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Household management practices and dietary diversity among the tribal communities in kerala

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ABSTRACT

The present study was conducted from 2020 to 2023 in the three tribal districts of Kerala. A sample of 500 respondents, including individuals from the Paniya, Irula, and Kanikkar tribes, was purposively selected from three districts: Wayanad, Palakkad, and Thiruvananthapuram. This study investigates the dietary diversity, living conditions, and food security status of tribal populations in Kerala, India. Through structured interviews and secondary data analysis, the study examines various aspects of food habits, household food availability, housing conditions, water scarcity issues, and livestock management among tribal communities. Results reveal disparities in housing infrastructure, access to safe drinking water, and availability of essential food items, highlighting the need for targeted interventions to improve food security and nutritional well-being among tribal populations in Kerala. The findings indicated that there is a substantial difference in the consumption of food items across various districts.

Keywords : Household management, Tribals in Kerala, Dietary diversity, Food availability

INTRODUCTION

Tribal communities in India are a diverse group, with significant differences in terms of language, culture, and traditions. They range from patriarchal to matriarchal, monogamous to polygamous, nature worshippers to polytheists, etc. The tribes in Kerala, represent only 5 percent of the total population Tribal communities in Kerala possess unique cultural traditions and practices that are often closely intertwined with their household management and food practices. These communities often face unique challenges related to food security, nutrition, and living conditions due to socio-economic marginalization and geographical isolation. Despite initiatives to improve living standards, disparities persist, impacting the health and well-being of tribal populations. Understanding the household management practices of tribal communities provides insights into their socio-economic conditions, livelihood strategies, and resource management techniques. Studying these practices helps in documenting and preserving indigenous knowledge systems, traditional agricultural techniques, culinary traditions, and rituals associated with food preparation and consumption. This study aims to explore the dietary diversities, household food availability, housing conditions, and water scarcity issues among tribal communities in three districts of Kerala:

Wayanad, Attapaddy, and Thiruvananthapuram. By employing a mixed-methods approach, including structured interviews and secondary data analysis, the study seeks to provide insights into the socio-economic determinants of food security and identify opportunities for intervention to enhance the nutritional status and well-being of tribal populations.

METHODOLOGY

The study involved 500 tribals from three districts in Kerala, encompassing both genders. Sampling included selecting 200 respondents each from the Paniya tribe in Wayanad (40 percent) and the Irula tribe in Attapaddy (40 percent), alongside 100 respondents of Kanikkars (20 percent) from Thiruvananthapuram district. This distribution aimed to ensure a diverse representation of tribal populations across various geographical areas within the state. The selection of locales (tribal hamlet areas) and respondents was done using purposive sampling, prioritizing voluntary participation to ensure cooperation during interviews and focus group discussions. A comprehensive interview schedule was crafted, incorporating questions on dietary practices, food habits, household food preparation, meal patterns, preferences, nutrition, cultural significances, cooking practices, and food practices during different physiological conditions and occasions. These inquiries aimed to provide a holistic understanding of the food-related behaviors and practices of the selected tribal communities. Data collection employed both primary and secondary sources. Primary data was gathered through interviews conducted directly with the respondents, allowing for an in-depth exploration of their dietary practices and food-related behaviors. This approach facilitated the collection of first-hand insights from the tribal communities, enabling a deeper understanding of their food habits and cultural practices. Secondary data, comprising existing literature, reports, and studies, complemented the primary data, providing additional context for analysis.

RESULTS AND DISCUSSION

Ensuring equitable and respectful treatment of participants is an ethical obligation for researchers. For research to be reliable, valid, and ethically conducted, the living conditions of research samples must be taken into account and documented. It enables scholars to responsibly contribute to the growth of knowledge, reach significant conclusions, and offer well-informed advice. The housing and living arrangements of the tribals selected for the study were analyzed in detail with the help of observations, informal discussions, participant interactions, and interviews. It includes data regarding ownership of the houses, housing conditions, cooking place, sources of cooking and drinking water, sanitary arrangements, electricity connection, and methods of garbage disposal.

Table No 1 : Housing and Living arrangements of the Respondents

Variables		Count (n=500)	Percentage (%)
Ownership of the house	Own	103	20.60
	Rent	2	0.40
	Govt provided	393	78.60
	Inherited	2	0.40
Housing condition	Thatched walls and thatched roof	16	3.20
	Mud walls and thatched roof	10	2.00
	Tiled House	63	12.60
	Concrete House	369	73.80
	Asbestos House	12	2.40
	Metal sheets	19	3.80
	Bamboo made	11	2.20
Cooking place	Outside house	273	54.60
	Inside house	222	44.40
	Others	5	1.00
Source of cooking*	Cooking source	488	98.19
	Fire chulha	150	30.18
Sources of drinking water	Own wells	34	6.80
	Public well	96	19.20
	Public tap/hand pump	217	43.40
	River/pond	135	27.00
	Bore well	2	0.40
	Tanker water	16	3.20
Scarcity of water	No	112	22.40
	Yes	388	77.60
Sanitary Arrangements	Own	382	76.40
	Community toilet	43	8.60
	Defecation in open field	75	15.00
Electricity connection to the household	Electrified	471	94.20
	Un-electrified	29	5.80
Garbage disposal	Available	490	98.00
	Not available	10	2.00
	Total	**500**	**100.00**

*Indicates Multiple Response

The data from the above table reveals distinct patterns in house-ownership among the respondents. Currently, 393 of the 500 families' occupants are in government-provided homes, demonstrating a significant reliance on public housing projects. Such houses have a kitchen, a bedroom, and a hallway. Outside the buildings, toilets were constructed. Some of the dwellings have been added to, including rooms and tiled floors. The majority of the government-built houses, however, still have unfinished construction some have even been abandoned for months. The majority of the houses just had their main doors, leaving the windows, floors, and kitchen slabs unfixed.

The government is expected to rebuild their deteriorating houses as part of the LIFE Mission housing initiative. Each chosen tribal family is expected to receive Rs 6 lakh, including Rs 3 lakh from the Tribal Development Department, to build a house. After demolishing their old houses, the construction work is turned over to a private contractor. A few houses have been finished. Those who have completed the construction works are also in a fix as they have undertaken the work after borrowing huge amounts from micro-lenders and moneylenders, expecting financial assistance from the government in a time-bound manner. Meanwhile, 20.60 percent of respondents own their houses outright. Only 0.40 percent of the population lives in inherited or rented housing.

The housing conditions of the respondents in the tribal areas across Thiruvananthapuram, Wayanad, and Attapaddy, reveal a diverse range of structures. The majority, 73.80 percent, reside in concrete houses. Notably, Attapaddy stands out with the highest proportion of concrete houses (80 percent), largely due to government initiatives in housing development. Wayanad follows closely with 79 percent and Thiruvananthapuram, on the other hand, has a significant portion (51 percent) of concrete houses but also a notable presence (17 percent) of alternative housing materials, metal sheet structures or low-quality plastic sheets raised on the foundation of their unfinished concrete houses and bamboo houses are more common, while tiled houses are predominant in Wayanad (14%), indicating regional variations in construction preferences and availability of resources.

