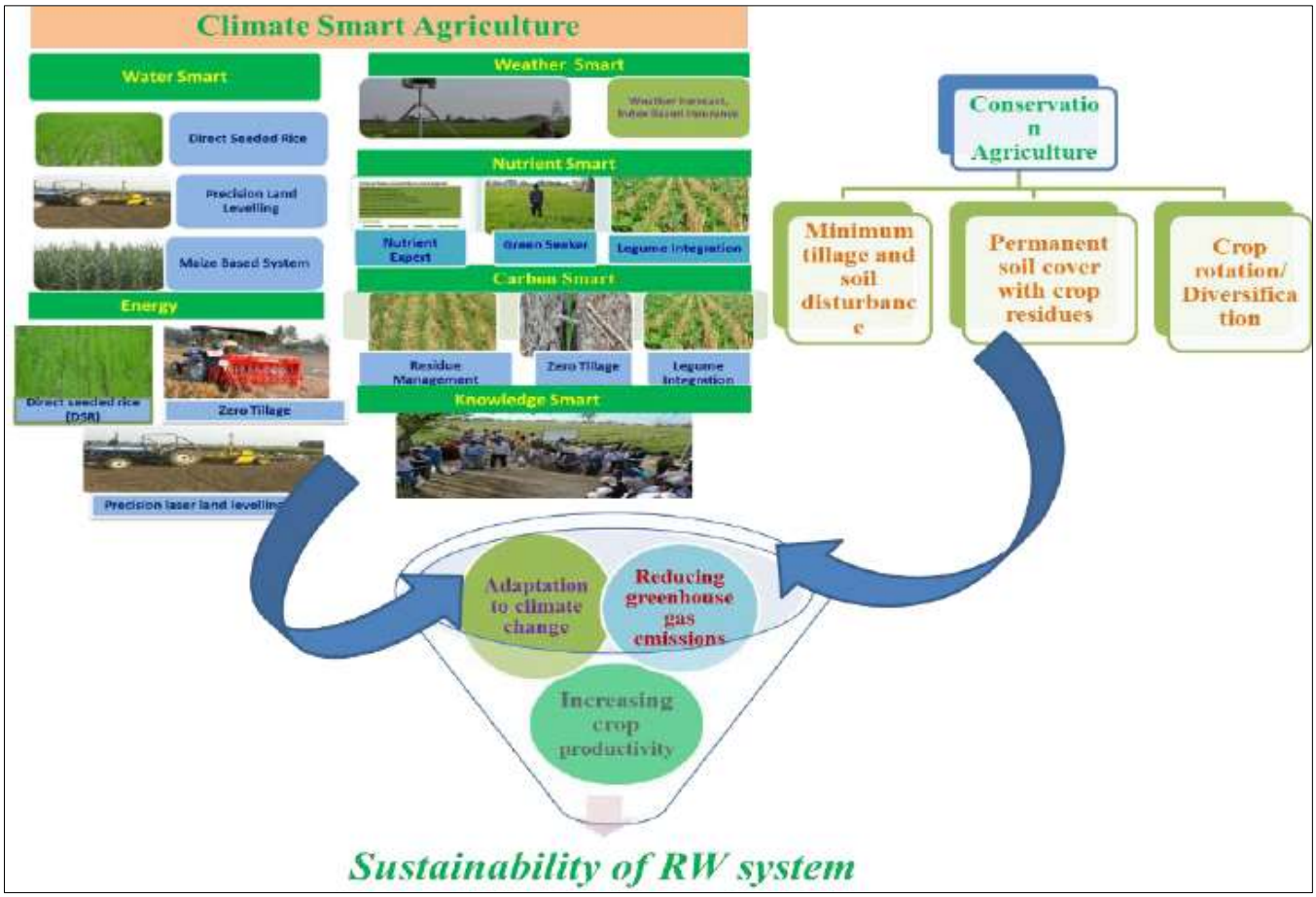
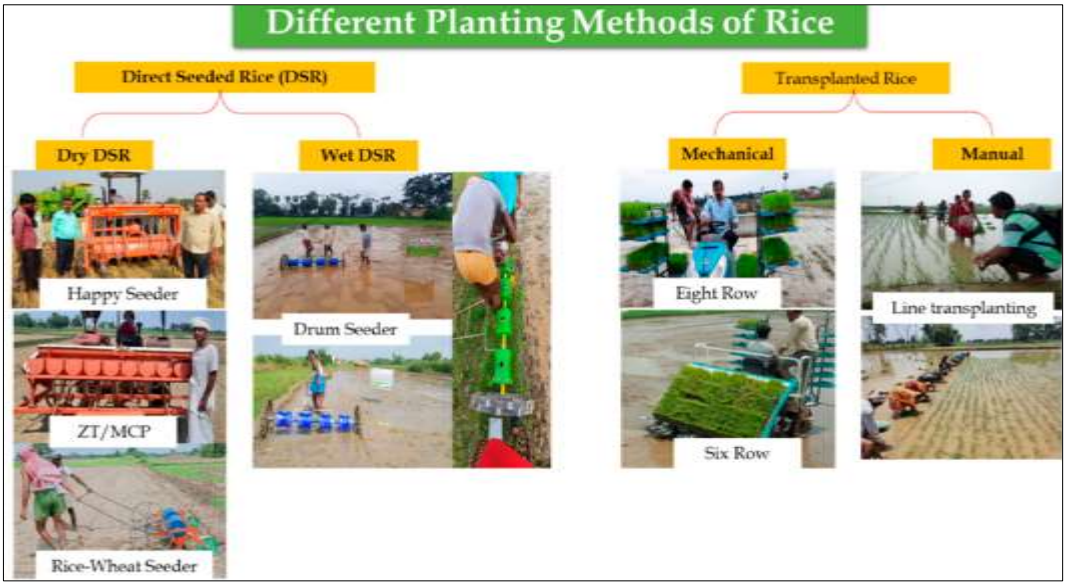


Fig 1: Climate smart agricultural and conservation agriculture based management practices for sustainability of RW system

Crop Establishments

The comparison of energy use pattern from different crop establishment methods of wheat revealed that the highest input energy consumption was(22164.8 MJ ha^{-1}) for CT and the lowest (18948.5 $MJha^{-1}$) was for PRB which was closely followed by ZT(19861.9 MJ ha^{-1}). The higher energy consumption under CT than ZT, attributed to more tillage operation. Residue retention proved 56% higher energy consuming than residue removal. The reason for higher energy use for R_R attributed to 40-cmresidues left in situ. Compared to zero N, the energy inputs were higher by 71% and 61% for 120 kg Nha^{-1} and 100 kg Nha^{-1}, respectively. The energy outputs for the TCE methods varied significantly. However, the highest energy output (75191 MJ ha^{-1}) was obtained from ZT followed by CT (66908 MJ ha^{-1}) and the lowest from PRB (64361 MJ ha^{-1}). Residue removal proved more energy output (7448 MJ ha^{-1}) than residue retention as residues added more to energy output. The abundant N (120 kg ha^{-1}) produced the highest energy output (88473 MJ ha^{-1}) followed by farmers' N (100kg ha^{-1}) of 83795 MJ ha^{-1} and the lowest (34192 $MJha^{-1}$) from zero N application. The energy use efficiency was 3.78, 3.4 and 3.02% for ZT, PRB, and CT, respectively. The higher energy use efficiency under ZT was mainly attributed to higher energy production with the use of relatively lesser energy utilization.

Sah *et al*. (2014) reported that with 100 kg N ha^{-1} and residue removal, CT consumed the highest energy input (19642.5 MJ ha^{-1}) followed by ZT (18314.4 MJ ha^{-1}) and the lowest from permanent raised bed (16866.5 MJ ha^{-1}). The maximum energy utilization was through fertilizers application in all the TCE methods. Conventionally grown wheat consumed energy on irrigation (24.3%), threshing and cleaning (18%), seeding (9.5%), tillage and crop establishment (9.1%), harvesting (1.8%), and the least (1.6%) on chemical application, while, PRB wheat consumed energy on threshing and cleaning (22.1%), irrigation(17%), TCE (8.2%), seeding (7.2%), harvesting (2.1%), and the least (1.8%) on chemical application. Zero-till wheat utilized energy on threshing and cleaning (27%), irrigation (17.9%), seeding (10%), TCE (3.2%), harvesting (1.9%), and the least (1.7%) on chemical application. Thus, the minimum TCE cost was associated with ZT as seed sowing was accomplished in one tractor-pass. About one-fourth of the total energy consumption was spent on irrigation applications in CT, while, they were 17.9 and 17% in ZT and PRB, respectively, as more water was required for CT than others.



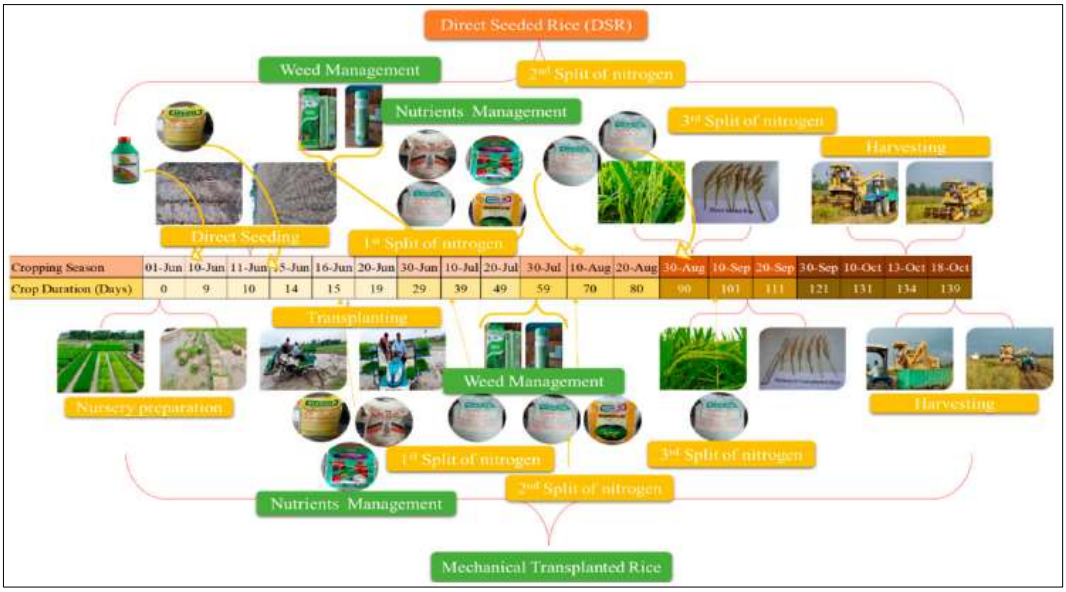


Fig 2: Different management practices under direct seeded and mechanically transplanted rice

Source and operation wise energy utilization pattern

Kakraliya *et al*. (2022) [17] revealed that an energy used in different field operations under various crop management activities was significantly affected by the rice establishment methods and was ranged from 422 to 436 MJ ha^{-1} (Fig. 1). Business as usual (Sc1) with high energy intensive practices consumed the highest (4336 MJ ha^{-1}) energy in seed bed preparation, whereas in Sc5 and Sc6 no energy was required

for seed bed preparation (Fig. 1). CSAP (mean of Sc4, Sc5and Sc6) consumed 57% less energy in crop establishment (transplanting/ sowing) operations compared Sc1 (978 MJ ha^{-1}). Irrespective of field operations, tillage consumed highest input energy in conventional management practice of RW system. This was due to repeated (5–6 passes) dry and wet tillage to prepare a seedbed for nursery raising and puddling consumed more diesel in machinery in Sc1. In addition to this, Sc1 and Sc2required 15–20 additional manual labour for transplanting rice seedlings.

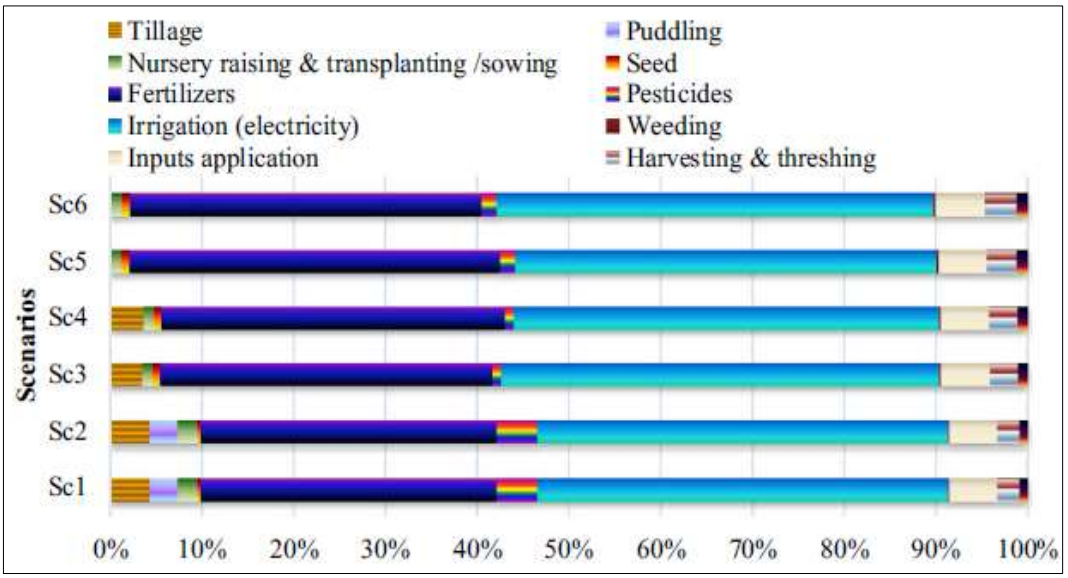


Fig 1: Operation-wise input energy-use pattern (%) under different management practices in rice. Where; Sc1, business as usual-conventional tillage (CT) without residue; Sc2, CT with residue; Sc3, reduce tillage (RT) with residue + recommended dose of fertilizer (RDF); Sc4, RT/Zero tillage (ZT) with residue + RDF; Sc5, ZT with residue + RDF + Green Seeker + Tensiometer; Sc6, Sc5 + Nutrient expert.

Zhang *et al*. (2023) reported that the total energy input under the surface irrigation method is 85.4 $GJha^{-1}$, of which the largest energy input is inorganic fertilizer, accounting for 48.31%, followed by electricity and labor, accounting for 13.74% and 12.19%, respectively. The smallest proportion of energy input is organic fertilizer, accounting for 2.58%. The total energy input under the water-saving irrigation method is 72.3 $GJha^{-1}$, showing a decrease of 15.42% compared with the total energy input of the surface irrigation method (Fig.2). The largest proportion of the energy input is still inorganic fertilizer, accounting for 35.7%, followed by the materials of the water-saving irrigation system and electricity. The proportion of the energy input is 16.06% and 13.73% respectively. The smallest proportion of the energy input is organic fertilizer, which accounts for 1.25%.

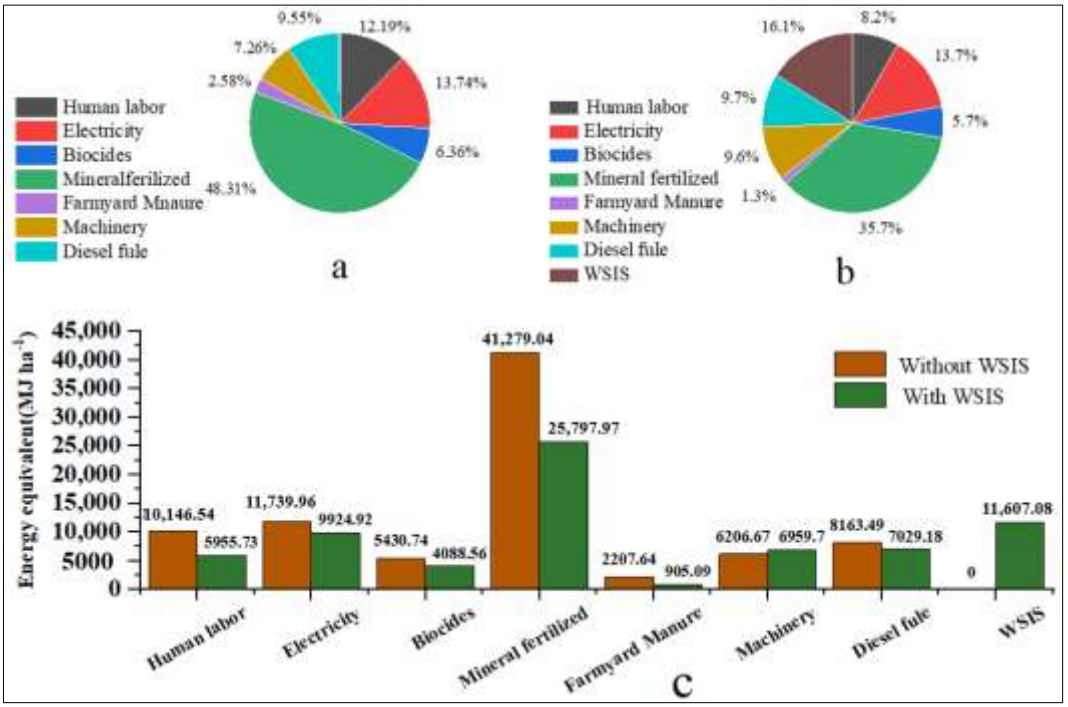


Fig 3: Composition of energy input under different irrigation methods

Kakraliya *et al*. (2022) [17] also found that in wheat, energy used under different management practices for seedbed preparations ranged from 892 to3078 MJ ha^{-1} and were significantly affected by crop establishment method. In seedbed preparation, Sc1 and Sc2 consumed highest energy (2228 MJ ha^{-1}) followed by Sc3 (1382 MJ ha^{-1}), whereas in Sc5 and Sc6no energy was required for seed bed preparation. Sc3-Sc6 consumed ~ 53% less energy in seedbed preparation and in sowing compared to Sc1 (Fig. 3).