The majority of the families stopped the construction work, anticipating the remaining funds from the government agencies. A smaller proportion, 3.20 percent, have thatched walls and roofs, while 2.40 percent live in asbestos houses. Additionally, 2.20 percent of the respondents dwell in bamboo-made structures, and 2 percent reside in houses with mud walls and thatched roofs.

A clear pattern in the respondents' cooking places was also studied. Surpassingly, 54.60 percent of the respondents choose outdoor cooking spaces. Further, 44.40 percent of households have an indoor kitchen. It is interesting to note that even among those who have an indoor kitchen, a notable proportion choose to construct an additional cooking area adjacent to their house. The mud and bamboo structure with a thatched roof with a door and no windows or holes, cow dung applied on the floors, and lacked even the most basic facilities, externally looks very small and compact but internally very spacious. Their mud kitchens are kept pristine. The traditional cooking medium “Chulah” is made of mud mixed with straw/dry grasses and stones. Out of 500 households, 488 use fire chulah (wood,

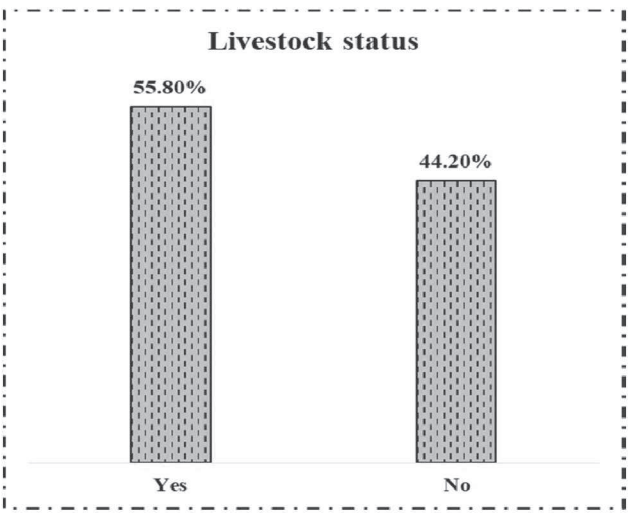
leaves, and straws) as a cooking medium, and 150 households have gas stove connections. All families opt for using chulah for cooking despite having a gas connection due to financial constraints.

The data also revealed that 77.60 percent of the respondents are experiencing extreme water scarcity, especially due to desertification-like situations in the regions of Attapaddy. Deficit rainfall during monsoon and massive deforestation had a significant impact on the availability of safe drinking water in these regions. The perennial rivers like Bhavani and Siruvani are their prime option. But ahead of the summer months, rivers have dried up. It has not only affected drinking water availability but also affected their cultivation and personal hygiene badly. Water is pumped directly without any effective treatment or purification, most of the households use untreated river water. Apart from that, the lack of proper water storage facilities in hamlets also makes the crisis severe.

Every tribal hamlet lacks proper pump facilities. Due to the lack of land availability digging wells is not possible for several families The Jalanidhi project of the government provides a drinking water supply to the hamlet but it is a big failure in the case of Attappady. A significant portion of households, 43.40 percent, rely on public tap/hand pumps as their primary source of drinking water. Further, 27 percent depend on rivers/ponds, while 19.20 percent utilize public wells. A smaller proportion, 6.80 percent, relies on their wells, and 3.20 percent depend on tanker water. Bore wells contribute the least.

Livestock and Poultry Management of the Respondents

Understanding the livestock possession of a household can be a useful tactic to increase and stabilize low-income families' incomes, diversify their sources of income, create job possibilities and manage hazards associated with drought. It will also help planners and policymakers to design livelihood opportunities based on that. An attempt was made to understand the status of livestock possession of the respondents which is depicted below.



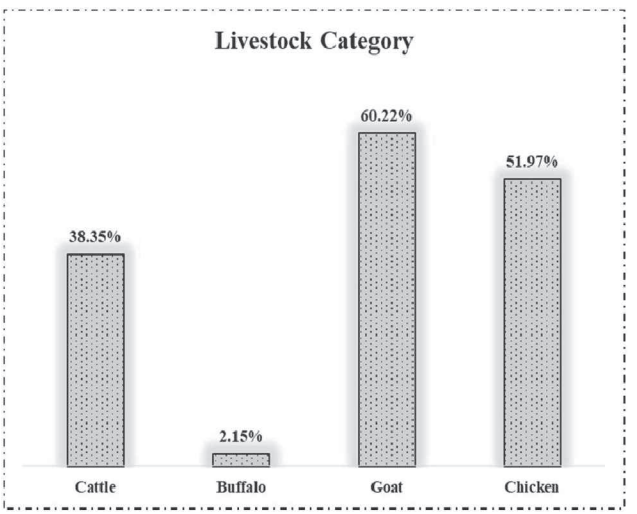


Fig. No. 1 & 2 : Livestock status and types among the respondents

The graphical illustration indicates that 55.80 percent of households own livestock. Among the respondents who own livestock, the distribution of livestock types is as follows: Goats/Sheep lead at 60.22 percent, followed by poultry rearing at 51.97 percent, Cattle at 38.35 percent, Buffalo at 2.15 percent, and Duck at 1.43 percent. A shift from agriculture to goat rearing is observed, due to its suitability for harsh environments and potential for high returns with low investment. Goats' ability to conceive and breed twice a year, often giving birth to twins, is a significant factor in households' income. However, despite being an income-generating activity, the potential for improving nutrition remains underutilized, as many tribal households rely on goat-rearing income. All households own goats, cattle, and poultry, but these animals' products are utilized for commercial rather than domestic purposes. So the diets were found to be grossly deficient in all nutrients. The challenge lies in addressing the severe shortage of fodder due to shrinking grazing lands.

Household Food Availability

The household food availability of the respondents was assessed and the details regarding the same are represented below.