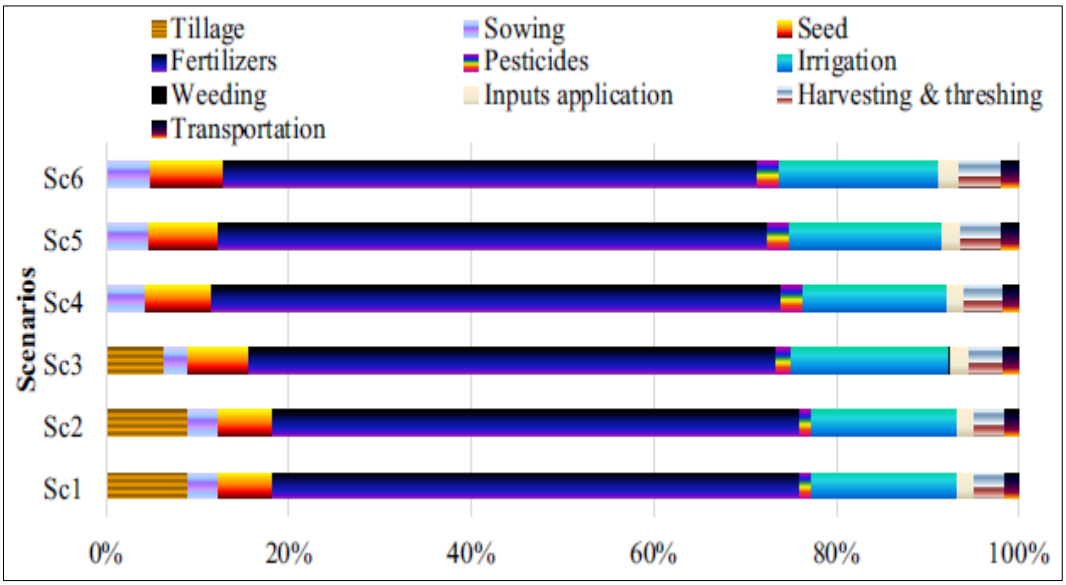


Fig 4: Operation-wise input energy-use pattern (%) under different management practices in wheat. Where; Sc1, business as usual or conventional tillage (CT) without residue; Sc2, CT with residue; Sc3, reduce tillage (RT) with residue + recommended dose of fertilizer (RDF); Sc4, RT/Zero tillage (ZT) with residue + RDF; Sc5, ZT with residue + RDF + Green Seeker + Tensiometer; Sc6, Sc5 + Nutrient expert

Diljun *et al*. (2023) observed that direct energy constituted 70% of total input energy in rice with a share of 44,613.28 MJ*ha*$^{-1}$. Direct energy made up 59% of input energy in wheat production with a share at 30,047.85 MJha^{-1}. The residual is indirect energy (30% and 41% for rice and wheat respectively). The renewable energy has a minor share and non-renewable energy accounted for 75% and 76% share in rice and wheat respectively. However, Energy use efficiency was estimated at 2.53 for rice and 2.15 for wheat. If energy use-efficiency is above 1, then the production system is generating energy and specific energy is estimated at 10.98 and 12.77 MJkg^{-1} for rice and wheat respectively (Fig.4a & 4b).

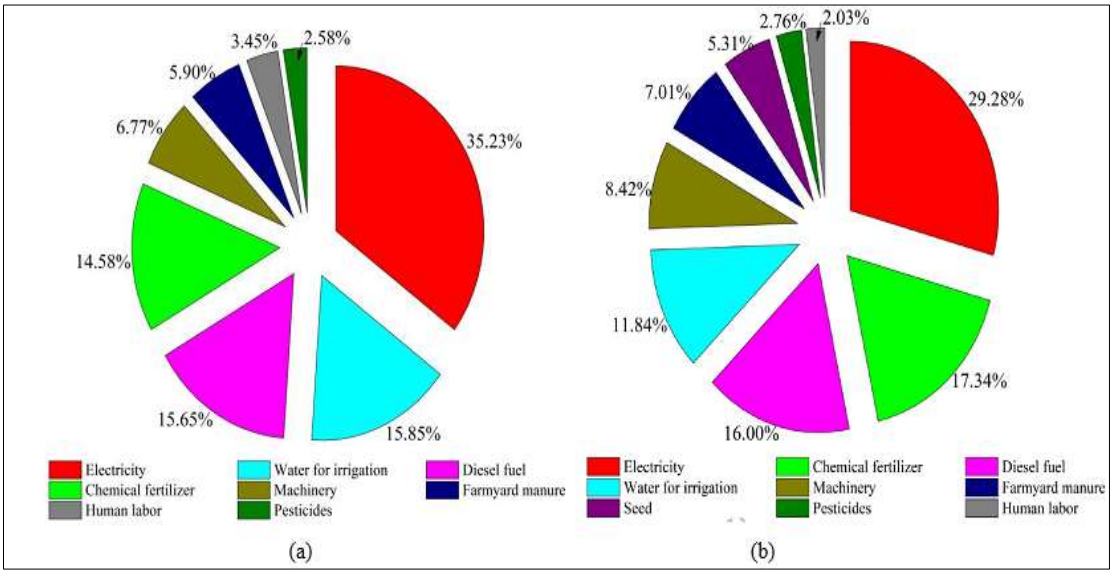


Fig 5: Percentage share of various constituents in total energy input in (a) rice production and (b) wheat production

Kakraliya *et al*. (2022) [17] observed that business as usual (Sc1) consumed more energy because of it required more tillage operations in seedbed preparation. However, in CSAP, tillage is not required for seeded preparation and energy is used only for seed sowing. On the system basis, CSAP consumed 76% less energy in seed bed preparation compared to Sc1 (7416 MJ ha^{-1}) (Fig. 5). The higher energy consumption in tillage could be due to fewer usages of modern agricultural machineries and higher use of human & animal power in conventional RW production (Fig. 3).

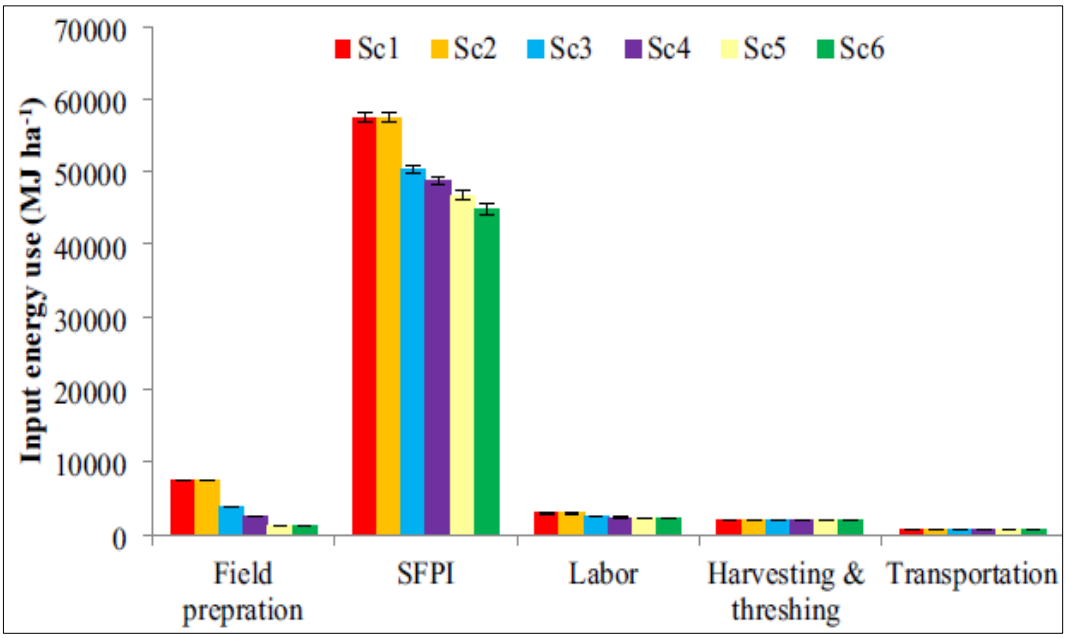


Fig 6: Operation-wise input energy-use (%) of RW system under different management practices. Where; SFPI are seed, fertilizer, pesticides and irrigation. Sc1, business as usual-conventional tillage (CT) without residue; Sc2, CT with residue; Sc3, REDUCE tillage (RT) with residue + recommended dose of fertilizer (RDF);Sc4, RT/Zero tillage (ZT) with residue + RDF; Sc5, ZT with residue + RDF + Green Seeker + Tensiometer; Sc6,Sc5 + Nutrient expert

Diljun *et al*. (2023) reported that the net energy gain is estimated at 97487.82 and 58476.09 MJha^{-1} for rice and wheat respectively. Per kilogram net energy gain for rice is 16.70 which is significantly higher than wheat (14.70) thereby implying that the production of rice leads to higher energy gain for every unit of production. The combined net energy gain of the rice-wheat cropping system is estimated at 155963.91 MJha^{-1} which is well within the range estimated by (Soni *et al*., 2018) [35] for fertile Indo-Gangetic Plains (1537900 to 2685100 MJha^{-1}). The agrochemical energy ratio for rice is 17% and for wheat its 20%. A high ratio implies large agrochemical footprint and negative environmental effects as nitrogen leaching, pollution in air and water and greenhouse gas emission (Pishgar *et al*., 2013) [24]. The higher consumption of nitrogen in the total input energy is the reason for the higher ratio in wheat. However, the ratio for both rice and wheat is lower than comparable studies in Iran which estimated the ratio in the production of corn as 40% which illustrates a chemical-intensive production system. The energy productivity for rice and wheat is estimated at 0.09 and 0.08 kgMJ^{-1} respectively.

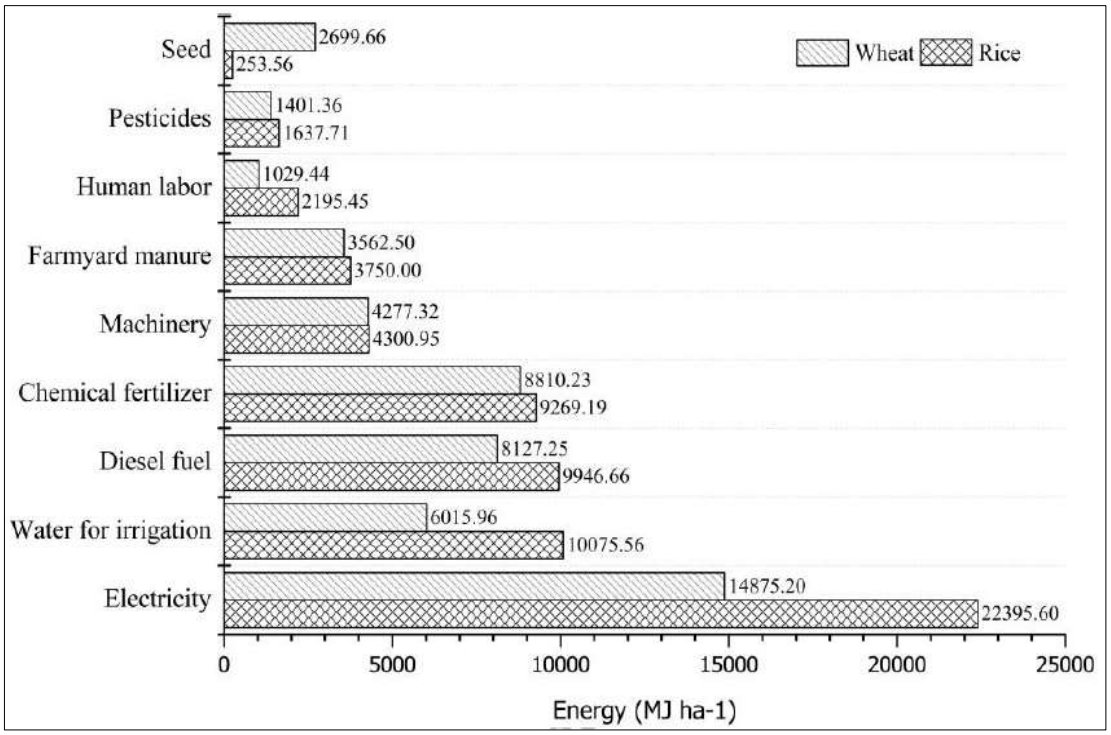


Fig 7: Input wise energy consumption for rice and wheat

Global Warming Potential

Agricultural activities contributes to the emission of three important greenhouse gases leading to the global warming–carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O). The share of agriculture to the emission of N_2O, CH_4, and CO_2 are 60%, 39%, and 1%, respectively (OECD, 2001). Rice based cropping system plays the major role to the emission of greenhouse gases (Fig. 5). Conventional flooded rice culture with puddling and transplanting is the major source of CH_4 emissions as prolonged flooding creates an anaerobic soil conditions accounting for 10–20% (50–100 Tg yr^{-1}) emission. Methane formation depends on the metabolic activity of a group of bacteria and activity of methanogen bacteria increases in anaerobic condition. The major pathways of CH_4 production in flooded soils are the reduction of C compounds to CH_4 due to restricted oxygen supply. Anaerobic condition is the pre-requisite for the activities of methanogenic bacteria and CH_4 production. Thus, CH_4 is low under aerobic condition. In the conventional transplanted rice field standing water is kept throughout the crop growing season and thus the methane emission is higher in this case while DDSR field is not continuously submerged and therefore, CH_4 is less in the DDSR field (Joshi *et al*., 2013) [38].

Atmospheric carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) had been accepted as the potential source of greenhouse gases (GHGs) that had significantly contributed to global warming due to their great radiative forcing (IPCC, 2007). Global agriculture contributed 10-12% to the net anthropogenic greenhouse gases (GHG) emissions estimated as 5.1-6.1 Pg CO_2-eq yr^{-1} in 2005 (IPCC, 2007). However, there could be a great potential to reduce total GHGs emission in agriculture by improving soil organic carbon (SOC) storage and/or decreasing CH_4, N_2O and CO_2 emissions through improving crop production techniques (Smith *et al.,* 2008) [34]. The emission of methane from the soil in puddled transplanted rice ranged from 0.8 to 1.9 t CO_2 equivalent ha^{-1} in various districts of Punjab compared to only 0.1–0.3 t CO_2 equivalent ha^{-1} in DDSR (Gartaula *et al*., 2020) [10]. The average global warming potential due to all the three greenhouse gases (carbon dioxide, methane, and nitrous oxide) in transplanted rice was 2.91 t ha^{-1} compared to 1.94 t ha^{-1} in DDSR (Gartaula *et al*., 2020) [10]. Gupta *et al*. (2016) [12] reported significantly low methane emission (82–87.2%) in the DDSR as compared to the puddled transplanted rice. DDSR leveraged with short or medium duration rice varieties/hybrids with early maturity and faster field vacation helps in conserving residual soil moisture useful for crops in rotation, widening the time window for effective residue management, and also facilitates in early or timely sowing of long-duration wheat varieties ultimately leading to enhanced system productivity, profitability, and sustainability.

The crop establishment method, cropping system followed and management of nutrient, water and pests are the key agronomical components responsible for remittance of greenhouse gases from agricultural fields. Methane is the second most important greenhouse gas after carbon dioxide (CO_2) and a single molecule of methane (CH_4) traps nearly 28 times as much heat, as does the CO_2. The studies on methane emission measurement indicated that CH_4 emission is primarily dependent on parameters such as frequency of water drainage, soil types, soil temperature (Parashar *et al*., 1991) [40] along with organic and inorganic fertilization (Singh and Benbi 2020). The formation of plough-pan or hard pan in wet tillage under conventional rice cultivation holds the water and blocks the soil pores, resulting in increased CH_4 emission. Further, these all processes depend upon decomposition rate of soil organic matter and soil redox potential (Saini and Bhatt 2020; Singh and Benbi 2020) [28, 32]. For instance, the production of 1 kg of rice returns 2.6 times more CO_2 equivalent emission to the environment than other cereals. Singh and Benbi (2020) [32] reported emission of 0.2 kg CO_2 equivalent per kg grain in rice–wheat system as compared to 0.1 kg CO_2 equivalent per kg grain in maize-wheat system. In puddled transplanting fields, intermittently flooding with single and multiple aerations reduced methane emission and lower global warming potential by about 18.1 and 27.6%, respectively, as compared to continuously flooded fields (Singh and Benbi 2020) [32]. The conventional rice cultivation showed higher CH_4 emission (50–250 mg m^{-2} d^{-1}) than direct seeded rice (<50 mg m^{-2} day^{-1}). The total cumulative soil flux of CO_2, nitrous oxide (N_2O) and CH_4 emissions in terms of CO_2 equivalent was 27% more in conventional rice–wheat system than direct seeded rice followed by zero-till wheat along with residue retention (Sapkota *et al*., 2014) [29]. A 34% reduction in global warming potential was observed on substitution of puddled transplanted rice with direct seeded rice. Irrigation and nutrient management systems followed by farmers and conventional tillage make significant contribution to greenhouse gases emission (Sapkota *et al*., 2014; Naresh *et al*., 2021) [29, 20]. Rice residue burning is largely practiced in northern India and burning of 1 Mg rice straw releases about 280 kg CO_2–C, 3 kg CH_4 and 0.07 kg N_2O-N with a global warming potential of 1118 kg CO_2 equivalent. In the scenario of climate change, global warming potential or emission intensity of various crops should be assigned as key factor, responsible for long-term sustainability of crop production and environment. It is evident from that rice crop poses extreme high global warming potential (0.50–5.65 kg CO_2-eq kg^{-1}) over others, *viz.* maize (0.18–0.45 kg CO_2-eq kg^{-1}).