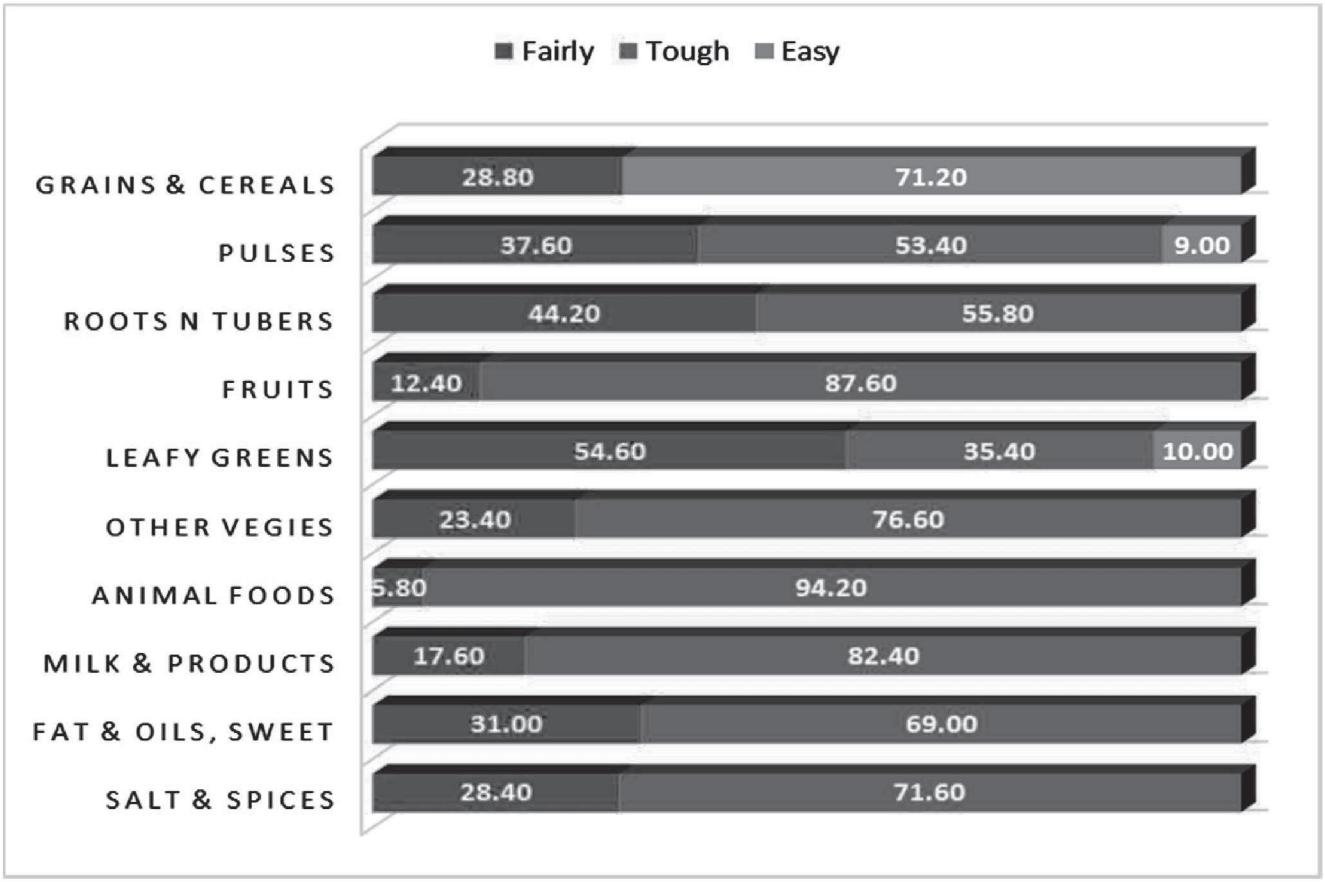


Fig No. 3 : Distribution of the respondents by level of household food availability

Based on the data provided, the distribution of household food availability varies across the districts. While comparing the tribal settlements from the three districts of Thiruvananthapuram, Attappady-Palakkad, and Wayanad certain trends emerged in terms of the non-availability of food items.

Regarding the consumption of fruits, Palakkad exhibited the highest non-availability of fruits (96.5 percent), followed by Wayanad (84.5 percent) and Thiruvananthapuram (76 percent). This suggests a significant lack of access to fruits, particularly in Attappady(Palakkad) and Wayanad. It also shows the scarcity of fruits grown in the forest regions. The price of the fruits in the market may also be one of the reasons for the non-incorporation of fruits in the daily diets of the tribals.

Regarding the consumption of animal-based foods, all three regions exhibited a significant scarcity, with both Attappady (Palakkad) and Wayanad at 93 percent, and Thiruvananthapuram slightly lower than that. Economic constraints compel individuals to restrict their intake of animal-derived food items.

In the case of consumption of Milk & its products, it was found that the Attapady region in Palakkad again demonstrated the highest non-availability (86.5 percent), followed by Wayanad (85.5 percent) and Thiruvananthapuram (68 percent). Attappady also reported the highest non-availability of other vegetables (70.5 percent), followed by Wayanad (82 percent) and Thiruvananthapuram (78 percent).

Overall, the Attapady region of Palakkad consistently shows higher percentages of tough accessibility across various food categories, indicating potential food security concerns in the district. Thiruvananthapuram and Wayanad, while facing challenges in specific items, generally have more balanced distribution patterns. This suggests a more pronounced food scarcity situation in the Attapady regions of Palakkad compared to the other two districts. The comparison highlights the need for targeted interventions to improve the availability of essential food items, especially fruits and animal food sources, in these regions to ensure better food security and nutrition for households.

Table No. 2 : Analysis of Variance (ANOVA) and Multivariate analysis of variance (MANOVA) for various food categories across three districts in Kerala

Food items	District			ANOVA		MANOVA	
	Thiruvananthapuram	Palakkad	Wayanad	F	Sig.	F	Sig.
Cereals and its products	29.71	29.71	29.71	0.000	1.000		
Pulses and legumes	28.44	22.33	8.16	168.674	0.000		
Roots and tubers	4.18	1.66	2.88	15.391	0.000		
Green leafy vegetables	14.14	11.80	15.83	5.201	0.006		

Other vegetables	14.40	12.06	14.40	2.104	0.123		
Fruits	0.95	0.68	0.79	1.877	0.154		
Fish	2.25	2.20	2.78	1.779	0.170		
Flesh meat	0.94	0.81	1.13	4.745	0.009		
Eggs	2.76	1.30	1.86	16.903	0.000		
Milk	5.57	3.14	3.88	3.576	0.029		
Curd	1.18	0.68	0.59	11.854	0.000		
Ghee	0.53	0.54	0.53	0.081	0.922	100.334	0.000*
Fats and oils	25.55	27.14	27.50	1.879	0.154		
Sugar	30	30	30	0.000	1.000		
Jaggery	0.88	1.02	0.59	1.371	0.255		
Honey	0.90	0.76	0.87	1.663	0.191		
Tea	28.81	29.11	1.26	2609.093	0.000		
Coffee	0.37	0.30	22.18	417.919	0.000		
Soft drinks and other energy drinks	0.21	1.11	0.23	49.048	0.000		
Fresh fruit juices	0.03	0.06	0.05	1.424	0.242		
Bakery food products	1.67	1.23	1.89	4.031	0.018		
Fast foods	1.06	1.02	1.16	0.768	0.465		

*Significant at 0.001 percent level

The table presents the results of the analysis of variance (ANOVA) and multivariate analysis of variance (MANOVA) for various food categories across three districts in Kerala. It also provides an insight into the variations in food consumption patterns among tribal communities across different districts of Kerala. Regarding the consumption of cereals and products, the ANOVA results indicate a significant difference ($F = 29.71$, $p < 0.001$) among the districts, suggesting variations in the consumption of cereals and their products. However, the MANOVA result is not significant ($F = 100.334$, $p = 1.000$), indicating that the overall consumption pattern across all food categories is not significantly different among the significant differences observed in the consumption of various food categories, including cereals, pulses, roots and tubers, green leafy vegetables, flesh meat, eggs, milk, curd, tea, coffee, soft drinks, bakery food products, and others. These differences may be attributed to socio-cultural preferences, dietary traditions, access to resources, and environmental factors prevalent in each district.

When the ANOVA significance level falls below 0.05, it indicates a substantial difference in the consumption of food items across various districts. Consumptions of Pulses and legumes, Roots and tubers, Eggs, Milk, and Curd exhibit a significant increase in Thiruvananthapuram. Consumptions of Tea and Soft drinks show a significant rise in Palakkad. Furthermore, the consumption of Green leafy vegetables,

Flesh meat, Coffee, Bakery food products, and Fast foods experiences a significant surge in Wayanad. The MANOVA results, with a significance level below 0.05, indicate that the consumption patterns of food items significantly differ among the sampled districts.

The significant findings underscore the importance of considering regional disparities and contextual factors in designing targeted interventions and policy measures to promote dietary diversity, food security, and nutritional well-being among tribal communities in Kerala. Furthermore, future research should explore the underlying determinants of food consumption patterns and their implications for health outcomes, livelihood sustainability, and community resilience among tribal populations in the region.

Factors determining Household Food Consumption

Household food consumption is influenced by an interplay of diverse factors. Paramount among these is family income, which dictates purchasing power. Food cost directly impacts choices, while individual preferences, likes, and dislikes shape dietary decisions. The availability of diverse food options, coupled with nutritional requirements, plays a pivotal role. Cultural and ethnic considerations contribute to food choices, as does the convenience of preparation and consumption. Together, these factors intricately shape the dynamic landscape of household food consumption.

Table No. 3 : Distribution of the respondents based on factors determining the household food consumption

Factors determining household food consumption	Districts						Total		Ranking
	Thiruvananthapuram		Palakkad		Wayanad				
	n	%	n	%	n	%	n	%	
Economy	100	100.00	199	99.50	198	99.00	497	99.40	2
Food cost	100	100.00	200	100.00	200	100.00	500	100.00	1
Likes/dislikes	9	9.00	36	18.00	18	9.00	63	12.60	4
Food availability	99	99.00	199	99.50	198	99.00	496	99.20	3
Food requirement	0	0.00	0	0.00	2	1.00	2	0.40	6
Ethnic concern	0	0.00	0	0.00	0	0.00	0	0.00	7
Convenience	5	5.00	19	9.50	34	17.00	58	11.60	5

Figures indicate Multiple responses

The table represents the distribution of factors influencing household food consumption across three districts: Thiruvananthapuram, Attappady (Palakkad), and Wayanad. In Thiruvananthapuram, family income and food cost are dominant factors, each constituting to 100 percent. Food availability follows closely at 99 percent. Food Likes/dislikes and convenience play minor roles at 9 and 5 percent, respectively.

In Attappady (Palakkad), food cost is again at 100 percent, with the economy closely following at 99.50 percent. Likes/dislikes and convenience contribute at 18 and 9.50 percent, respectively. In Wayanad district mirrors the pattern of the other districts, emphasizing family income and food costs. However, convenience is notably higher here, at 17 percent, while food requirement is at 1 percent, and ethnic concern is absent. Overall, the data underscores that food likes/dislikes, food requirements, ethnic concern, and convenience receive minimal attention across districts, with varying degrees of neglect, while economic factors and food cost in shaping household food consumption patterns.

Dietary Diversity of the Respondents

Studying the dietary practices of communities offers valuable insights into their culture, health, and sustainable living. Understanding what people eat, how they prepare their food, and the significance of certain dishes can shed light on historical traditions, economic factors, nutritional habits, and nutritional needs. By examining their diet, we can identify health risks, promote culturally appropriate interventions, and preserve indigenous knowledge.

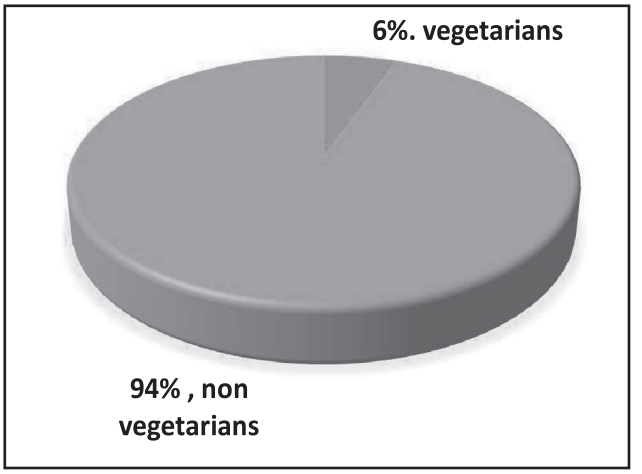


Fig No : 4 Dietary habits of the respondents

The above figure reveals a stark contrast in dietary preferences, with a vast majority, 94 percent, favoring a non-vegetarian diet compared to a mere 6 percent adhering to a vegetarian lifestyle. However, despite the prevalence of non-vegetarianism, the intake of animal-based foods such as fish and meat among respondents is remarkably low.

CONCLUSION

Tribal communities often experience food insecurity and nutritional deficiencies due to various factors such as limited access to resources, geographical isolation, and socio-economic marginalization. Studying their

food practices helps assess the availability, accessibility, and utilization of food resources within households. It also enables the identification of dietary patterns, nutritional gaps, and culturally appropriate interventions to address malnutrition and promote food security among tribal populations. Household management practices and food habits directly affect the health and well-being of tribal communities. Researchers can identify health risks, prevalent diseases, and hygiene-related issues by examining their dietary practices, cooking methods, and sanitation practices. This information is crucial for designing health promotion programs, sanitation initiatives, and interventions to improve overall health outcomes among tribal populations.

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Exploring the Relationship between Choice of Study Medium and Information Seeking Behaviour of Students

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ABSTRACT

The primary objective of this research was to evaluate the connection between the information-seeking behaviour (ISB) of students of post-graduation and the medium of their study. The sample size was 378 post-graduate students randomly taken through a lottery method from the selected faculties of Rajasthan University, Jaipur. Descriptive survey design was used for the research. Data collection was done by utilizing a self-structured questionnaire (online). The study's results, revealed that there is a significant effect of the medium of study on the way of information- searching by PG students. Specifically, the medium of study showed a significant association with library-related aspects, such as purposes of library use, library usage, consulted sources of information and time spent at library by students. However, the medium of study is not significantly influence the factors like the purpose of seeking information, purpose-wise internet usage, browsing of various e-resources, and the extent of use of internet.