Datta *et al*. (2022) also found that lower GHGs emission ($F_{CA\text{-}High\ Fertilizer}$: 1474 kg CO_2-eq ha^{-1}) -compared to conventional practices ($F_{CP\text{-}High\ Fertilizer}$: 2400 kg CO_2-eq ha^{-1}). The intensity of GHG emissions was higher in FCP-High Fertilizer (0.37 kg CO2-eq kg^{-1}) over $F_{CA\text{-}High\ Fertilizer}$ (0.10 kg CO_2-eq kg^{-1}). Crop residue burning in conventional practices resulted higher CH_4 (788 kg CO_2 eq ha^{-1}) and N_2O emission (179 kg CO_2-eq ha^{-1}); whereas in CA, there were no GHG emissions as no burning took place. Higher N_2O emissions were estimated in $F_{CA\text{-}High\ Fertilizer}$ (559 kg CO_2-eq ha^{-1}) over $F_{CP\text{-}High\ Fertilizer}$ (518 kg CO_2-eq ha^{-1}) from fertilizer-induced field emission. A large amount of C was sequestered in soil under $F_{CA\text{-}High\ Fertilizer}$ (899 kg CO_2-eq ha^{-1}) compared to $F_{CP\text{-}High\ Fertilizer}$ (172 kg CO_2-eq ha^{-1}) wheat. In FCA-Medium Fertilizer, lower GHG emissions (1296 kg CO_2-eq ha^{-1}) were observed over $F_{CP\text{-}Medium\ Fertilizer}$ (2062 kg CO_2-eq ha^{-1}). The GHG emission intensity was also lower in the former (0.06 kg CO_2-eq kg^{-1}) than in $F_{CP\text{-}Medium\ Fertilizer}$ (0.41 kg CO_2-eq kg^{-1}), although the fertilizer dose was same. Also N_2O emissions were higher in $F_{CA\text{-}Medium\ Fertilizer}$ (501 kg CO_2-eq ha^{-1}) than $F_{CP\text{-}Medium\ Fertilizer}$ (452 kg CO_2eq ha^{-1}) practices. Due to burning crop residues in $F_{CP\text{-}Medium\ Fertilizer,}$ 665 and 151 kg CO_2-eq ha^{-1} CH_4 and N_2O were emitted, respectively. Significantly higher quantities of SOC were sequestered under FCA-Medium Fertilizer (929 kg CO_2-eq ha^{-1}) than FCP-Medium Fertilizer (122 kg CO_2 eq ha^{-1}) wheat. Conventional practices with application of 250

kg urea and 125 kg DAP ha^{-1} ($F_{CP\text{-}Low\ Fertilizer}$) caused additional GHG emissions of 1827 kg CO_2-eq ha^{-1} with an intensity of 0.37 kg CO_2-eq kg^{-1}. Similar quantities of CH_4 and N_2O were emitted due to crop residue burning as in $F_{CP\text{-}Medium\ Fertilizer}$ with conventional practices. Field induced emissions of CH_4 and N_2O were 151 and 375 kg CO_2 eq ha^{-1}, respectively, under $F_{CP\text{-}Low\ Fertilizer}$. The main source of variation in GHG emissions between CA and conventional agricultural practices was the management practices. Conventional practices in $F_{CP\text{-}High\ Fertilizer}$ registered about 63% higher total GHG emissions than $F_{CA\text{-}High\ Fertilizer}$, which were due to less soil disturbance (zero tillage), residue retention instead of burning, green seeker and Nutrient Expert-based N applications to soil in later stages, leading to lower emissions (Kakraliya *et al*. 2018) [15]. In CA-based practices, higher N_2O emissions might occur due to denitrification from soil under residue retention conditions developing anaerobic micro-pockets in the presence of high soil moisture content at soil surface where microbes use nitrate and nitrite as terminal electron acceptor and produce N_2O (Brady and Weil 2007) [4]. Bhatia *et al*. (2010) [3] and Gupta *et al*. (2016) [12] also observed higher N_2O emissions under CA-based agricultural practices in northern India. Sapkota *et al*. (2017) [31] pointed out that the source and amount of N fertilizer also influences GHG emissions from soil. Lower GHG emissions were observed upon application of lower doses of N fertilizer to soil. In conventional wheat, about 12% less N_2O emissions were observed than in zero tilled wheat in northern India (Bhatia *et al*. 2010) [3]. Higher N_2O emissions from zero tilled wheat than conventional were also observed by Sapkota *et al*. (2015) [30] in rice–wheat cropping systems of north-western Indo-Gangetic plains.

Datta *et al*. (2022) [7] also found that dry direct seeded rice culture reduced 24 to 79% and 43 to 75% CH_4 emission under continuous flooded and intermittent irrigated system compared with the puddle transplanted continuous flood irrigated rice field (Kumar and Ladha, 2011) [18]. Pathak *et al*. (2013) [23] reported that CH_4 emission in dry seeded field was 0.6–4.9 kg ha^{-1} and puddled trans-planted field was 42.4–57.8 kg ha^{-1} in different areas of Punjab, India. Although dry direct seeding can re-duce CH_4 emission under aerobic soil condition, the relatively more soil aerobic state may increase N_2O emission. N_2O is produced as by-product during soil microbial nitrification and de-nitrification processes. N_2O emission in DDSR and PTR–CI field was0.95 kg N^2O N ha^{-1} and 0.65 kg N_2O N ha^{-1}, respectively (Liu *et al*., 2014). In India, the N_2O emission was 0.31–0.39 kg N ha^{-1} under PTR–CI which increased to 0.90–1.1 kg N ha^{-1} and1.3–2.2 kg N ha^{-1}, in conventional tillage dry direct seeded rice and zero till dry direct seeded rice, respectively (Kumar and Ladha, 2011) [18]. Pathak *et al*. (2013) [23] estimated that N_2O emission in 2009 in DDSR was 0.9–1.2 kg ha^{-1} and 0.8 to 1.1 kg ha^{-1} in PTR field's in Punjab, India while that was 2.0–2.2 kg ha^{-1} in DSR and 1.6–1.8 kg ha^{-1} in TPR in 2010. Methane emission starts at redox potential of soil below −150 mV and is stimulated at less than −200 mV (Wang *et al*., 1993) [39].

Fuller *et al*. (2011) [9] also found that rice based cropping system plays the major role to the emission of greenhouse gases (Fig.8). Methane formation depends on the metabolic activity of a group of bacteria and activity of methanogen bacteria increases in anaerobic condition. The major pathways of CH_4 production in flooded soils are the reduction of C compounds to CH_4 due to restricted oxygen supply. Anaerobic condition is the pre-requisite for the activities of methanogenic bacteria and CH_4 production. Thus, CH_4 is low under aerobic condition.

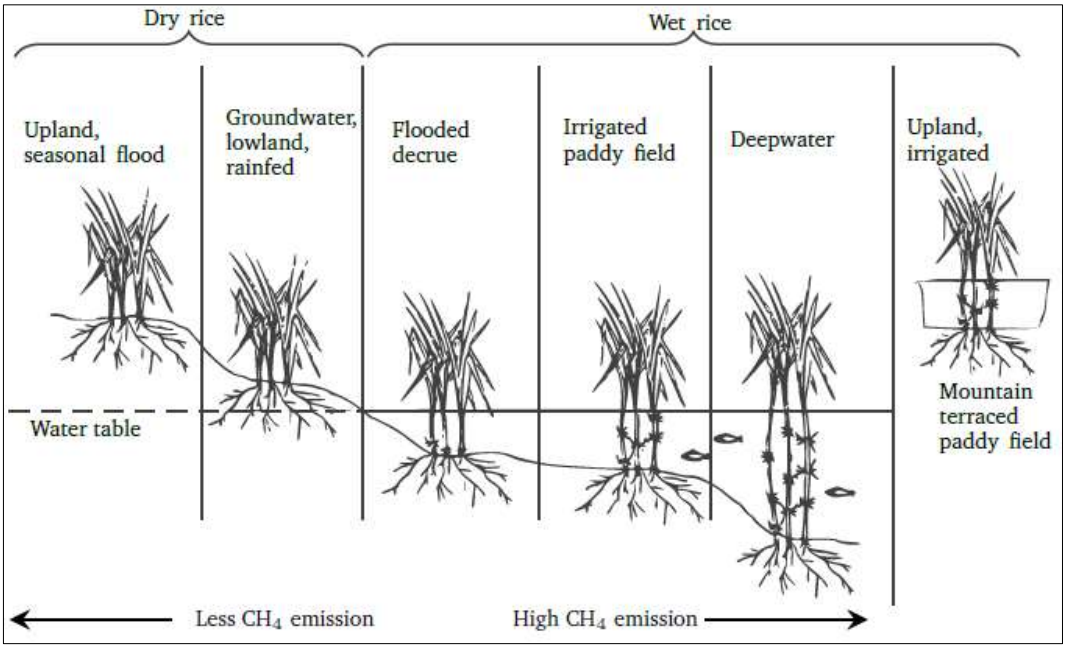


Fig 8: Methane gas emission from rice field as a function of water management in the field

Bijarniya *et al*. (2020) [2] reported that the crop management scenarios, S1 recorded the highest GWP and CO_2 emission intensity followed by S2 and the lowest was in S6 and following overall trend of S6 > S5 > S4 > S3 > S2 > S1 (Fig.9). The higher GWP and CO_2 emission intensity in farmer practices scenarios (S1 and S2) reflects the more contributed in carbon footprints. The mean CSAPs recorded lower GWP by 1598, 1749 and 1876.3 kg CO_2 eq. ha^{-1} yr^{-1} compared to S1 (3652.7 kg CO_2 eq. ha^{-1} yr^{-1}), respectively. Input like diesel fuel (for land preparation, seeding and irrigation water application), fertilizers constitute and puddling in rice, the major share of the total emissions of

GHGs (N_2O and CH_4) estimated for the system (Fig. 9). The CSA based scenarios (S4 S5 and S6) related to low inputs and no puddling in rice contributed to low emissions of GHGs compared to farmers practice (S1), whereas higher input used and followed repeated tillage in wheat and puddling in rice.

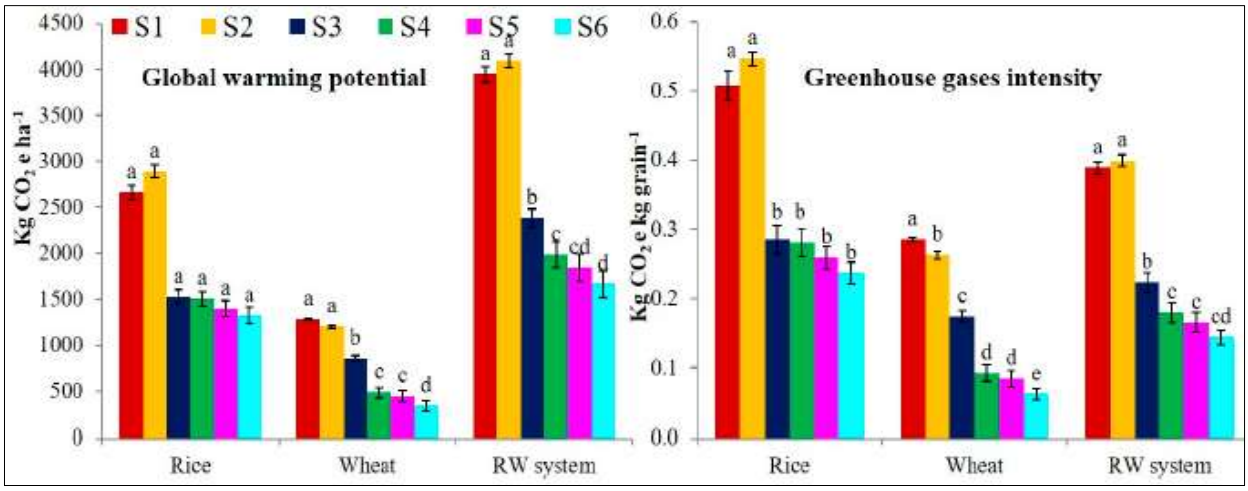


Fig 9: Mean annual global warming potential (GWP) and greenhouse gases intensity of rice-wheat system under divergent crop management scenarios. S1- Conventional tillage (CT) without residue; S2- CT with residue, S3- Reduced tillage (RT) with residue + Recommended dose of fertilizer (RDF); S4- RT/zero tillage (ZT) with residue + RDF, S5-ZT with residue + RDF + green seeker + tensiometer +Information & communication technology +crop insurance and S6- S5 + site specific nutrient management

Conclusions

Climate Smart agricultural practices such as CA with zero tillage, residue retention with diversified crop rotation resulted in a decrease in soil pH in wheat compared to conventional agriculture practices. Lower GHG emissions were estimated from CSA than from conventional practices. These CSA practices provide an excellent alternative to conventional agriculture practices in north-west IGP for adaptation to climate change irrespective of farm type and size. Rice–wheat cropping system in north-west IGP has contributed immensely to fill the increasing empty stomachs but has consequently led to many sustainability issues *viz*. declining water resources, degrading soil health and environment degradation which is further responsible for stagnating/decreased land and water productivity. There is need to refine the agronomical practices for direct seeded rice along with genetic tailoring for anaerobic emergence from deeper depth, higher vigour with more source to sink translocation of photosynthates during the grain filling stage and promotion of mechanical-based weed control. The large scale adoption of short-duration and stature rice varieties may bring significant decline in groundwater draft besides producing the optimum biomass and providing the enough time for sowing of succeeding crop with effective *in-situ* residue management. Moreover, rescheduling the transplanting time considering the changes happening in monsoon arrival time in the region and technological support with the aspects of short-duration varieties, better performing genotypes under late transplanting would be helpful to increase the water and nutrient productivity in conventional rice–wheat system.

The total energy input for rice and wheat was valued at 63825 and 50799 $MJha^{-1}$ respectively. Combine energy input and output for combine crop rotation 114624 and 270588 $MJha^{-1}$ respectively. Primary contributors in the input energy are electricity for water pumps and water for irrigation followed by nitrogen fertilizer and diesel fuel. The input-wise energy estimates can be used to estimate GHG emissions and the global warming potential (GWP) of the rice-wheat cropping cycle in north India for larger policy-relevant interventions. Energy use efficiency in rice-wheat system is low (2.53 for rice and 2.15 for wheat) and the specific energy ratio is high 10.98 $MJkg^{-1}$ for rice and 12.77 $MJkg^{-1}$ for wheat). This implies that there is a need to optimize energy use, implement energy efficiency measures and improve productivity per unit of energy consumed in the system. There is a close association between and GHG emissions, global warming potential (GWP) and non-renewable energy input. Our estimated share of non-renewable sources was 75% for rice and 74% for wheat. Therefore, there is a need to curtail the use of non-renewable energy resources. There is considerable scope in energy savings through improvement in energy efficiency in agriculture water pumps, minimum tillage and harmonizing sowing season with the monsoon season. Optimizing fertilizer management by reducing synthetic fertilizer inputs and increasing organic compost and improving water management is vital. The state departments should converge to introduce energy-efficient practices which will go a long way in ensuring the sustainability of production system in the country.

Hence, alternate tillage and establishment methods must be invented and recommended for the sustainable establishment of rice–wheat cropping system as a whole including the intervening period so that soil health and environment must be improved for overall lifting of the livelihoods of the farmers of north-west IGP. Performance of these technologies is, however, site-specific and changed depending upon the soil textural classes and agro-climatic conditions. This suggests that farmers must pick them up from the many as per their soil texture and agro-climatic conditions. Conventional indigenous age-old practices are responsible for all the earlier discussed un-sustainability issues which must be replaced with more advanced and sustainable climate smart agriculture practices (CSA). Therefore, the role of these CSA to achieve sustainable food production with minimal impact on the soil, underground water and the atmosphere and in improving the

declining land and water productivity become more important now than ever. Apart from cropping system perspective, adoption of soil/water conserving technologies like conservation tillage, recycling of crop residue back to soil, micro-irrigation systems, integrated nutrient management, etc., would be helpful to lessen the burden on natural resources and to uphold the agricultural sustainability amidst the rising risk of climate change.

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APPLICATION OF YOGA PRACTICE FOR PREVENTION OF DOPING IN SPORTS AND ATHLETICS

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ABSTRACT
T0 apply forbidden substances and methods used to improve the performance are collectively called as Doping. The International Sports federation is taking effort to stop the spread of such misbehavior by using applying various tests, methods, educational programs and support medical treatment by applications of Yoga.

Yoga is a prehistoric put into practice that aims to make sense of balance and physical condition to that physical, intellectual, emotional and religious individuality. Yoga is fundamentally a religious restraint based on an enormously slight Science which focuses on bringing harmony between mind and body. It is an art and science for a healthy livelihood. The word "Yoga" is derived from the Sanskrit root yuj meaning "to join", "to yoke" or "to unite".

Sport pertains to any form of physical motion or sport, habitually competitive and well thought-out that aims to use, sustain, or develop physical ability and skills while as long as a pleasure to participants and in some cases, entertaining to listeners.

The yoga poses enhances equilibrium and management as well as meditation. By improving stability, you can decrease the hazard of falls and improve your technique when exercising, leading to better performance overall.

Effects of Yoga on the Soul: Yoga may help you strengthen your relationships. Yoga has a calming effect on the mind. Yoga assists us in achieving inner serenity

KEY WORDS: Yoga, Performance enhancing substances, doping and athletes.

INTRODUCTION
"Doping represents the use of substances or physiological intermediates, which do not normally exist in the human body, presented as an external resource to increase the athletes' performance during the competition. (Detlief & Hemmersbach, 2010.)

The extend use of performance enhancing substance in the athletic games and Olympics has increased since last half century. The governing body WADA (World anti-doping agency) annually restructured their codes and anti-doping standards to organize the use of performance enhancing substance (Morente & Zabala, 2013).In the modern sport world the trainer and athletes are taking risks to take the performance enhancing substance to improve the endurance and performance at large need for winning at every level of competition (Bamberger, 1973). Due to international endorsement, Multimillion dollar contracts, sports merchandising and appearance fees, nowadays modern athletes and athletic organizations have grown to use of the performance enhancing substance to improve the performance in the professional games and athletics. It is an ethical, medical and legal problem (Baron *et. al*., 2007). In the adolescent t of athletes the efforts are taken to improve their behavioral skills through organization of behavioral skills training and educational information about the dangers of substance use rarely change adolescent substance use behaviors (Skiba *et.al*. 2004). The motivation is required to avoidance of use of performance enhancing substance in the athletes and practitioners. Yoga and meditation is one of the approaches which received growing attention involves mind–body practices. It put forward an effective complement to existing substance use prevention programs (Pentz,

2014). The behavioral skills may diminish risk factors by improving mood, anxiety and enhancing self-regulation (Fishbein *et.al.*, 2015).

The Main objective of this research is to determine the impact of yoga in the prohibition of doping and enhancing performance, attitude and strength of athletes.

METHODOLOGY:

The present study will be based on primary and secondary sources of data. The primary data is collected though field survey, questionnaires and personal interviews method and secondary data is collected through various government reports and available sources.

RESULT & DISCUSSION

The sutras represent the eight limbs of yoga such as Yama, Niyama, Asana, Pratyahara, Pranayama, Dharana, Dhyanaand Samadhi. It guides us in various aspects to enhance the harmony in mind, body and spirit. The eight limbs of yoga are now considered as acrucial foundational aspect of the Indian yogic tradition.Yoga is self-possessed of many layers, all of which can improve physical performance.

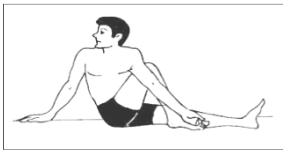
Doping Substances & Adverse effect of Doping

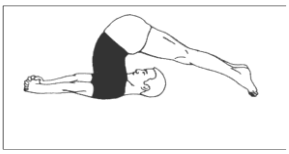
The Most commonly used doping substances aresteroids like closterbol, androstendiol, boldenose and dihydroxytestosterone and testosterone. The hormones such as EPO, *hGH*, insulin-like growth factors, insulin and gonadotropins. The antagonists like beta-2 agonists like terbutalineanti-estrogenic agent that is anastrozole, the diuretics furosemide. The substances causes adverse effects like Dizziness and Nausea, Cardiac arrhythmia and angina, Weakening of the heart muscle, Palpitations, Tachycardia, Nervousness, Headaches and Insomnia (World Anti-Doping Agency. *2006)*.

Yoga and their benefits against doping substances

The various types of yoga improve core vigor,balance, flexibility, endurances, vital capacity and focus(Butzer *et.al.*, 2016).

Aasana and Pranayama and their benefits

 **Pachimotanasana**	Increases backbone mobility and a whole-body flexibility with peace of mind and reduces nervousness.
 **Vakrasana**	Increases the blood flow, nutrients flow, and oxygen flows in the pelvic region thus good for reproductive system andbeneficial for spondylosis, menstrual disorders, anorexia, constipation, and cervical pain.
 **Garudasana**	Helps to Prevent pains, cramps, and inflexibility in the joints. Advantages to improve intellectual and physical condition and elasticity.

 **Halasana**	Strengthens back muscles and prevent in reduction tension in your neck, and shoulders Which strengthens shoulders, arms, and legs, improves strength and joint mobility.
 **Ardhchandrasana**	It decreases anxiety from the lesser stomach by escalating the vertebrae, ankles, and thighs; opening the hips; increasing stretching, hamstrings, calves, and shoulders.
 **Sarvangasan**	Helps to accomplish a sense of balance, better posture, and peacefulness in a diversity of habits. It known as the "Queen of Asanas," is extremely good for both mental and physical wellbeing.
 **Bhujangasana**	Helps to relieve anxiety and tiredness. This pose opens the upper body and helps to clear the passages of the heart and lungs and it helps improves the movement of blood and oxygen, especially throughout the spinal and pelvic regions.
 **Mayurasana**	Improve digestion and increases digestive power. It makes easy good progress and avoidsconstipation.
 **Kapalbharti Pranayam**	It enhances the functioning of the kidneys and liver. Furthermore, since this pranayama exercise takes practice and patience to perfect, working with it increases our inner strength and stamina, leading to a sense of empowerment.
 **Suryabhedan Pranayam**	Improves blood circulation and purification It generates lots of energy by enhancing your metabolism

CONCLUSION:

Meditation gives mental strength and stability to the sportsman and different aasanas build physical strength in sportsmen making sportsmen more confident to participate in various states, national, and international sports competitions. With this study, it is to be advised that the doping test should be compulsory for state and national-level competitions.

Counseling should be provided to sportsmen and other athletes about the hazardous effects of doping drugs on the body.

LIMITATION:

The qualitative data analysis techniques should be applied for this yoga practices for prevention of doping test in sport activity.

RECOMMENDATIONS:

The effect of particular yoga should be needed to study on use of specific doping substance. It should be necessary to measure the level of endurance of particular asana at selected sports and using gender. There should use the alternative assessment methods to measure more valid and realistic data.

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APPLICATION OF YOGA PRACTICE FOR PREVENTION OF DOPING IN SPORTS AND ATHLETICS

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TO INVESTIGATE THE SUPPRESSION OF FUNGAL AND BACTERIAL BIOFILMS BY A BIOSURFACTANT PRODUCED BY THE BACTERIUM PSEUDOMONAS AERUGINOSA.

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ABSTRACT:

Pseudomonas aeruginosa is the primary producer of rhamnolipids, which are extracellular secondary metabolites with surface-active properties. The main goal of this research was to look at Pseudomonas' potential to produce biosurfactants. When compared to commercially available surfactants, their low toxicity, biodegradability, efficiency, and specificity drew more attention, making them suitable candidates for use in new generations of microbial dispersal agents and as an adjuvant to control surface-growing communities of microorganisms. Biosurfactant dispersal properties have been shown to rival those of conventional inhibitory agents against bacterial and yeast biofilms. There is mounting evidence that this biosurfactant plays a role in various stages of this bacterium's biofilm development.Furthermore, rhamnolipids have significant anti-adhesive and disrupting potential against established biofilms formed by a variety of bacterial and fungal species.The purified biosurfactant's inhibitory activity against Candida and bacterial biofilms, which are groupings of microorganisms that may stick to inanimate surfaces, resulting in the spread of various illnesses, was the second focus of the research.

Keywords: Rhamnolipids, Anti-adhesion, biosurfactant, Biofilm producers.

INTRODUCTION

Biosurfactants are extracellular or cell membrane surfactants produced by bacteria, yeasts, and fungi(Karanth, Deo, and Adi 1999; Mulligan 2005; Tabatabaee et al. 2005).They are a structurally varied collection of surface-active compounds produced by microbes. Where the hydrophobic moiety can be a carbohydrate, an amino acid, a cyclic peptide, a phosphate, a carboxylic acid, or alcohol, among others, and the hydrophilic moiety can be a carbohydrate, an amino acid, a phosphate, a carboxylic acid, or alcohol, among others (Satpute et al. 2010). These compounds diminish surface and interfacial tension in aqueous solutions as well as hydrocarbon mixtures, making them promising candidates for improving oil recovery and demulsification processes (Desai and Banat 1997; Muthusamy et al. 2008; Youssef et al. 2004).They are amphipathic molecules that can form specialized structures that are essential to their action. (Christofi and Ivshina 2002).They increase the area at the aqueous hydrocarbon interface, which improves hydrocarbon bioavailability to microbial cells. This accelerates hydrocarbon dissolution and utilization by microorganisms. (Tuleva, Ivanov, and Christova 2002).

Bacterial attachment to surfaces and biofilm formation have serious consequences in the food, environmental, and biomedical fields. Biofilm is a bacterial community that adheres to biotic and abiotic surfaces and is embedded in a polymeric matrix that is primarily composed of polysaccharides, proteins, and nucleic acids. (Flemming and Wingender 2010). Bacteria found in natural, clinical, industrial and food-processing environments are referred to as biofilms. Biofilms are now thought to be the source of many pathogen outbreaks. (Aarnisalo et al. 2007; Lapidot, Romling, and Yaron 2006)and bacterial biofilms are responsible for more than 80% of microbial infections in the body and bacterial biofilms account for more than 80% of microbial infections in the body (Moreau-Marquis, Stanton, and O'Toole 2008; Wu et al. 2016). In biofilms, bacteria are typically well protected from the effects of antibiotics, disinfectants, and the host immune system. Biofilms are very challenging to eradicate because biofilm bacteria are up to 1000 times more resistant to antibiotics and host immune responses than planktonic bacteria. (Burmølle et al. 2010; Kamali et al. 2021). Therefore, searching for novel compounds or strategies to inhibit biofilm formation or disperse preformed biofilm is needed. The presence of biofilm in food processing environments can result in spoiling and disease transmission, posing a health concern to consumers. (Zezzi do Valle Gomes and Nitschke 2012). There is sufficient data to suggest that the biofilm mode of life leads to greater resistance to antimicrobial agents. (Satpathy et al. 2016).

C. Albicans is a fungus that commonly occurs as a commensal in the gastrointestinal and vaginal flora of many healthy individuals. It is also an opportunistic pathogen: this microorganism can cause severe infections in case of a deteriorated immune

system of the patient (Tournu and Van Dijck 2012). For instance, cancer patients, HIV patients, elderly people, and patients who take immunosuppressive drugs or antibiotics, have a higher risk of uncontrolled Candida proliferation (Ramage et al. 2001).

Pseudomonas rhamnolipid, a glycolipid biosurfactant, has numerous applications in these fields. The application of biosurfactants to a surface changes its hydrophobicity, interfering with microbial adhesion and desorption processes; in this respect, biosurfactant production by Pseudomonas bacteria in vivo can be viewed as a defense against other colonizers. (Dafforn, Lewis, and Johnston 2011; van Hoogmoed et al. 2000; Joseph et al. 2001).

The current study sought to assess the antibacterial and anti-adhesive effects of this biosurfactant against a variety of pathogenic microorganisms. Biosurfactants have been demonstrated to prevent pathogenic organism adhesion to solid surfaces or infection sites; consequently, prior attachment of biosurfactants to solid surfaces may constitute a new and effective method of countering pathogenic microorganism colonization. (Harshada 2014; Singh and Cameotra 2004).The amount of biofilm generated by Salmonella typhimurium, Salmonella enterica, E. coli, and Proteus mirabilis was reduced by pre-coating vinyl urethral catheters with a surfactin solution before inoculation with the medium. (Mireles, Toguchi, and Harshey 2001).

2. MATERIALS AND METHODS

2.1. MICROORGANISM COLLECTION

The biosurfactant-producing Pseudomonas aeruginosa NDBS strain was identified from oil-contaminated soil used for production in the Latur district. The isolated organism is morphologically, biochemically, and genetically identified using 16s rRNA sequencing. Similarly, hemolytic activity, oil displacement, and drop collapse tests are used to screen biosurfactant producers.

2.2. PREPARATION OF INOCULUMS FOR BIOSURFACTANT SYNTHESIS

Isolates were grown in 100 ml of sterile minimum media (MM) containing 2% vegetable oil as a carbon source. The suspension was incubated for 48 hours at 37 °C. The culture flask inoculum was subcultured in nutrient agar. (Sharma and Saharan 2016). The isolates were kept in nutrient broth. 2% (v/v) glycerol stock before use in the current study. For the formation of biosurfactant, a 5% inoculum of a 24-hour-old culture was employed. Before use, the optical densities of pseudomonas were read at a wavelength of 600nm, and the growth of pseudomonas was recorded at 24-hour intervals.

2.3. PRODUCTION MEDIA AND CULTIVATION CONDITIONS

The defined medium's composition was (per liter) minimal agar medium with dextrose (1gm/lit), dipotassium phosphate (7.0gm/lit), mono potassium phosphate (2.0gm/lit), sodium citrate (0.5gm/lit), magnesium sulphate (0.1gm/lit), ammonium sulphate (1.0 gm/lit) with 2 percent (v/v) cheap carbon source such as vegetable oil with PH 7.0 ±0.2 Inoculated production media with 5% (v/v) of selected isolate pure culture and incubated for 72 hours at 37 °C at 150 rpm min^{-1}.

2.4. BIOSURFACTANT PURIFICATION AND EXTRACTION

2.4.1. ACID PRECIPITATION

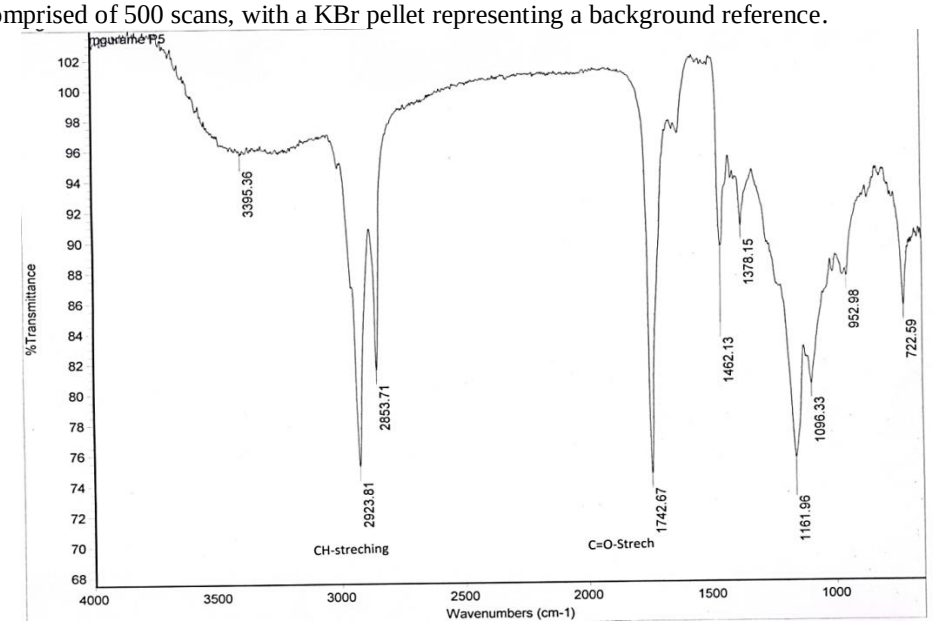
To remove cells, the culture was centrifuged at 7000g for 15 minutes. The supernatant was then acidified with hydrochloric acid to a pH of 2.0. After 30 minutes of centrifugation at 7000g, the precipitate was extracted with a chloroform-ethanol solvent (2:1). Extraction was used to collect and weigh the precipitate. The partially purified biosurfactant was dissolved in 10 mL chloroform and purified further using column chromatography. The column was packed with silica G-500, and different fractions were collected with $CHCl_3$:CH_3OH in 50:3, 50:5, and 50:20 ratios. The resulting product was used to characterize and investigate biofilm inhibition.

2.5. BIOSURFACTANT CHARACTERIZATION

The presence of aliphatic hydrocarbon chain groups was revealed by FTIR analysis of the biosurfactant's wave numbers for C-H bonds. The presence of alkanes was confirmed by C-H bonds of the CH3, CH2, and CH groups observed at wavenumbers 3,355 and 2923 cm1, 2853, 1462, and 1378 cm1. The presence of C-O bonds was indicated by the wavenumber 1,762,1142 cm1. The above data from the respective wave numbers confirmed the biosurfactant's glycolipid nature. The biosurfactant's mass spectrometric analysis supports the biochemical and FTIR results, with peaks observed at m/z = 597, 521, 332, 323, & 242 indicating the presence of carbohydrate and lipid moieties.

2.5.1. FOURIER TRANSFORM INFRARED SPECTROSCOPY

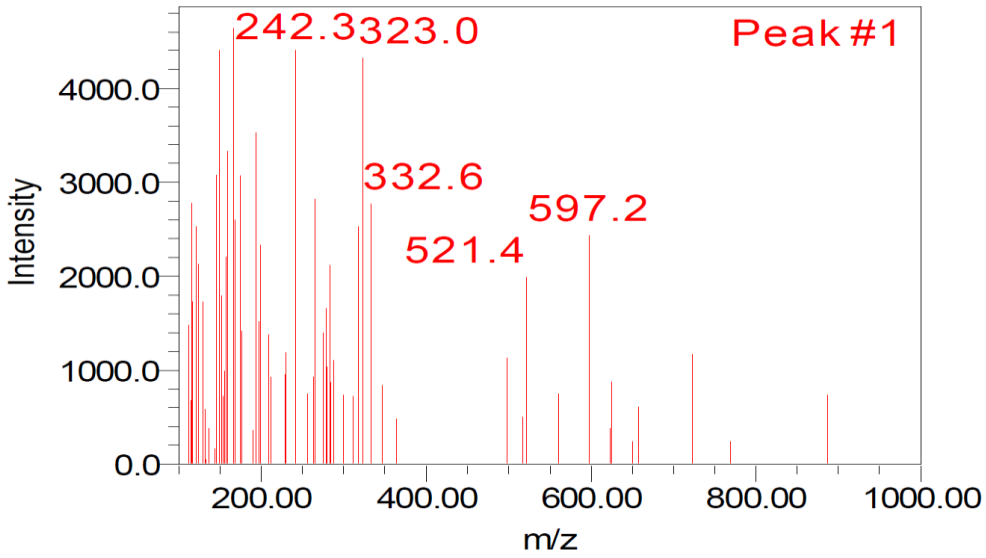
Fourier transform infrared spectroscopy (FTIR) is most useful for identifying distinct types of chemical bonds (functional groups), and can thus be used to identify some components of an unknown mixture. To obtain translucent pellets, freeze-dried crude biosurfactant (10 mg) was ground with 100 mg of KBr and pressed with 7,500 kg for 30 seconds. Infrared absorption spectra were recorded using a Thermo Nicolet AVATAR 330 FTIR system with a spectral resolution of 4 cm1 and a wavenumber accuracy of 0.01 cm^{-1}. All measurements were comprised of 500 scans, with a KBr pellet representing a background reference.



FTIR spectrum of *Pseudomonas aeruginosa* biosurfactant.

2.5.2. MASS SPECTROMETRIC ANALYSIS OF BIOSURFACTANT

The biosurfactant was dissolved in methanol and thoroughly mixed. The biosurfactant was mass spectrometrically analyzed using UV-MS in an LCMS Quadrupole ion-trap mass spectrometer. At a flow rate of 10 µl min^{-1}, standard solutions and samples under investigation were infused into the mass spectrometer. The nitrogen and auxiliary gas flow in the UV-MS were kept at 50 and 5 ml/min, respectively, and are arbitrary values set by the software. The scanning was done in negative ion mode and at 2,000 *m/z*.



Pseudomonas aeruginosa biosurfactant LC-MS spectrum.

2.6. FORMATION OF BIOFILM

Biofilm growth quantified using crystal violet measurement of biofilm formation is one of the most often used techniques for evaluating the efficacy of biosurfactants and biofilm inhibitory agents (O'Toole 2011).In a 96-well microtiter plate, a microbial biofilm is stained with 1% crystal violet. Instead of only staining the biofilm's component cells, the crystal violet also discolors the biomass of the entire biofilm, including EPS and extracellular proteins.(Banat, De Rienzo, and Quinn 2014).