The implications of these findings extend to research and practice in psychology, particularly in the domains of education, library science, cognitive development, performance appraisal etc.

Key words : Exploring Information, Internet Surfing, Library, University Students, Hindi & English Medium Students, Influence, Evaluation

INTRODUCTION

In contemporary society, information stands as a pivotal tool for individual development, indispensable for both personal and professional growth. The manner in which individuals employ various methods, approaches, and patterns to seek and utilize information is termed as information seeking behaviour (Manjunath and Babu, 2018). This behaviour reflects people's intention to bridge knowledge gaps (El Maamiry, 2016), influenced by a myriad of personal, emotional, educational, and social factors (Kumari & Sharma, 2014). Given the diverse information needs and seeking methods among different demographic groups, it becomes imperative to understand the specific requirements and behaviours of various users in order to efficiently provide them with tailored information.

The proliferation of information across different domains has ushered in an 'era of information' characterized by an unprecedented 'information explosion'. Consequently, there is a pressing need to

identify the underlying needs driving information seeking behaviors among diverse user groups. Such insights can equip educationists and policymakers with a comprehensive understanding of behavioral patterns, facilitating the timely dissemination of need-based information.

Moreover, information serves as a cornerstone for the academic and holistic development of students. Ismaila (2019) underscores its significance as a vital resource in this regard. Chayal and Vatta (2021) conducted a study on academic information needs of the PG students, study revealed that majority of students required information at very high extent related to curricular activities (70%), career counseling and employment (65%) followed by competitive examinations (57.75%) and research work (47.50%) etc.. However, students' information needs vary due to distinct attributes and requirements. Students adopt multiple approaches for searching, researching, selecting, and sharing information, often drawing from diverse sources including libraries (Khan and Khan, 2020).

The information-seeking behavior of students is influenced by a multitude of factors such as personality traits, educational background, field of study, academic environment, and the medium of instruction (Ahmed and Sheikh, 2019; Pinto *et al.*, 2014). For instance, studies by Ozowa and Aba (2017) and Desta et al., (2017) highlight the impact of course of study and personality traits on students' information-seeking behaviour. Recognizing students as pivotal stakeholders in educational institutions, it becomes paramount for universities to deliver pertinent information in a timely and accurate manner.

Research conducted by Chodha and Gupta (2017) at the National Institute of Technology in Jalandhar, India, underscores the importance of assessing students' information-seeking behaviour to enhance resource relevance and user satisfaction. Such endeavors aid in developing strategies and improving information services to meet students' needs effectively. By furnishing accurate and reliable information promptly, educational institutions contribute significantly to students' overall development while optimizing resource utilization.

Therefore, the present study endeavors to evaluate students' information-seeking behaviour, focusing particularly on the influence of the medium of instruction. Given the pivotal role of the medium of instruction in shaping academic experiences, this study is essential for refining information services and catering to the specific requirements of students.

MATERIALS AND METHODS

Present research was conducted at University of Rajasthan, Jaipur. This university is a largest and oldest state University of Rajasthan. Students enrolled in Post graduates' degree programmes of Humanities, Social Science and Law streams for the academic year 2019-2020 of Rajasthan University of Jaipur were the respondents of the study. Present study was completed in 2022. 464 students were selected from the selected faculties of the university by applying random sampling method. Sample size was calculated through Yamane's formula. Sampling was done in 3 stages i.e., selection of the university (1st stage), selection of the faculties (2nd stage) and selection of sample (3rdstage). At the first stage University of Rajasthan was selected purposively and faculties from this university were also selected through purposive sampling method. For the sample selection,

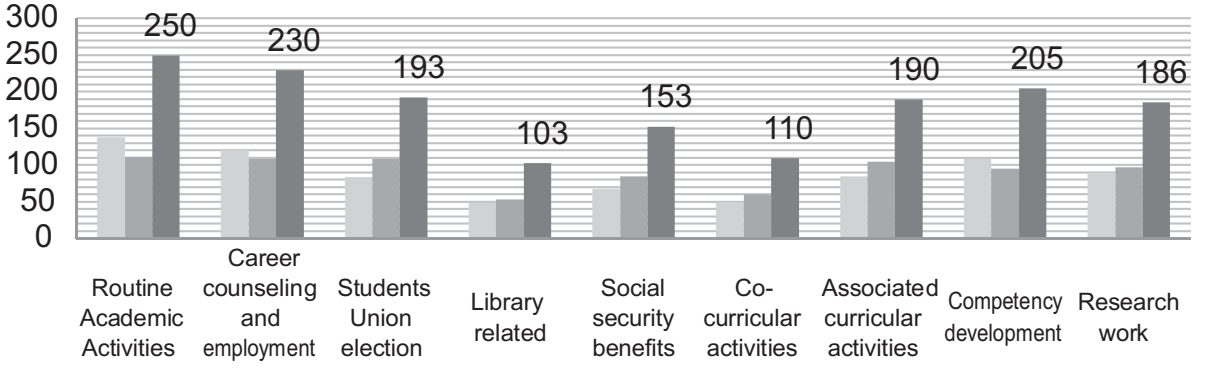
first of all, a list of admitted students for the session 2019-2020 was taken from all the selected faculties/departments of the university. Afterwards, two separate lists of boys and girls were prepared, and then the random sampling technique (lottery method) was applied to ensure an equal number of boys and girls in the selected sample. Self-developed tool (online questionnaire) was used for collection of the data. Reliability and Validity of the tool was also tested. Tool was given to 25 subject experts for content validation and to check the accuracy and language of questions. After validation of questionnaire, pre- testing of the tool was done with identified sample (not included in final sample) to check the understanding level of questions, formatting and clarity of language etc. This was also found reliable as the reliability coefficient for the structured questionnaire was 0.85. Tool was distributed via whatsapp and e-mails to 478 students. 378 questionnaires (completed) were returned, with an 81.46 per cent response rate. Descriptive survey research design was used for the study. Medium of the study was independent variable and Information seeking behaviour was dependent variable of the study. Hypothesis made in null form. Chi-square test was used to assess the association between information seeking behaviour and medium of the study.

RESULTS AND DISCUSSION

The association between independent variable (medium of study) and information seeking behavior (dependent variable) is presented below. Purpose of seeking information, information sources consulted, purpose of use of library, time spent in library, browsing of different e-resources (e-books, e-journals, digital library), and extent of usage of the internet were the major aspects of the information seeking behaviour of student.