2.6.1. GROWING A BIOFILM

1. Grow a pathogenic strain of *E. coli*, *S. aureus*, *P. Vulgaris*, *S. Typhi*, and *Candida albicans* in a rich medium (i.e. Muller Hinton /PDB) overnight.
2. For biofilm assays, dilute the overnight culture at 1:100 in a fresh medium. A standard biofilm assay medium that promotes planktonic growth and the formation of a more robust biofilm.
3. The dilution in each well of a 96-well dish Incubate the microtiter plate at 37°C for 4-24 hours.

2.6.2. STAINING THE BIOFILM

1. After incubation, turn the plate over and shake out the liquid to remove the cells.
2. Place the plate in a small tub of water and gently submerge it. Shake the water out. Repeat this procedure a second time. This step assists in the removal of unattached cells and media components that can be stained in the following step and significantly reduces background staining.
3. Fill each well of the microtiter plate with 125 L of a 0.1% crystal violet solution in water. Incubate the microtiter plate for 10-15 minutes at room temperature.
4. Rinse the plate 3-4 times with water by immersing it in a tub of water as described above, shaking it out, and blotting vigorously on a stack of paper towels to remove all excess cells and dye.

2.7. RESULTS

Biosurfactant acts as an anti-adhesive agent, preventing the growth of biofilms produced by *Proteus Vulgaris*, *Staphylococcus aureus*, and *Candidaalbicans* wherever planktonic cell proliferation occurs.

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Biosurfactant Production by *Pseudomonas aeruginosa* Strain LTR1 and its Application

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Abstract: On the circumstances of the growing market of biosurfactants all over the globe, the presented study focused on the characterization of biosurfactants produced by newly isolated and reported as biosurfactant producer strain *Pseudomonas aeruginosa* LTR1. Additionally, production media and physiological factors are optimized using a one factor at a time (OFAT) approach for maximum biosurfactant production. Its efficacy for antimicrobial and emulsification activities was determined. P. aeruginosa LTR1 utilized all the carbon sources provided, and a maximum of 9.5 g/L of biosurfactant was produced in soybean oil supplemented minimal salts medium (MSM). The critical micelle concentration (CMC) was determined as 12 mg/L and can reduce the surface tension of the medium from 72 mN/m to 31 mN/M. The biosurfactant was characterized by biochemical analysis and Fourier transform infrared spectroscopy (FTIR) and was not a protein in nature, possibly a glycolipid type of biosurfactant. It has shown the good emulsification activities for various hydrocarbon tested here. In addition, it acts as an antibiofilm agent with the minimum inhibitory concentration required for 50 % biofilms reduction was 30.95 µg/mL suggesting the antimicrobial potential. The biosurfactant produced by *Pseudomonas aeruginosa* strain LTR1 has shown good surface-active properties, good emulsification, and antimicrobial activities, demonstrating its potential for application in various areas like the oil industry, especially in tank cleaning, bioremediation of spills at sea or soil, and candidates as antimicrobial.

Keywords: *Pseudomonas aeruginosa;* petrochemical contamination; biosurfactant; critical micelle concentration; antibiofilm

1. Introduction

Biosurfactants are natural surface-active compounds produced biologically, mostly by bacteria, yeast, and fungi [1]. Glycolipids, liposaccharides, and proteins produced extracellularly are the biosurfactants that possess hydrophilic and hydrophobic domains [2]. Such properties render these molecules to reduce the surface and interfacial tension of compounds with different phases and form a microemulsion. Thus, biosurfactants and synthetic surfactants can act as excellent detergents, emulsifying agents, and dispersants [3,4].

Synthetic surfactants are organic compounds commonly used in various sectors such as soaps, detergents, shampoos, cosmetics, kinds of toothpaste, and drugs [5]. High production & energy costs, less solubility, charge, types, and physicochemical properties of synthetic surfactants are challenging criteria for selection and use for industrial purposes. The frequent uses of such chemical compounds increase their deposition at the user site and remain persistent

forever, increasing the environmental burden [6]. Moreover, toxicity is a major concern that may affect the flora and fauna of the ecosystem, disturbing the balance of the ecosystem [7]. On the other hand, biosurfactants are eco-friendly, biodegradable, better compatible, less toxic, and potentially active compared to synthetic surfactants [6]. Biosurfactants are a structurally diverse group of compounds that include simple fatty acids, glycolipids, peptides, lipids, siderophores, and polymeric surfactants [8]. Biosurfactants are now popular in various fields includes bioremediation, antimicrobials, food processing, and cosmetics. From an economic point of view, the biosurfactants market size was 1736.7 Million USD in the year 2019, and 5.5 % CAGR is expected for 2020–2026, with European countries as major customers [9].

Various anthropogenic activities are the major reason for hydrocarbon pollution in marine and terrestrial ecosystems [10]. Although hazardous and non-hazardous hydrocarbons pollute most of the oil filling station and highway side soil, such soil consortia are found rich in hydrocarbon degraders [11]. In natural settings, hydrocarbon degraders experience different interactions with other microorganisms, requiring more time for hydrocarbon degradation [12]. Interestingly, most isolated hydrocarbon degraders are efficient biosurfactant producers [13,14]. Producing biosurfactants by microorganisms using various substrates is more expensive; hence, various research groups commonly use agriculture products and waste as substrate [15]. Every year, tons of organic waste is generated worldwide from various industries, i.e., fruit processing industries, coffee processing industries, oil processing mills, and agronomic crops, which can be used as a renewable substrate source to produce biosurfactants [15]. Environment-friendly and surface activity make the biosurfactant an excellent agent for oil extraction, cosmetics, antimicrobial, and medicine [16]. *Pseudomonas spp.* usually found in oil-contaminated soil, oil-spill site, and hydrocarbon-containing soil can produce biosurfactants especially ionic glycolipids biosurfactants such as rhamnolipid [17]. In this research work, physiological conditions and important media components of MSM [18] are optimized for the production biosurfactant by *P. aeruginosa* strain LTRI using the OFAT approach. Biosurfactant produced was further characterized, and its various activities, including surface emulsification activity against various hydrocarbons, were studied. Antimicrobial activities against the human pathogen *Candida albicans* were also studied.

2. Material and Methods

The *P. aeruginosa* strain LTR1 used in this study was previously isolated from the oil-contaminated soil sample from Latur, Maharashtra [19]. The strain was maintained on nutrient agar slant until further use. A single colony from 24 hrs old culture plate was inoculated in nutrient broth. 24 hrs old culture broth was used as an active culture for further uses.

2.1. Optimization of media components and physicochemical parameters for growth of biosurfactant producers.

Biosurfactant production is directly proportional to the growth of biosurfactant producers [20]. The maximum growth of bacterial isolate was optimized by using different parameters like carbon, nitrogen, temperature, pH, potassium dihydrogen phosphate, ferric chloride, and magnesium sulfate.

2.2. Screening of carbon and nitrogen sources.

The *P. aeruginosa* strain LTR1 was grown on various carbon sources such as glucose, soybean oil, kerosene, petrol, and diesel at 2 % (for glucose wt/v, rest v/v) concentration in MSM. Flasks were incubated at 37 °C for 12 to 120 hrs and 150 rpm on a rotary shaker incubator. The growth in each flask was determined by measuring optical density at 610 nm on a UV-double beam spectrophotometer (Systronics computer-based double beam spectrophotometer 2202). The maximum growth was considered positive for the utilization of a carbon source.

2.3. Optimization of temperature and pH.

To study the growth at different temperatures, *P. aeruginosa* strain LTR1 was inoculated in the MSM broth, which contains 2 % soybean oil. Culture inoculated media flasks were incubated at various temperature ranges for 12 to 120 hours at 150 rpm on a rotary shaker. The temperature range selected was 27 °C, 32 °C, 37 °C, 40 °C, and 45 °C. Similarly, after temperature optimization, pH optimization was carried out at 37 °C. The pH range used to optimize growth was 6, 6.5, 7, 7.5, 8, and 8.5. The growth in each flask was determined by measuring optical density at 610 nm on a UV-visible double beam spectrophotometer.

2.4. Optimization of potassium dihydrogen phosphate ferric chloride and magnesium sulfate.

For obtaining optimum growth of *P. aeruginosa* strain LTR1 at various concentrations of potassium dihydrogen phosphate (KH_2PO_4), ferric chloride ($FeCl_3$), and magnesium sulfate ($MgSO_4$) culture was inoculated in the mineral salt broth, which contains 2 % of soybean oil. Concentrations used were in the range of 1 to 5 g/L for KH_2PO_4, 10 to 60 mg/L for $FeCl_3$, and 100 to 500 mg/L for $MgSO_4$. Flasks were incubated at 37°C for 24 to 48 hrs at 150 rpm on a rotary shaker. The growth was determined by measuring optical density at 610 nm on UV-Vis double beam spectrophotometer [21].

2.5. Production of biosurfactant in batch culture.

MSM medium supplemented with 2 % Soybean oil as carbon source, includes 100 mg/L NH_4NO_3/$(NH_4)_2SO_4$, 3 g/L KH_2PO_4, 2 g/L K_2HPO_4, 200 mg/L $MgSO_4$, 10 mg/L $CaCl_2$ and 20 mg/L FeCl3, was used as production medium for biosurfactant production. Five percent 24 hrs old *P. aeruginosa* strain LTR1 inoculum was transferred in a 1000 ml production medium, and the flask was incubated at 37 °C and 150 rpm for 3 days. The pH of the medium was initially adjusted to 7.0 ± 0.2 by 0.1 M HCl.

2.6. Biosurfactant extraction and recovery.

Extraction was carried out by the acid precipitation method. The bacterial cells were removed from the broth by centrifugation at 5000 rpm for 30 min. at 4 ºC. Acid precipitation was used to remove lipid and proteins, adding 6 M HCl to achieve a final pH of 2.0, and the flask was kept in a refrigerator at 4 ºC overnight. The white precipitate was collected, and the supernatant was discarded after centrifugation at 5000 rpm for 20 min. To extract the biosurfactant, the precipitate was dissolved in chloroform:ethanol (2:1) solvent system for 10 min. The organic phase was collected and concentrated by evaporation in a hot air oven at 45 ºC temperature yielding a viscous, honey-colored crude biosurfactant. Crude biosurfactant weight was recorded on the analytical balance. The concentrated crude biosurfactant was dissolved in methanol/chloroform and was used for further analysis [22].

2.7. Characterization of biosurfactants.

The produced biosurfactant was analyzed for the qualitative detection of carbohydrate, protein, and lipid and was determined by the anthrone, Bradford & saponification method, respectively [23,24].

2.8. Purification of biosurfactant.

Analytical column chromatography was used to purify the biosurfactant. The column was packed by adding 50 gm slurry in 26 × 3.3 cm^2 glass chromatographic column of activated silica gel G-500 prepared in chloroform ($CHCl_3$). One-gram crude biosurfactant sample dissolved in 5 mL of chloroform was loaded onto the top of the prepacked chromatography column. The column was washed with chloroform to elute the neutral lipids. The various fractions of biosurfactant were collected stepwise by using chloroform/methanol in different ratio in sequence: 50:3 v/v (250 mL), 50:5 v/v (200 mL) and 50:50 v/v (100 mL) by maintaining a flow rate of 1 mL/min at room temperature. All the eluted fractions of biosurfactant were dried in a hot air oven at 45 ºC to obtain the pure product.

2.9. Surface tension measurement.

The purified biosurfactant was diluted in deionized water, and the surface tension was measured using a Du Nouy ring type, Data physics (Angeltoni-DCAT11) tensiometer. Deionized water was used for standardization, and SDS was used as a standard surfactant. Biosurfactant was diluted in the range of 1.0 to 200 mg /L in distilled water. The average value of triplicate readings was considered the surface tension of respective solutions.

2.10. Determination of CMC.

Critical micelle concentration (CMC) of the biosurfactant was calculated by plotting surface tensions versus biosurfactant concentrations. Concentration at which no significant variation in surface tension was observed and considered CMC of the biosurfactant [25].

2.11. Fourier Transform Infrared (FTIR) analysis.

The column purified biosurfactant was characterized by Fourier Transform Infrared spectrophotometer (FTIR) spectroscopy to detect functional groups. The IR spectrum was recorded on Prestige- 21 FTIR (Shimadzu, Japan) in the spectral region in the range of 400 to 4000 cm^{-1} at a resolution of 2 cm^{-1} using 0.2 mM KBr pellet method [24].

2.12. Emulsification test.

The emulsification activity of the biosurfactant produced by *P. aeruginosa* strain LTR1 was determined [26]. 3 mL of strain LTR1 grown supernatant was mixed with 3 ml of various hydrocarbons in separate tubes. Tubes were mixed vigorously mixed for 2 minutes and kept still for a further 24 hrs. Percent emulsification activity was determined by using the following formula:

$$EI24\% = \frac{\text{Height of the emulsified layer}}{\text{total height of the liquid column}} \times 100$$

2.13. Antibiofilm activity.

Antibiofilm activity of biosurfactant produced by LTR1 was studied against *Candida albicans* biofilms. In brief, 1×10^6 cells/mL in phosphate-buffered saline (PBS) were distributed in each well of 96 well microtiter plates. The plate was incubated at 37 °C for 90 minutes on a rotary shaker incubator for adhesion. After completion of the incubation period, wells were washed with PBS. Various concentrations of biosurfactant ranging from 100 to 6.25 µg/ml in 200 µl of RPMI-1640 were distributed in prewashed cell adhered plate. The plate was incubated at 37 °C for 24 hrs and 120 rpm in a shaking incubator. After 24 hrs incubation, all the liquid media were removed. Wells were washed thrice with sterile PBS to remove unadhered cells/biomass. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) viability assay was performed to determine the biomass formed [27] . Light microscopic pictures were taken on Olympus inverted microscope (Model-CK40).

2.14. Statistical analysis.

In triplicate, media component and physiochemical optimization, emulsification, and antibiofilm activity experiments were performed. Means and standard deviations are calculated and represented graphically. Shapiro-Wilk test was conducted to test non-normality. One-way ANOVA tests were performed at 0.05 *p*-value.

3. Results and Discussion

3.1. Screening of carbon and nitrogen substrate for the growth of biosurfactant producers.

There is a direct relation of growth with biosurfactant production [20]. Different carbon sources were screened in the present study to obtain optimum growth at various time intervals (Figure 1A and 1B). Four different carbon substrates such as petrol, kerosene, diesel, and soybean oil were taken for the study. Among all these, maximum growths were obtained in soybean oil as a sole carbon source in 72 hours incubation followed by petrol. The moderate growth was observed in diesel which showed maximum growth on the third day. The least growth was observed in kerosene oil at all different time points taken for the study.

3.2. Optimization of physicochemical parameters and metal constituents.

Temperature, pH, KH_2PO_4, K_2HPO_4, $MgSO_4$, $CaCl_2$ & $FeCl_3$ concentrations were optimized to obtain maximum growth of *P. aeruginosa* strain LTR1 (Figure 1). Effect of temperature on the growth of *P. aeruginos*a strain LTR1 was studied in the range of 27 °C to 45 °C. The maximum growth increment was observed at 37 °C in soybean oil within 24 hrs. The trend continued up to 72 hrs and started declining after that (Figure 1C). In the case of hydrogen ion concentration, *P. aeruginosa* strain LTR1 showed maximum growth at pH 7 in soybean oil as the sole carbon source within 72 hrs (Figure 1D). KH_2PO_4 and K_2HPO_4 are the best sources of potassium and phosphate, which show an effect on the growth and synthesis of biosurfactants. *P. aeruginosa* LTR1 showed maximum growth at 2 g/L and 3 g/L concentrations of KH_2PO_4 and K_2HPO_4, respectively, in the flask containing soybean oil as sole carbon source and pH 7.0 incubated at 37 °C, 150 rpm in shaking incubator (Figure 1E). Increasing the concentration of KH_2PO_4 and K_2HPO/ increases the growth of organisms till the concentration reaches 2 g/L and 3 g/L, respectively; beyond that, increasing concentration decreases the growth of the microorganism. The divalent cations such as Mg^{++} and Ca^{++} affect

the emulsification activity of the biosurfactant synthesized by *P. aeruginosa*. Increasing concentration of Mg^{++} from 1.1 mM to 2.5 mM concentration increased emulsification activity [28]. Furthermore, $MgSO_4$ as a divalent cation and trace element affects growth and emulsification activity [29]. Maximum growth was observed at 200 mg/L concentration of $MgSO_4$ in the flask containing soybean oil. Increasing the concentration of $MgSO_4$ increases the growth of organisms till the concentration reaches 200 mg/L, and beyond that, increasing concentration decreases the growth (Figure 1F). Similarly, $CaCl_2$ also showed a concentration-dependent change in the growth. Maximum growth was observed at 10 mg/mL at 72 hours. Later concentrations of $CaCl_2$ showed a decrease in the growth. Ferric chloride as a source of iron is also one of the important constituents of MSM and essential for biosurfactant production media[30]. The optimum concentration of $FeCl_3$ required for maximum growth was 20 mg/L after 72 hours incubation containing soybean oil as sole carbon source, pH 7 at 37 °C (Figure 1G).

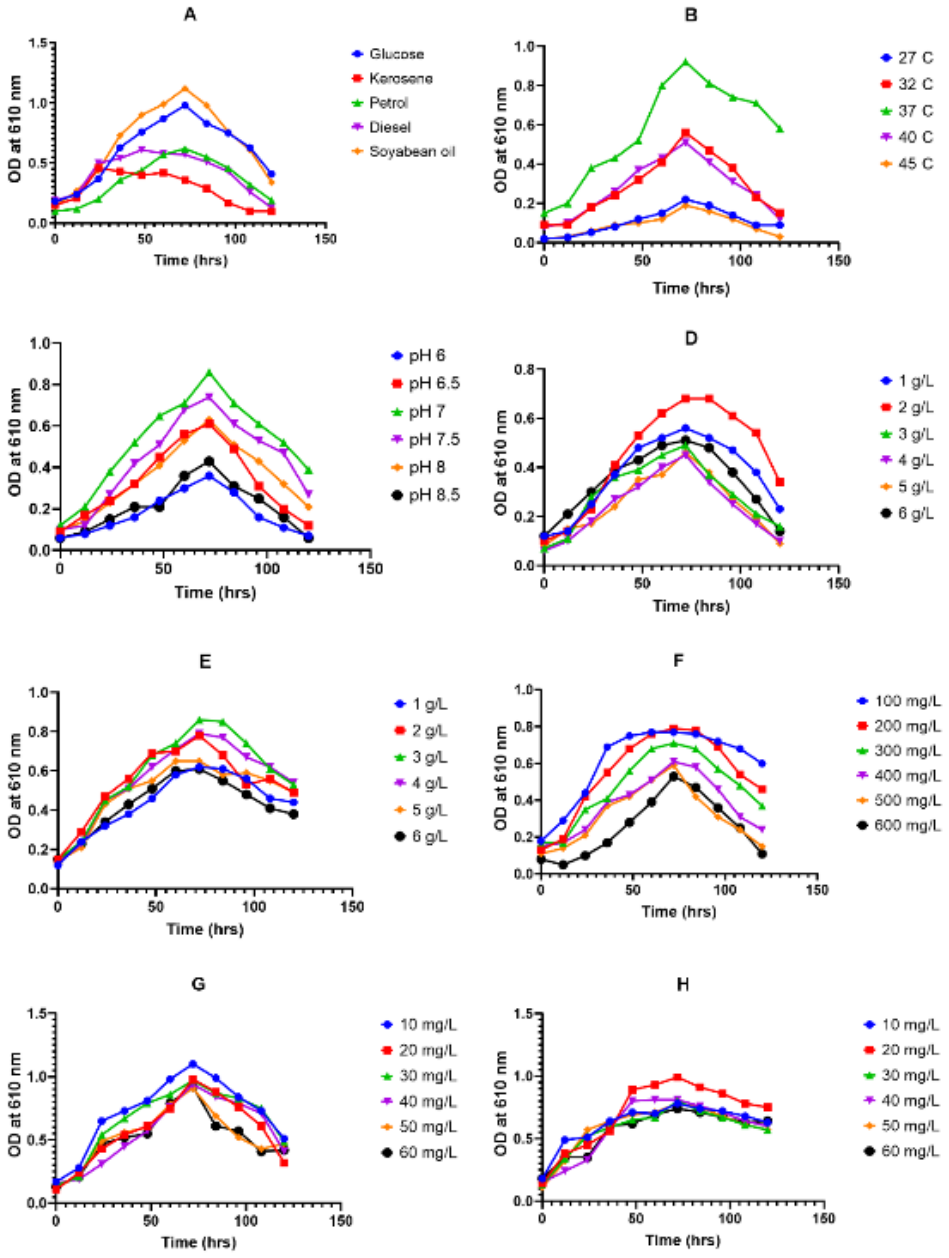

Figure 1. Figure: effect of carbon source, Temperature pH, and various metal sources on growth of biosurfactant producing strain LTR1. Growth at **A**: various carbon sources; **B**: Temperature; **C**: pH; **D**: KH_2PO_4 **E**: K_2HPO_4; **F**: $MgSO_4$; **G**: $CaCl_2$; **H**: $FeCl_3$, $p \leq 0.05$.

3.3. Production of biosurfactants by batch culture.

In the optimized production medium *P. aeruginosa* strain, LTR1 showed pigmented and turbid growth after 48 hours of incubation. About 9.5 g/L of biosurfactant yield was obtained within 72 hours.

3.4. Biochemical characterization of biosurfactant.

The extracted biosurfactant was biochemically characterized for detecting carbohydrates, protein, and lipids using qualitative tests. Anthrone and saponification tests for carbohydrates and lipids, respectively, were positive for the extracted biosurfactants. Bradford test performed for protein was negative. Biochemical tests indicate that the biosurfactant produced by *P. aeruginosa* strain LTR1 is glycolipid. Most of the biosurfactant glycolipids produced by *Pseudomonas* are rhamnolipid. So, we further analyzed the biosurfactant for rhamnolipids.

3.5. Purification of biosurfactants by column chromatography.

The biosurfactant produced by *P. aeruginosa* strain LTR1 was extracted by acid precipitation method, and the crude extract was subjected for further purification using silica gel column chromatography. The fraction of rhamnolipid eluted at 50:50 v/v methanol: chloroform was restored. The purified biosurfactants were light yellow to brownish and viscous liquids with solubility in most organic solvents but sparingly soluble in water and methanol. The recovered biosurfactant was dried by using a rota-evaporator to the powder.

3.6. Identification of functional group by FTIR.

FTIR is a powerful technology for the functional group's determination of chemical compounds. Purified biosurfactant was analyzed for functional group detection using FTIR. The significant peaks were observed at 3240 cm^{-1}, 2923 cm^{-1}, 2854 cm^{-1}, and 1745 cm^{-1} vibrations indicating stretching for –CH and $–CH_2$. Vibration at 2923 cm^{-1} and 2854 cm^{-1} indicates $–CH_3$ stretching. In addition, strong stretching of –C=O of the ester carbonyl group was observed at 1745 cm^{-1}. The FTIR spectra of biosurfactants from *P. aeruginosa* LTR1 also revealed the presence of lipid moiety in the purified glycolipids showing peaks at 1458 cm^{-1}, 1378 cm^{-1}, 1147 cm^{-1}, 1073 cm^{-1}, and 1013 cm^{-1}. The intensity band in the region of 1455–1386 cm^{-1} shows bending of the hydroxyl (-OH) group, which reflects the presence of the carboxylic acid as a functional group in the compound [31]. Similarly, the biosurfactant showed an intensity band at 1458 cm^{-1}indicated bending of the hydroxyl (-OH) group, which reflects the presence of the carboxylic acid functional group in the compound. The band in the region of 1378 cm^{-1} was the result of deformations and bending vibrations of $–C-CH_3$ and $–C-CH_2$ groups (Figure 2).

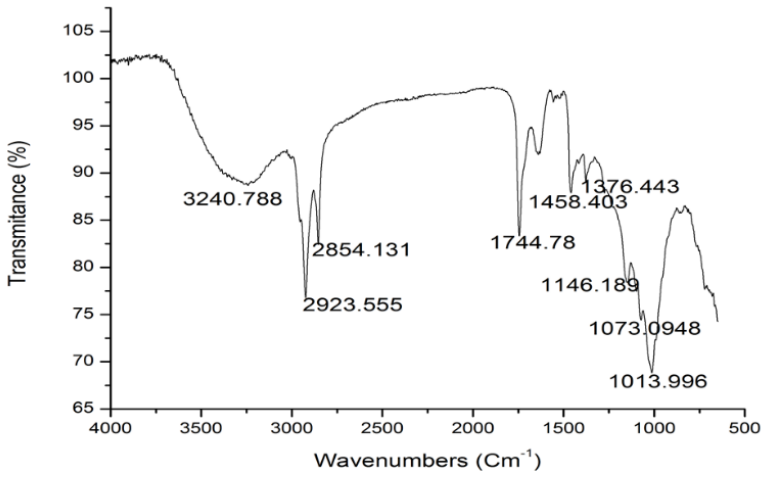

Figure 2. Determination of surface tension & CMC of biosurfactant produced by *P. aeruginosa* strain LTR1 in optimized production media containing Soybean oil as sole carbon source.

3.7. Surface tension & determination of CMC.

The reduction in surface tension measurement and determination of critical micelle concentration (CMC) of crude biosurfactant was carried out to measure extracted biosurfactants' tension-active properties and effectiveness. The biosurfactant obtained from *P. aeruginosa* strain LTR1 was able to reduce the surface tension of water from 72 to 31 mN/m. The point of deflection obtained from the surface tension reduction curve at the lowest concentration of biosurfactants was 12 mg/L called critical micelle concentration (CMC). Further increase in the concentration of biosurfactants showed a very negligible impact on the lowering of surface tension (Figure 3).

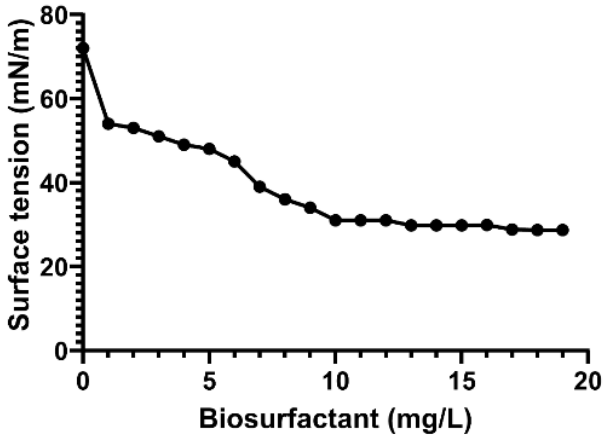

Figure 3. FT-IR spectra of biosurfactant produced by *P. aeruginosa* strain LTR1 using Soybean oil as the sole carbon source.

3.8. Emulsification activity.

Emulsification activity of the biosurfactant produced in the supernatant culture of *P. aeruginosa* strain LTR1 determined for kerosene, diesel, petrol, and soybean (Figure 4). The supernatant containing biosurfactant, emulsification index for soybean was maximum (74.66 ± 3.05) and minimum for diesel (38.33 ± 7.03). Emulsification indices for kerosene and petrol were 50 ± 5.56 and 48 ± 3, respectively (Figure 4). A higher emulsification index for a biosurfactant of the bacteria against hydrocarbon indicates the stronger hydrocarbon-degrading capacity of bacteria [32].

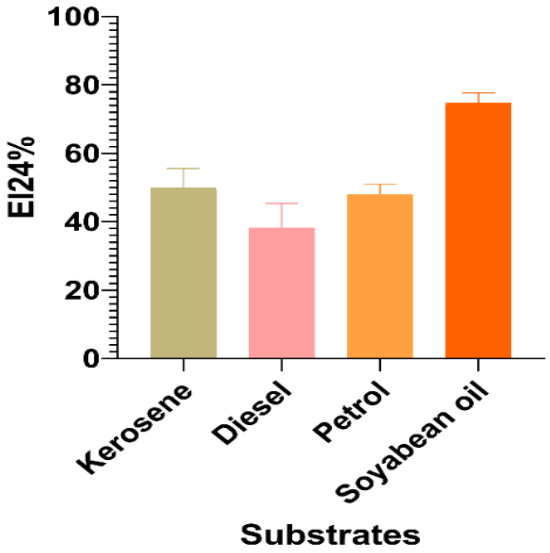

Figure 4. Emulsification activity of a supernatant culture of *P. aeruginosa* strain LTR1 against various hydrocarbons, $p \leq 0.05$.

3.9. Antibiofilm activity of purified biosurfactant.

Biofilms are challenges for clinicians and researchers due to their high resistance against antimicrobials, especially antibiotics [33,34]. Biosurfactants have shown their potential as antimicrobials against drug-resistant microorganisms [35]. Antibiofilms activity of purified biosurfactant was determined by broth dilution method and estimated by MTT assay. There was a concentration-dependent decrease in biofilm formation when treated with various concentrations of biosurfactant (Figure 5 and 6). The minimum inhibitory concentration was recorded to be 30.91 µg/mL. The inhibition concentration was higher than other standard antifungals like caspofungin (0.25 µg/mL) and other antifungals [36], but they can be potential drug development candidates.

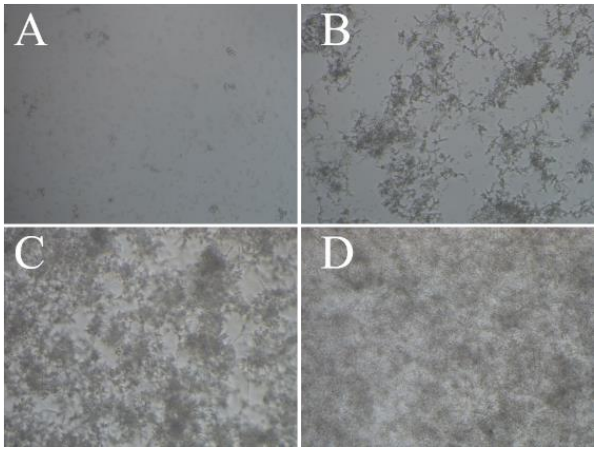

Figure 5. Light microscopy picture of biofilms treated with different concentrations of purified biosurfactant produced by *P. aeruginosa* strain LTR1, **A**: 100 µg/mL, **B**:50 µg/mL, **C**:25 µg/mL, and **D**: Control (no biosurfactant).

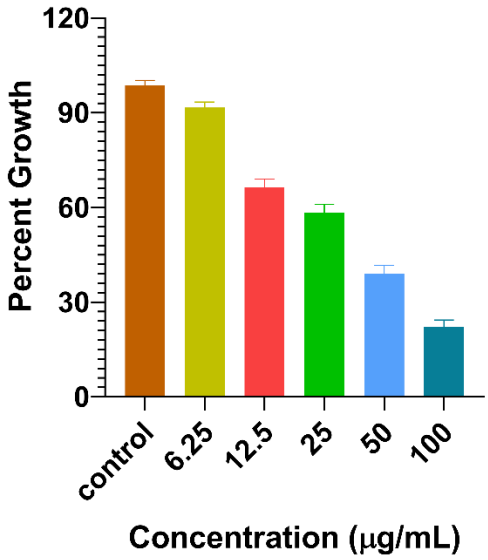

Figure 6. Antibiofilms activity of purified biosurfactant produced by *P. aeruginosa* strain LTR1, $p \leq 0.05$.

Various carbon sources such as olive oil and distillery wastes, processed wastewater from food industries, kerosene, diesel, petrol, *n*-hexadecane, paraffin oil, whey, molasses, palm oil, canola oil, vegetable oil, and corn oil refinery wastes were successfully used to produce biosurfactant [37]. Due to their easy availability throughout the year, easy transportation, and low cost, these raw materials locally grasped more attention to use as a substrate for the production of biosurfactants [38]. The soybean is one of the major vegetable oil crops produced worldwide, and supply is more than that the demand [39]. It is a rich source of unsaturated fatty acid consisting of oleic, linoleic, and linolenic acid [40]. India's Marathwada and West Maharashtra region has a high cropping pattern of soybean as a major cash crop cultivated to produce soybean oil [41]. Here in this research communication thus we used soyabean oil as one of the carbon sources and kerosene, petrol, and diesel.

Pseudomonas species have been reported as a prominent biosurfactant producer and produce glycolipids type biosurfactants, mainly rhamnolipid. Functional group analysis by FTIR and biosurfactant characterizations studies indicate that isolated biosurfactant is not a protein in nature. NMR and MS studies may explain the exact structure of the glycolipid type of biosurfactant. Mercade *et al*. reported the 6.4 g/L of rhamnolipid biosurfactant production from *Pseudomonas* spp. JAMM using olive oil mill effluents which were extracted by acid precipitation method [38]. Rahman *et al*. reported 4.31 g/L rhamnolipid production using soybean oil from *P. aeruginosa* DS10-129. With 12 days of fermentation [42]. In the comparative study of the production of biosurfactants using various vegetable oils at 2 % concentration such as olive oil, palm oil, and coconut oil in defined mineral salt medium from *P. aeruginosa* A41 strains [43]. The optimum yields of 6.58 g/L, 2.91 g/L, and 2.93 g/L biosurfactants production were reported in 2 % olive oil, palm oil, and coconut oil, respectively. In rhamnolipid production by *P. aeruginosa,* DS10-129 exploited soybean oil as a substrate with 12 days of incubation. There was no significant growth detected within three days of incubation. Later, a progressive increase in growth was observed up to 12 days. Later, there was no significant increase in the growth and production of rhamnolipid [42]. This exhibited that biosurfactants were produced as secondary metabolites.

In comparison to *P. aeruginosa* DS10-129 to produce biosurfactants using soyabean oil, *P. aeruginosa* LTR1 were the best candidates having a one-day lag phase with a higher growth within 72 hrs. Biosurfactant yield by *P. aeruginosa* strain LTR1 was sufficiently more

and showed a reduction of surface tension of water. The biochemical constituent of biosurfactant produced by *P. aeruginosa* strain LTR1 is Rhamnolipid type. Rhamnolipids are glycolipids shown to be efficient metal chelating agent that interacts with metals through a polar glycosidic bond [44]. In the rhamnolipid treated calcareous soil, biomass production was increased as it helps in the absorption of Zinc by the plant [45]. The chelating metal property of the biosurfactants has made these molecules a potential candidate for heavy metal remover from water and soil [46]. Biosurfactants are also have been utilized for enhanced crude oil extraction transportation and recovery [8]. Biosurfactant produced by *P. aeruginosa* strain LTR1 showed emulsification activity in the range of 38 to 74 against vegetable oil and petrochemical hydrocarbon, suggesting its use in oil refiners and bioremediation of soil and water bodies contaminated by oils due to spillage. Antibiofilm activity at 30.95 µg/mL is an indicative figure as a potential candidate for antimicrobial drug development. Thus, the rhamnolipid produced from hydrocarbonoclastic bacteria *P. aeruginosa* strain LTR1 with promising tensioactive properties and antimicrobial may find applications in various industries.

4. Conclusions

The biosurfactant could be produced from various carbon compounds, including an agricultural product like soybean oil using *P. aeruginosa* LTR1. Physiochemically optimized MSM medium showed 9.5 g/L of biosurfactant production within 72 hours. The biochemical characterization of biosurfactant revealed the presence of carbohydrate and lipid, which had tension-active properties of 31 dyne/cm with a critical micelle concentration of 12 mg/mL. Culture supernatant of *P. aeruginosa* strain LTR1 showed emulsification activity in the range of 38 to 74 against vegetable oil and petrochemical hydrocarbon, suggesting its use in oil refiners and bioremediation of soil and water bodies contaminated by oils due to spillage. Antibiofilm activity at 30.95 µg/mL is an indicative figure as a potential candidate for antimicrobial drug development. Thus, the rhamnolipid produced from hydrocarbonoclastic bacteria *P. aeruginosa* LTR1 with promising tensioactive properties and antimicrobial may find applications in various industries.

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Conflict of interest

The authors declare no conflict of interest.

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HISTOLOGICAL CHANGES IN FEMALE GONADS OF *MERETRIX MERETRIX* UNDER THE INFLUENCE OF CHLORPYRIFOS

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ABSTRACT

Chlorpyrifos exert adverse effect on Female gonad of estuarine clam *Meretrix meretrix*. The clams treated with Chlorpyrifos showed considerable damage to the female gonad in summer season. The animal showed swelling of the follicle walls and deterioration of cellular material. Follicle wall was ruptured at places. Cytoplasm of oocytes showed prominent vacuoles. A few oocytes showed kariolysis and appeared to undergo degeneration. The vitellogenic oocytes showed fragmentation of cytoplasm.