Medium and Purpose-wise seeking information

Figure1 shows the association between medium and the purpose-wise seeking information by the respondents.



	Routine Academic Activities	Career counseling and employment	Students Union election	Library related	Social security benefits	Co-curricular activities	Associated curricular activities	Competency development	Research work
English	138	121	84	49	68	50	85	109	89
Hindi	112	109	109	54	85	60	105	96	97
Total	250	230	193	103	153	110	190	205	186

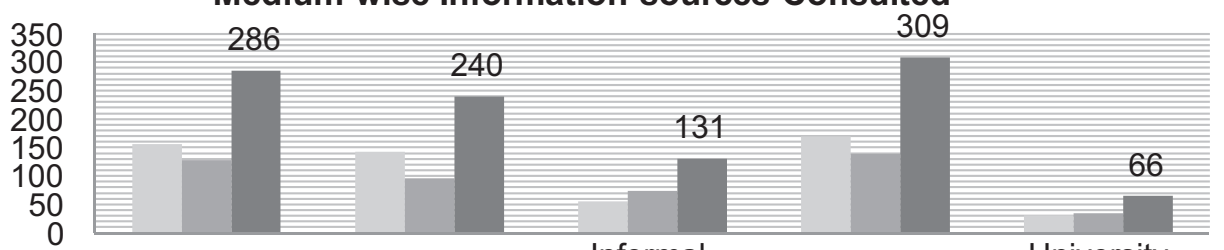
Fig.1 Medium and Purpose-wise seeking information

X^2 value=12.1746, df=8, P-Value -.143579. *Not* significant

According to Fig. 1, Students who were from English medium were more inclined to find information about their daily academic work (55.2%), job and career-related advice (52.6%), and competency potential (53.2%). The majority of those respondents desired information regarding student elections (56.5%), extracurricular activities (54.6%), social security benefits (55.7%) and library facilities (52.4%) were from Hindi medium. The Studies revealed that there was not any significant difference between the medium of instructions to the participants and the various objectives. It's a good test because the P value (0.143579) is greater than the 8 degrees of freedom significance level (0.10). Therefore, the null hypothesis must be accepted. It indicates a relationship between different comparisons. Therefore, we conclude that the medium of study did not influence participants' information-seeking goals.

Medium-wise information sources consulted

Figure 2 shows the comparison of medium and type of information sources consulted by the respondents.



	Library	Faculty	Informal sources	Internet	University administration
English	157	143	56	170	31
Hindi	129	97	75	139	35
Total	286	240	131	309	66

Fig.2 Medium-wise information sources consulted

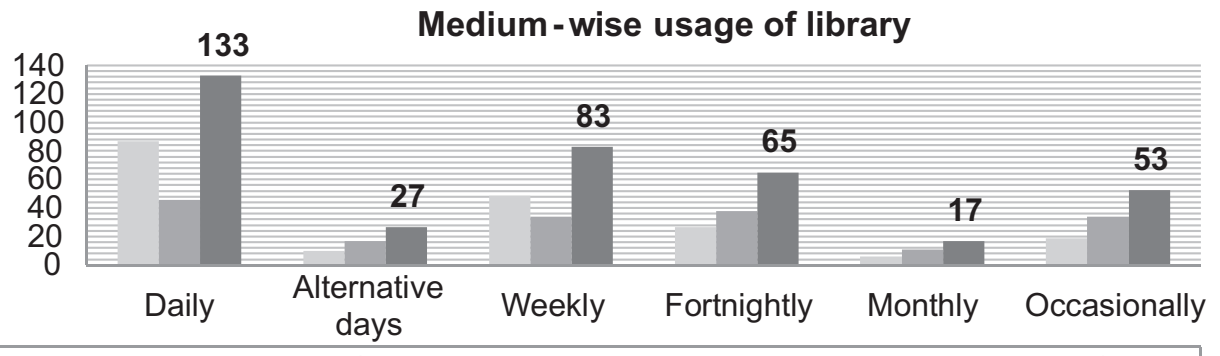
X^2 value= 11.2214 df=4, P Value - .024185. Significant (p < .05.)

Fig. 2, shows that out of 286 respondents who preferred academic libraries, 54.9 per cent were studying in English and 45.1 per cent were studying in Hindi. 240 respondents who consulted teachers 59.6 per cent were studying in English and 40.4 per cent were studying in Hindi. The percentage of Hindi and English medium respondents who consulted personal contacts such as family and friends were 57.2 per cent and 42.6 per cent respectively. 55 per cent of internet users were from English medium. In comparison, 53 per cent of college survey respondents were educated in Hindi. A further finding conclusively indicates that there is a significant difference between the medium of instructions and such data sources referred to. As the P value (.024185) is less than 4 degrees of freedom (0.05). Therefore, the null hypothesis must be rejected. Results showed that teachers (faculty) were more consulted by English-medium students and informal sources were more consulted by Hindi-medium students. This might be due to the different personality characteristics of the students. English-

medium students could be more confident or have less hesitation in communicating with faculty and other university administration sources than Hindi-medium students.

Medium-wise usage of library

Figure 3 explores the relation between medium of study among the responders and periodicity of libraries usage in a university environment.



	Daily	Alternative days	Weekly	Fortnightly	Monthly	Occasionally
English	87	10	49	27	6	19
Hindi	46	17	34	38	11	34
Total	133	27	83	65	17	53