Key Words : Histology, Chlorpyrifos, *Meretrix meretrix,* female gonad

Introduction:

In India, pesticides are widely used in agriculture on variety of crops for pest control. Indiscriminate use of pesticides has seriously endangered aquatic fauna, man and its environment. Pesticides in water pose severe problems, because their residues induce adverse effect on the health of human and domestic animals. Unlike other chemical contaminants, pesticides are poisonous substances which are deliberately introduced into the environment in order to exploit their toxic properties to kill unwanted organisms and they become serious pollutants. Environmental scientist and engineers now consider pesticides as a source of pollution. Although there is great deal of concern about pesticide pollution, only the few past years have witnessed pesticide toxicity to animals.

Organophosphate has acute toxicity impact on different animals. These are major group of insecticides that are broadly applied to control various insect pests. The main toxic effect of organophosphate is due to the inhibition of acetylcholine esterase-9 which may block the nerve ending (Coppage*et al.*, 1975). The organophosphates also affect the respiratory and circulatory system causing muscular twitching. Basically, they block the activities of choline esterase, which play an important role in the transmission of nerve signals. The actions of this enzyme are affected by the organophosphate insecticides, which in turns results in the dysfunctioning of cardiac cycle (Hart *et al.*, 1993). Acute organophosphorus poisoning may induce multisystem toxicity leading to severe toxicity and death (Darren *et al.*, 2007).Among organophosphorus compounds, malathion and parathion are mostly used. Mukhopadhyay *et al.* (1978) have reported an elaborative work of pesticide and their effects on freshwater animals from different physiological aspects.

Water and mud at the bottom of rivers, streams, lakes and ponds are the major depositories of pesticides. The suspended mud particles, bottom sediments and organic matters may adsorb and hold-up a large amount of pesticides from the water column. It is believed that the wet soil molecules compete for the adsorption site with non-polar pesticide molecules (Edwards, 1973), so that the insecticides get released and move in the direction of flow of water.

The persistence of some pesticides in the environment, coupled with repeated or multiple uses of the different chemical agents to increase their effectiveness can result in hazardous effects on aquatic species (Schubert *et. al.*, 1978). Chlorpyrifos (trade names include Dursban TM and Lorsban TM), one of the most widely used insecticides in the U.S. and major part of world with 20 to 24 million pounds applied annually, has been linked to thousands of pesticide poisoning incidents. This Dow Agro-Sciences, previously Dow-Elanco, product is a broad-spectrum chlorinated organophosphate insecticide (Aspelin, 1997). Chlorpyrifos is one of the pesticides encountered in the marine environment. It is a crystalline organophosphate insecticide. The IUPAC name of chlorpyrifos is O, O-diethyl *O*-3, 5, 6-trichloro-2-pyridyl phosphorothioate and with molecular formula $C_9H_{11}C_{13}NO_3PS$. Chlorpyrifos is moderately toxic and chronic exposure has been linked to neurological effects, developmental disorders, and autoimmune disorders.

In Ratnagiri district, Chlorpyrifos is widely used to control pests of mango and paddy. Majority of farmers are not aware about the proper use of chlorpyrifos and its adverse effects. They just focus on the productivity and protection of plant from insect pest. This ignorance is commonly responsible for excess use of chlorpyrifos which leads to the pollution of nearby water reservoirs and finally estuaries. Because of its misuse chlorpyrifos, gets concentrated in aquatic bodies like rivers and estuaries and might get biomagnified in the food chains in the near future.

Molluscs (particularly bivalves) and fish are usually proposed as good bioindicators for environmental contamination of marine ecosystems (Claude, 1999). Clams are sessile organisms and can't move from unfavorable to favorable environment. Clams have been extensively utilized in the past as a biological indicator of pollution in monitoring programs. The reason for this choice is that the mussel is a sessile, filter-feeding organism, able to accumulate within its tissues many of the contaminants (pesticides, hydrocarbons, metals, etc.) present in sea water. In addition, mussels show a wide geographical distribution, thus permitting the survey of extensive coastal areas. The contaminants accumulated in the tissues of mussels may cause a "stress syndrome" with alteration to their physiology (Viarengo*et al.*, 1991).

The study of histology provides a very important and useful data, concerning changes in cellular and sub-cellular structure of an organ. Any particular alteration of cell may indicate the presence of disease or toxic substance. The extent of damage induced by the toxicant to particular organ can also be judged at a cellular level. According to Virchow, the German pathologist, the normal functioning of any organ is possible when its structure is normal. Histopathology deals with the study of patho-chemical changes induced in the microscopic structure of tissues. Brown and Jordan (1968) suggested that there is a clear correlation between pathological condition of cell or tissues and its affected functions. These studies beneficial to investigate the extent of pollution and the nature of lethality of pollutants.

Materials and methods :

The Kalbadevi estuary is 8 Kms away from Ratnagiri in the Kokan region and this is the major estuary on the west coast of Maharashtra state. The experimental clam, *Meretrix meretrix*, used for the present study was collected from Kalbadevi estuarine region, During low tide clams are handpicked. In the shallow estuaries and sandy beaches fishermans usually remove the sand by their feet or by wooden or metal plates and pick out the buried clams (Joseph *et al.*, 2000).

The clams of size 5.5 to 6 cms were selected, brought to the laboratory and stocked in the plastic containers containing filtered, aerated estuarine water, for 48 hours. Clams well acclimatized to the laboratory condition were grouped in ten animals each and kept in plastic containers containing 5 l filtered estuarine water. Static bioassay tests (Finney, 1971) were conducted for 96 hours by using Chlorpyrifos. For every experiment, a

control group of clams was also run, simultaneously. For the formulation of test concentration, pilot experiment was conducted and range of concentration was selected such that it resulted in zero to total mortality.

The toxicity tests were repeated for three times and LC_0 and LC_{50} values were determined. The regression equation between log of concentration (X) and probit mortality (Y) were determined statistically for acute toxicity using the formula $Y= \alpha + \beta \log (X)$ and 95% fiducial limits were established (Finney, 1971).

Based on the LC_{50} values, $1/10^{th}$ concentration of the LC_{50} of chlorpyrifos was selected forsublethal toxicity (30 days) studies. Sublethal exposure was done in static system where water and pesticide medium were renewed every 12 hours to obtain the desired pesticide concentration. A control, free of pesticide, was maintained in each experiment.

After studying for 96 hours (acute) and 30 days (chronic) toxicity of Chlorpyrifos to *Meretrix meretrix* in different seasons, female gonad of control, LC_0 and LC_{50} groups from acute exposure and $1/10^{th}$ concentration of LC_{50} groups from chronic exposure were removed and fixed in neutral buffer formalin for 48 hours for proper fixation. Female gonads were washed in distilled water, then dehydrated in ethyl alcohol; cleared in xylol and embedded in tissue mat (at 58-60°C melting point) and then they were sectioned at 5 to 6 µm thickness on a rotary microtome (Erma, Japan). These sections were stained with Harris Hematoxylene and alcoholic Eosin stain and mounted in DPX. All the observations for microphotography were done under trinocular research microscope attached with camera (Carl Zeiss, model: Axiostar Germany).

Results and Discussion

The female gonad showed a developmental condition of gonad in summer and winter. The female follicle showed follicle wall with prominent germ cells and vitellogenic oocytes of different sizes. Many free mature eggs possessed dense cytoplasm and distinct nucleus located in centre having prominent nucleolus. The staining of these different parts in female follicle was distinct. In monsoon, female gonad showed few relict gametes in the female follicle containing very few mature eggs with distinct nucleus and nucleolus. Very few vitellogenic oocytes and germ cells appeared along the follicle wall (Plates 1, 2, 3).

In summer, as compared to control, LC_0 group of clams showed considerable damage to the female gonad. The follicle wall ruptured at places with shrinkage of germ cells along the wall. Deterioration of ooplasmic material, the nucleus and nucleoli was observed in mature eggs. The germ cells and vitellogenic oocytes lost their shape and detached from follicle wall. As compared to control, in LC_0 group, swelling of follicle was considerable in summer. More or less similar types of effects were observed in LC_0 group during three different seasons, but the effect was comparatively more severe in summer followed by monsoon and winter (Plates 1, 2, 3).

As compared to control, LC_{50} group of clams showed considerable damage to female follicles. The damage was more severe in summer than in winter and monsoon. The female follicle wall was distorted but mostly showed prominent nuclei and nucleoli and the cytoplasm was opaque. These are likely to undergo degeneration. Small sized previtellogenic oocytes also showed such conditions. Nature of damage was more severe in summer than the other seasons (Plates 1, 2, 3).

In summer, chronic group showed swelling of the follicle walls and deterioration of cellular material. Follicle wall was ruptured in places. Cytoplasm of oocytes showed prominent vacuoles. A few vitellogenic oocytes showed kariolysis and appeared to undergo degeneration. The vitellogenic oocytes showed fragmentation of cytoplasm. The damage was more severe in summer followed by monsoon and winter (Plates 1, 2, 3).

Reproductive cycle of M. meretrixwas divided into 5 stages: proliferation stage,

growing stage, maturation stage, breeding stage and suspensive stage. Gonad of female have light brown, gonad of male have milky white when sexually mature (Nguyen,2013). In the present study, the tissue damage was observed in LC_{50} and chronic group than control and LC_0 group. Severe damage was observed in summer.

Comparing the effects of chlorpyrifos on female gonads of clams, it was observed that, effects were more prominent in summer, followed by monsoon and winter. Follicular shrinkage and distortion was observed in LC_{50} and chronic groups.Muley (1985), while studying the effect of pesticides on the gastropod, *Viviparus bengalensis*, observed growth retardation and damage to germ and sex cells.Bagchi (1990) studied the effect of quinolphos on testicular steridogenesis in fish *Clarius batrachus*. Their observations are in good agreement with present study.

In LC_{50} and chronic group, vacuoles in cytoplasm and degeneration of oocytes were observed. Muley (1985) observed similar changes in both LC_0 and LC_{50} groups of *V. bengalensis* after acute exposure to folithion and lebaycid. Rastogi and Kulshrestha (1990) studied the histopathological changes from ovary due to pesticidal stress for *Rasborodani conius.*Prabhupatkar (2004) observed similar type of histological alterations in female gonad of *Meretrix meretrix* after acute exposure to Cypermethrin. Dutta and Dalal (2008) while studying the effect of endosulfan in blue gill sunfish (*Lepomis macrochirus*) observed that, histopathological changes like damage to stroma and cytoplasm and nuclear retraction of oocytes cells. Tendulkar (2012) observed similar type of histological alterations in female gonad of *Marcia (Katelysia) opima* after acute exposure to Cypermethrin. In a study conducted by Gormley and Teather (2003), Japanese Medaka (*Oryzia slatipes*) was exposed to different concentrations of endosulfan for 24 hours exposure times and eggs were found to take longer to hatch. The resulting fry were smaller at one week of age and had decreased mobility at two weeks of age.

The histological study of Female gonad of clams showed more pronounced effects in chronic and LC_{50} group than LC_0 and control group. Severe damage in female gonadwas observed in summer than that in Monsoon and winter. The hierarchy of female gonad damage is in descending order is Summer> Winter > Monsoon.

Plate No. 1

Summer Season

Female Gonad

(250X)

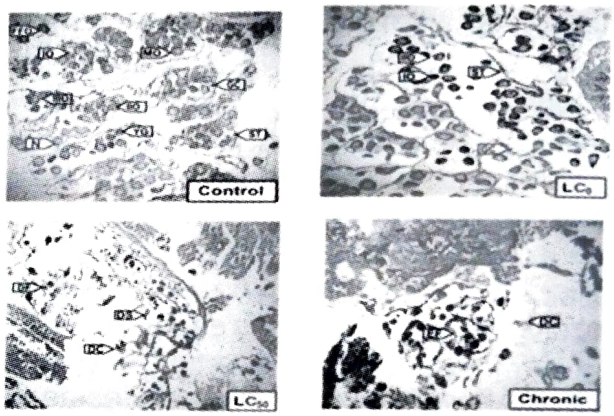

Plate 1:Section of Female Gonad of *Meretrixmeretrix*from Control, LC_0, LC_{50} and Chronic exposure to Chlorpyrifos during summer (250X).

Plate No. 2

Monsoon Season

Female Gonad

(250X)

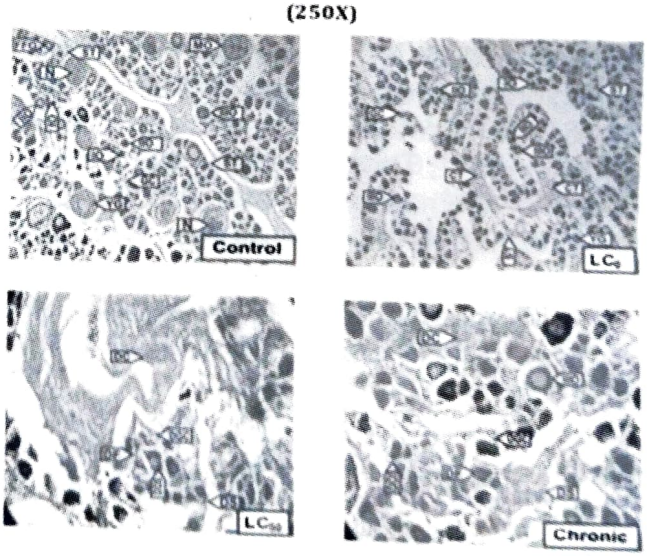

Plate 2:Section of Female Gonad of *Meretrixmeretrix*from Control, LC_0, LC_{50} and Chronic exposure to Chlorpyrifos during Monsoon (250X).

Plate No. 3
Winter Season
Female Gonad
(250X)

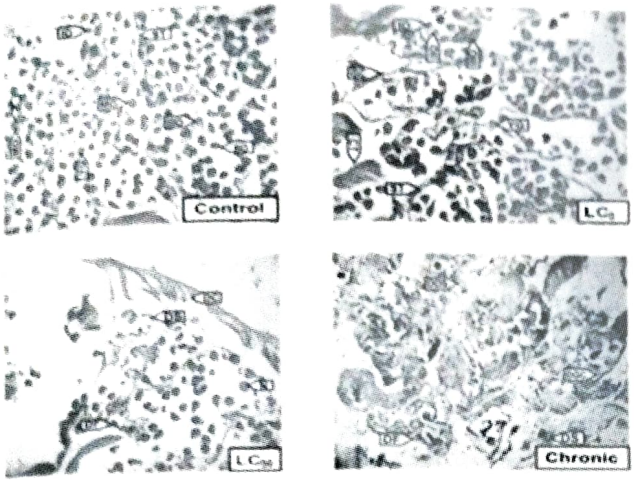

Plate 3:Section of Female Gonad of *Meretrixmeretrix*from Control, LC_0, LC_{50} and Chronic exposure to Chlorpyrifos during Winter (250X).

Abbreviations : FFO: Fully Mature Follicle, MO: Mature Ovum, GC: Germ Cell, N:Nucleus, ST : Stroma, YG: Yolk Granules, IO:Primary Oocyte, IIO: Secondary Oocyte, IIIO:Tertiary Oocyte, DF: Distorted Follicle, DS: Degenerated Serosa, DC: Damaged Connective Tissue, EF: Empty Follicle

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HISTOCHEMICAL STUDIES ONTHE EFFECT OF SODIUM FLUORIDE ON THE PRODUCTION OF INTESTINAL MUCIN IN FRESHWATER FISH, *RITA RITA*

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ABSTRACT

Fluoride toxicity adversely affects various organs of freshwater fishes when those are exposed to their excess concentration. It was observed during present histochemical investigation that sodium fluoride exposure significantly increased mucin secretion and goblet cell proliferation in freshwater fish, *Rita rita* in order to protect the intestinal mucosa.

Key words : Sodium fluoride, Mucin , Intestine , *Rita rita*

Introduction

The main source of fluoride in ground water is fluoride-bearing rocks such as cryolite,fluorite, fluorspar, fluorapatite and hydroxylapatite(Meenakshi *et al.,* 2004). Fluoride enters into the human body through water, drugs, food, industrial exposure, (Susheela *et al.,* 1993), and it causes adverse effects on the health causing fluorosis. Fishes are important creatures of aquatic food chain, and through them some toxicants may reach human beings as well (Gopalkrishnan, 1990).

Accumulation and increased mucus secretion in fluoride exposed fish may be an adaptive and protective response, in order to avoid the absorption of toxicant by body surface (Dasand Mukherjee, 2003; Yilmaz *et al.,* 2004).

Intestinal goblet cells maintain protective epithelial barrier through mucin production (Gustafsson *et.al.,* 2021) . Present investigation was carried out to investigate the effect of sodium fluoride on histochemical changes due to the secretion of mucin produced by goblet cells of the fish. .

Material and Methods

Fresh water fishes, *Rita rita* were collected from Ujani Dam, Solapur Maharashtra. Those were allowed to acclimatize for 10 days in laboratory conditions. The fishes were divided into two groups, one served as control group while another the experimental group. The fishes from experimental group were exposed to 7 mg /l sodium fluoride. After an interval of 10 and 20 days, fishes from both groups were dissected and the tissue from intestine was fixed in Carnoy's fixative. The tissues were dehydrated in alcoholic grades and cleared in xylene. The paraffin blocks of the tissue were prepared for section cutting, and sections were subjected to Alcian Blue 1.0 pH stain. Microphotographs of stained sections were observed for histo-chemical observation of mucin producing goblet cells.

Results and Discussion

The histo-chemical changes caused in intestinal mucin due to the treatment with Sodium fluoride have been shown in figures 1-3.It was noticed that proliferation of goblet cells and mucin production increased in sodium fluoride treated group, which indicated a defense mechanism against toxicant. In control group goblet cells secreted mucin in comparatively small amount. The increase in the number of goblet cells and proportion of mucin production increases considerably after 10 and 20 days of exposure when compared with the control group.