Fig. 3 Medium-wise usage of library

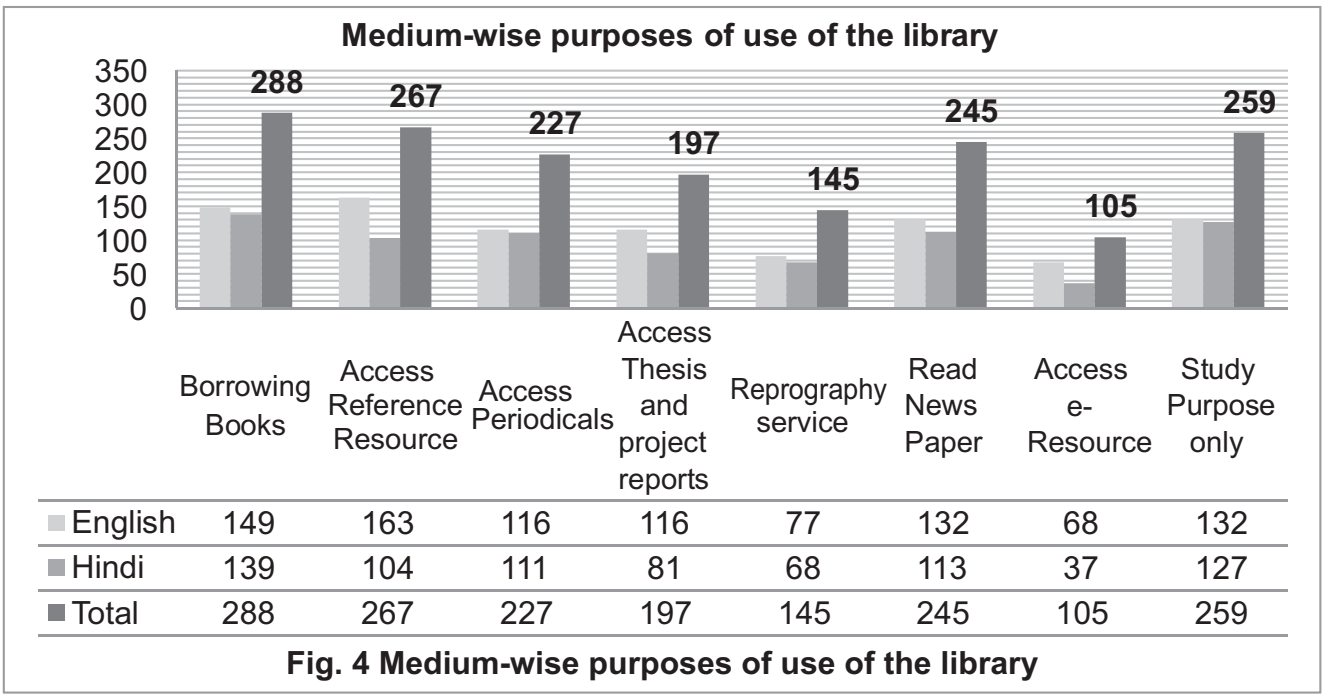
X^2 value= 23.9393 df=5, P-Value .00022. Significant (p < .01.)

Fig. 3 shows that out of 133 respondents, who use the library on a daily basis, 65.4 per cent were from English medium and 34.6 per cent were Hindi medium students. Of the 27 participants who use the library every day, 62.9 per cent were study in Hindi and 40.9 per cent were study in English. Further of the 83 participants, who use the library every week, 59 % were learning in English and 40.9 per cent are learning in Hindi. The proportion respondents intend to visit the library bi-weekly, monthly and occasionally is skewed towards Hindi-medium respondents. The information in Fig. 3 reveals clearly that English-medium student's visits the library more than Hindi medium students. This may be because more availability of English books than Hindi. In addition, the outcomes further indicated that there is a large difference between the average and frequency of library usage, because the P value (.00022) is less than 5 degrees of freedom (0.01). Therefore, the null hypothesis must be rejected. Results related to the frequency of library visits showed that daily and weekly visits were done by English-medium students more than Hindi-medium students. Therefore, the study area revealed that, as compared to Hindi-medium students, a large number of English-medium students visit the library more than Hindi-medium students. This might be due to the increased availability of books and other literature in English in the library.

Medium-wise purposes of use of the library

As can be seen from Fig-4, most of the 288 participants (51.7%) who borrowed books from the library were studying in English. Of the 227 respondents who used the library to access journals, 51.1 per cent were studying in English and 48.9 per cent were studying in Hindi. Of the participants who went to the library to assess reference materials, 61.1 he

per cent were studying in English and 38.9 per cent were studied in Hindi. Among the 197 respondents who visited the library for articles and publications, the percentage of those using English was higher on average (58.9%) than those using Hindi (41.1%). Identical effects were noted when reading newspapers and using online resources. In addition, the number of students in both environments is similar and they visit the library only to study.



	Borrowing Books	Access Reference Resource	Access Periodicals	Access Thesis and project reports	Reprography service	Read News Paper	Access e-Resource	Study Purpose only
English	149	163	116	116	77	132	68	132
Hindi	139	104	111	81	68	113	37	127
Total	288	267	227	197	145	245	105	259

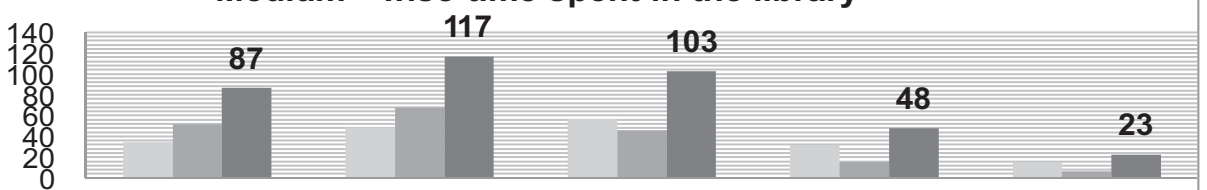
Fig. 4 Medium-wise purposes of use of the library

X^2 value=13.86, df=7, P-Value - .0537. Significant ($p < .10$.)

As can be seen from the above Fig.4, Additionally, there a marginally significant difference between the medium of instruction in terms of their different purposes of use of the libraries. The P-value (0.0537) is less than the significance level (0.10) at 7 degrees of freedom, hence it is statistically defensible. As a result, the null hypothesis must be rejected. It implies that there is a link between the variables being compared. As a result, study found that the medium is linked to the respondents' purposes for visiting libraries. Chayal et al. (2023) also stated that stream of the study influence the library use behaviour of the PG students..

Medium-wise time spent in the libraries

Fig.5 clearly shows that there was a considerable difference in the medium of instruction and the total number of hours spent in the library. These findings indicate that most of the respondents (59.7%) who made use of the library longer than an hour per day were studying in Hindi. Of the 117 respondents who spent one to two hours visiting the library every day, 58.1 per cent were Hindi students and 41.9 per cent were English medium students. Of the 103 participants who spent two to three hours in the library every day, 55.3 per cent studied in English and 44.7 per cent studied in Hindi. The total number of students of English is above the average (66.6%), in comparison, Hindi medium respondents (33.3%) spent three to four hours a day going to the library. A similar trend emerged for spending more than four hours a day at the library.



	0-1 hour	1-2 hours	2-3 hours	3-4 hours	>4
English	35	49	57	32	16
Hindi	52	68	46	16	7
Total	87	117	103	48	23

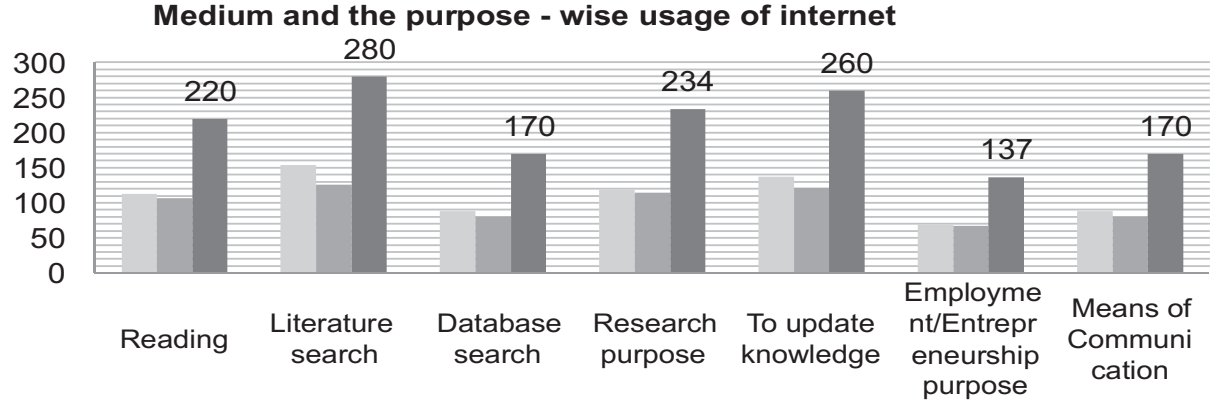
Fig. 5 Medium-wise time spent in the library

X^2 value=16.43, df=4, P-Value- .00248. Significant (p < .01.)