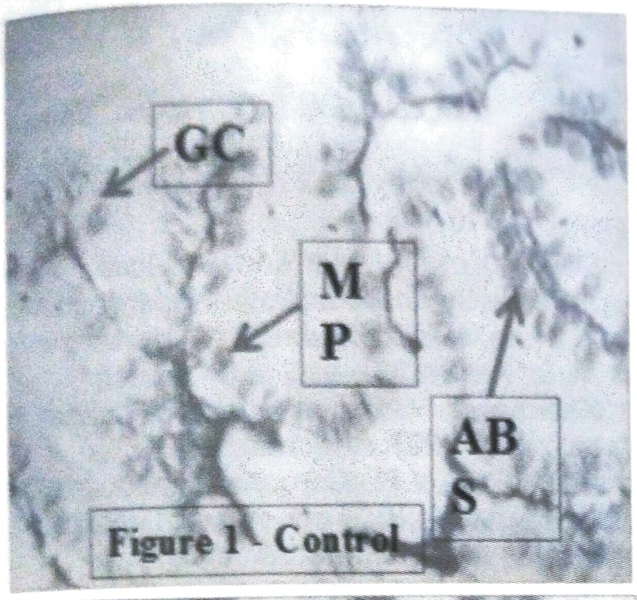

Figure 1 - Control

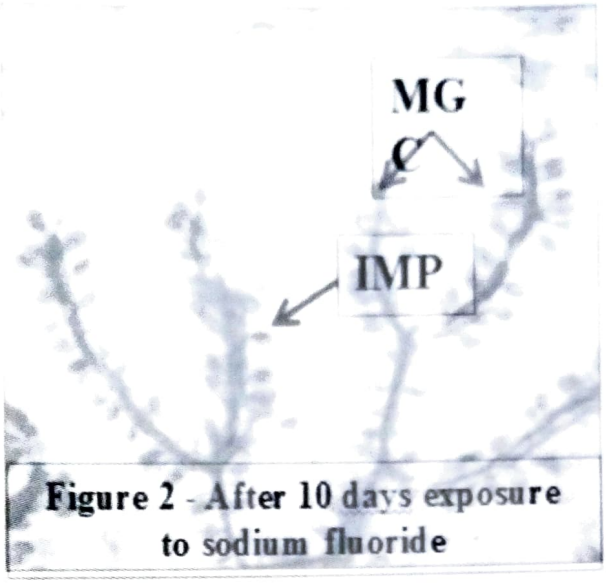

Figure 2 - After 10 days exposure to sodium fluoride

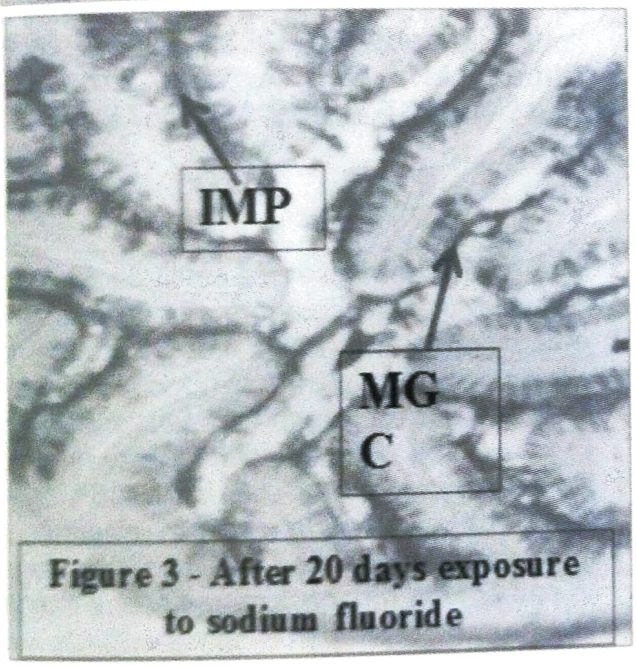

Figure 3 - After 20 days exposure to sodium fluoride

GC- Goblet cells ,
MP- Mucin Proportion,
ABS - Alcian Blue pH 1.0 stain
MGC-Multiplied goblet cell
IMP- Increased mucin proportion

It was reported by Bagale *et al.*, (2020) that the muco-substances and glycogen content in the intestine of *Tilapia mossambica* increased over time of exposure. The intestinal mucosa is the first line of defense for the host and thus plays a pivotal role for maintaining gut homeostasis (Gilloi*set al.*, 2018). Kshirsagar and Injal had earlier (2022) reported adverse effects of sodium fluoride on histological structure in testis of freshwater fish ,*Rita rita*. Abdel-Wahab *et al.*,(2020)observed that Lead Nitrate toxicity resulted in alterations of intestine in African cat fish (*Clariasgariepinus*) and recorded considerable proliferation and multiplication in number of mucin secreting goblet cells. Present study,thus, indicated that, chronic exposure to sodium fluoride causes significant increase in the production of mucin by goblet cells. The mucin content was found to be increased with the exposure time.

It can thus be concluded that the mucin secreting goblet cells proliferate due to sodium fluoride toxicity,as a mean of defense mechanism. Goblets cells and mucin concentration considerably increased to prevent absorption of Sodium fluoride and provide protection to gastrointestinal tract.

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।। संशोधक।।

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- **शके १९४४**
- **वर्ष : ९०**
- **पुरवणी अंक : ६**

✷ प्रकाशक ✷

श्री. संजय मुंदडा

कार्याध्यक्ष, इ. वि. का. राजवाडे संशोधन मंडळ, धुळे ४२४००१

दूरध्वनी (०२५६२) २३३८४८, ९४०४५७७०२०

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महाराष्ट्र राज्य साहित्य आणि संस्कृती मंडळाने या नियतकालिकेच्या प्रकाशनार्थ अनुदान दिले आहे. या नियतकालिकेतील लेखकांच्या विचारांशी मंडळ व शासन सहमत असेलच असे नाही.

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21 वी सदी के हिंदी उपन्यास 'शकुंतिका' में चित्रित स्त्री विमर्श के नए आयाम

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प्रस्तावना :

21 वीं सदी के हिन्दी साहित्य को विभिन्न तरह से समझने हेतु नया दौर शुरू हुआ इसी दौर में नारी विमर्श, दलित विमर्श आदिवासी विमर्श, सांस्कृतिक ऐतिहासिक बोध जैसी विविध विमर्श धाराएं विकसित हुई। हिन्दी साहित्य में नारी हमेशा चर्चा का विषय रही है श्र भारत में नारीवादी आंदोलन की शरुवात नवजागरण के साथ हुई। स्त्री विमर्श उस साहित्यिक आंदोलन को कहा जाता है जिसमें स्त्री अस्मिता को केंद्र में रखकर संगठित रूप से स्त्री साहित्य की रचना की गई। हिंदी साहित्य में स्त्री विमर्श अन्य अस्मितामूलक विमर्शों के भांति हीं मुख्य विमर्श रहा है जो की लिंग विमर्श पर आधारित है। हिंदी साहित्य में भी स्त्री विमर्श अनेक धाराओं में विकसित हुआ और उसका मूल कारण लेखिकाओं का अपना अनुभव जगत और अपनी अलग-अलग सामाजिक स्थिति । नारी आदिकाल से ही पीड़ित एवं शोषित रही है श्र पुरूष प्रधान समाज ने स्त्री को मान मर्यादा के आड़ में सदा दबाकर रखना चाहा । कभी घर का इज्जत कहकर तो कभी देवी कहकर चार दीवारों के अन्दर कैद ही रखा । इन्हीं परम्परागत पितृसत्तात्मक बेड़ियों को लांघने की लड़ाई है स्त्री - विमर्श।

प्रस्तुत शोधनिबंध के निम्न उद्देश्य है :

- 21 वीं सदी केहिंदी साहित्य में स्त्री विमर्श को जानना।
- 'शकुंतिका' उपन्यास में स्पष्ट नारी विमर्श के विविध दृष्टिकोण को जानना ।
- नारी विमर्श में हो रहे बदलाव को जानना ।
- हिंदीउपन्याससाहित्य में नारी की भूमिका को जानना।

प्रस्तुत शोधनिबंध के लिए ग्रथालयीन पत्र-पत्रिकाएँ, संदर्भ ग्रंथ साहित्य,वृत्तपत्रियलेख,पाठ्यपुस्तके आदि का सहारा लिया गया है ।

स्त्री विमर्श वस्तुतः स्त्री के प्रति होनेवाले शोषण अन्याय अत्याचार के खिलाप संघर्ष है । स्त्री मुक्ति अकेले स्त्री की मुक्ति नही बल्कि संपूर्ण मानवता की मुक्ति है । नारी मुक्ति से जुड़े अनेक प्रश्न चाहे वह पारिवारिक हो, सामाजिक हो, आर्थिक हो या शैक्षणिक हो वह सुलझाना चाहती है ।आज की स्त्री पितृसत्ताक पद्धति को तोडकर परंपरागत सीमाओं को लांघकर पुरुष की तरह स्वतंत्रता चाहती है । अपने अधिकारों के लिए लढ़ रही है । खुले आकाश तले अपने स्वतंत्र अस्मिता के लिए जीना चाहती है ।

शकुंतिका भगवानदास मोरवाल का एक नवीनतम उपन्यास है। उपन्यास का कथानक संवेदनशीलऔर भाषा सहज - सरल है। यह छोटा सा उपन्यास भारतीय समाज की उस धारणा में आये बदलाव को रेखांकित करता है जो लड़कियों को लड़कों से कमतर आँकती रही है। लेकिन जिस सामाजिकबदलाव को लेखक दिखा रहा है और जितनी सहजता से चित्रित कर रहा है वह भारत के समाज का कितना वास्तविक चित्र है, यह विचारणीय है।

शकुंतिका नारी विमर्श पर लिखा महत्वपूर्ण उपन्यास है इस उपन्यास में स्त्री जीवन की विविध छटाओ का परिचय होता है तीन पीढ़ियों से लेकर नारी जीवन में हो रहे बदलाव को लेखक ने बखूबी से इस उपन्यास में जांचा परखा है। भारतीय समाज व्यवस्था पितृसत्ताक है। किंतु इस उपन्यास में नए परिवर्तन वादी विचारों को स्पष्ट कर स्त्रीसत्ताक पद्धति को तथा स्त्री के जीवन पद्धति को सजीवता से उभारा गया है । बेटी का जन्म परिवार के लिए भार न होकर वह उस परिवार का आधार बन जाता है बेटे से भी ज्यादा खुश बेटियों से भरा परिवार है जिसमें कोई चिंता नहीं कोई किसी प्रकार की कमी नहीं बल्कि खुशियां ही खुशियां समाई है । जो समाज व्याप्त होते हुए भी समाप्त होने का नाम नहीं ले रही है बल्कि दिन-ब-दिन बढ़ती जा रही है। शकुंतिका नारी जीवन पर आधारित उपन्यास है। सिर्फ बेटे ही कुलदीपक नहीं होते बल्कि बेटियां भी कुलदीपक बनकर कुटुंब का उद्धार करती है और अपने परिवार का सहारा बन जाती है। आज भी कितने परिवार हैं जिनमें बेटियों के जन्म का स्वागत

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नहीं किया जाता बल्कि बेटे के जन्म का स्वागत ढोल बजाकर मिठाइयां बांटकर किया जाता है। खुशिया बाटी जाती है।

प्रस्तुत उपन्यास में भगवानदास मोरवाल ने भगवती और दुर्गा के माध्यम से परंपरागत विचारधारा वाली मध्यमवर्गीय महिलाओं का चित्रण किया है । दुर्गा को पोता होते ही थाली बजाकर आनंद व्यक्त किया जाता है किंतु भगवती के घर एक के बाद एक दोनों पोतियों के होते हुए भी तीसरे बच्चे का जन्म होनेवाला है । भगवती के मन में डर है कि कहीं बेटा पैदा नहीं हुआ तो वंश कैसे आगे बढ़ेगा और इसी विचार से वह निराश रहती है। आधुनिक युग में भी पुत्र जन्म की लालसा उनके मन में कम नहीं होती भगवती मन ही मन दुखी होती है । जब तीसरी पोती बुलबुल का जन्म होता है तो मानो भगवती का परिवार खुशियों से भर उठता है । गौरैया जैसी चहचहाहट उनके घर में बनी रहती है परंतु बेटिया परिवार पर बोझ नहीं बनती है। बड़ी पोती गार्गी डॉक्टर बन जाती है सिया वकील बन जाती है। दुर्गा का पोता विभोर दसवीं में फेल हो जाता है उसे लगता है कि सारे के सारे कौरव इसी घर में क्यों पैदा हुए। बड़े होने पर दुर्गा और उग्रसेन के सभी बेटे और पोते उनका घर छोड़ कर चले जाते हैं और दोनों अकेले उस घर में रहने लगते हैं। उनके मन में निराशा ही निराशा छा जाती है। दुर्गा भगवती से कहती है –भगवती, उनसे जाकर पूछो जिनके लड़कियाँ नहीं हैं । अब हमारे यहीं देख लो । सारी की सारी कौरवों की फ़ौज़ पैदा हो गयी । मैं तो ऊपरवाले से रात–दिन यही बिनती करती रहती हूँ कि बस हमें एक पोती दे दे । परिवार में इनका कोई विरोध भी नहीं है।

भगवती का परिवार गौरयों की चहचहाट से गूंज उठता है। दुर्गा हमेशा भगवती के घर आकर उनकी खुशी में शामिल हो जाती है गार्गी के डॉक्टर बनने पर वह खुद मिठाईयां बांटती है। एक दिन दुर्गा बीमार होती है तब गार्गी उसका इलाज करती है उनके घर खाना लेकर पहुंच जाती है अपनी दादी जैसी उनकी रखवाली करती है। भगवती का छोटा बेटा रूपेश और बहू जयंती शादी के छह साल बाद भी निसंतान है। सभी प्रकार का इलाज करने पर भी उन्हें बच्चा नहीं होता। उनके घर में अनाथ आश्रम से बच्चे को गोद लेने की चर्चा शुरू होती है। ऐसे में चाची दुर्गा लड़के के बजाए लड़की को गोद लेने की सलाह देती है तीन लड़कियों के साथ–साथ चौथी भी लड़की इस घर में आ जाए तो सुख का ही अनुभव आप करेंगे यदि किसी अनाथ आश्रम से लड़के को गोद लिया जाए तो इसकी क्या गारंटी है कि वह प्रॉपर्टी में हस्तक्षेप नही करेगा इन लड़कियों के साथ अन्याय करेगा इन्हें अपनी बहन नहीं मानेगा। ऐसा उसे लगने लगता है। इसतरह बड़ी सहजता से पूरा परिवार भगवती कि बात से सहमत होते हुए अनाथालय से लड़की गोद लेने के निर्णय को स्वीकार करता है। दुर्गा के दोनों पोते उसे छोड़कर प्रॉपर्टी में अपना अधिकार जमाने हेतु बार–बार झगड़ा करते हैं।यहाँ मोरवाल ने परंपरागत विचारो को छोडकर आधुनिक विचारधाराओ को स्पष्ट किया है । पहले ही घर में तिन लडकियों के होते हुए दुर्गा दादी के कहने पर चौथी लडकी को गोद लाना समाज में स्त्री की तरफ देखने में हो रहे परिवर्तन को स्वीकृत करना है ।

बेटे के जन्म पर कुआं पूजन की परंपरा होती है किंतु भगवती के घर पीहू को अनाथ आश्रम से लाने पर कुआं पूजन किया जाता है। लड़की होने पर भी उसके जन्मदिन पर घरवाले धूमधाम से कुआं पूजन करते हैं। इस प्रसंग में हमें परंपरागत विचारों से मुक्ति भगवती में दिखाई देती है। ठीक ही दोनों पोतिया गार्गी और सिया डॉक्टर और वकील बन जाती है। अपनी अपनी मर्जी से शादी करती है की बेटी बुलबुल की अपनी बिरादरी में शादी की जाती है उनके लोग उससे दहेज की अपेक्षा रखते हैं। उसके ससुराल वाले बहुत लालची होते हैं किंतु गार्गी और सीया उन्हें एक कानूनी नोटिस भेजते हैं जिससे वे सबक सिखाते हैं। उग्रसेन और दुर्गा के दोनो बेटे माता पिता को छोडकर चले जाते है। दोनों अकेलेपन को महसूस करते हैं।भगवती की पोती पीहू भी पढ़ाई हेतु ऑस्ट्रेलिया चली जाती है किंतु वह अपनी दादा–दादी के प्रति संवेदनशील होती है अपने दादा दादी की मृत्यु पर ऑस्ट्रेलिया से आ जाती है यहां दादा दादी के परिवर्तनशील विचारधारा को लेखक ने गर्व से महसूस किया है । पिहू अनाथालय को जिससे उसे माता पिता ने उसे गोद लिया था उस आश्रम को तीस लाख डोनेशन देती है। उसे डी बी फौडेशन अर्थात दशरथ और भगवती फाउंडेशन नाम देती है ।

निष्कर्ष :

समग्र रूप से इस उपन्यास के बारे में यह कहा जा सकता है कि उपन्यासकार इस बात का समर्थक है कि लड़कियाँ किसी भी तरह से लड़कों से कम नहीं हैं। अपितु संस्कारों में वे उनसे श्रेष्ठ ही हैं। पढ़ लिखकर वो भी अपना फर्ज निभा सकती हैं।आज की नारी चेतनशील है जिसे अच्छे–बुरे का ज्ञान है। इसलिये लड़के–लड़कियों के फरक को मिटाते हुए नई रोशनी समाज में क़ायम होनी चाहिए। हर एक व्यक्ति में योग्यता आत्मा का गुण है इसलिये जाति, धर्म, भाषा, रंग और लिंग के आधार पर सामाजिक भेदभाव समाप्त किये जाने चाहिए। भगवानदास मोरवाल ने 21 वी सदी में स्त्री विमर्श में हो रहे

बदलाव को 'शकुंतिका' उपन्यास के माध्यम से विविध उदाहरण देकर स्पष्ट किया है । पितृसत्ताक मानसिकता को लेकर आज भी समाज स्त्री को समानता का अधिकार नही देता। भगवानदास मोरवाल ने शकुंतिका में स्त्री जीवन से लेकर परिवर्तन वादी विचारों को हमारे सामने रखकर समाज में आ रहे बदलाव को बखूबी से चित्रित किया है।

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