From the Fig. 5 based on the findings there was a considerable difference in the medium of instruction and the number of hours spent in the library. In comparison to Hindi medium respondents, English medium respondents spent more time in the library. The P-value (.00248) is less than the significance level (0.01) at 4 degrees of freedom; hence it can be statistically supported. According to result, the null hypothesis must be rejected. It implies that there is a relation between the variables being compared. From above, English medium students spent more time in the library, probably because the free website provides unlimited Internet access. Since the curriculum is predominantly English, access to electronic resources is also available. The study shows that English-medium students spend more time in the library, possibly because of the availability of free internet services that provide unlimited access to e-content and literature in English language more. The study here recommended that the library be equipped with need-based resources for Hindi-medium students too.

Medium and the purpose-wise usage of internet

Fig.6 shows the association between the respondents' medium and the purpose-wise access to the internet by the respondents.



	Reading	Literature search	Database search	Research purpose	To update knowledge	Employment/Entrepreneurship purpose	Means of Communication
English	113	154	89	119	138	69	89
Hindi	107	126	81	115	122	68	81
Total	220	280	170	234	260	137	170

Fig. 6 Medium and the purpose-wise access to the internet

X^2 value= 1.3531, df=6, P-Value -0.9686. Not significant

Fig. 6, revealed that 51.4 per cent of the 220 participants who received online education were reading in English and 48.6 per cent were from Hindi medium. Of the 280 students who access to the internet for literature search, 55 per cent were studying in English and 45 per cent in Hindi. 170 respondents who access to the internet to search for information, 52.4 per cent were studying in English and 52.4 per cent were studying in Hindi. majority of respondents access to the internet for research, study, communication and information updating is almost similar in both reports. From above, it is clear that there is non-significant difference between medium of study and the different factors for which users access the Internet. It is as true as real because the P value (0.9686) is above at 6 degrees of freedom (0.10). Therefore, the null hypothesis must be accepted. Thus, it can be stated that the medium is not associated with different purposes of using the internet by the respondents. Results focused that there is not much difference in the purpose of the use of the internet by the both Hindi and English medium students , as both mediums of students are in need of information from the internet for updating of knowledge, communication, literature search, research purposes, etc. Khatri, D. (2022), revealed that students like to access various websites such as Google, Wikipedia, etc. These sources were considered the first source for research work and course reading etc. Although there is not significance difference in the use of e-resources by the Hindi and English medium students but study also revealed that for literature search and to update knowledge English-medium students used the internet and e-resources (e-books, e-periodicals, and journals) higher than Hindi-medium students, it might be due to available literature on the internet is more in English with current updates as compared to Hindi. It could be due to not getting content, e-books, e-journals, or required information in Hindi on the internet.

Medium-wise browsing of E-resources by the respondents

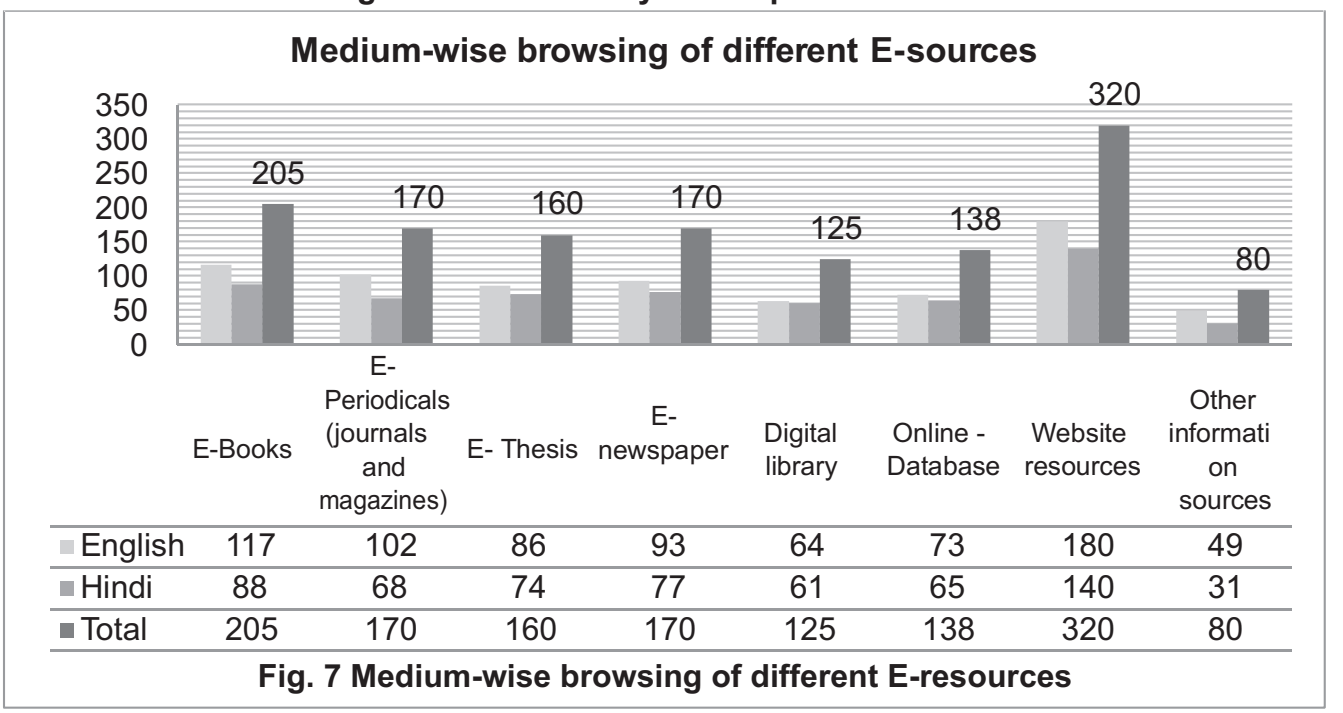
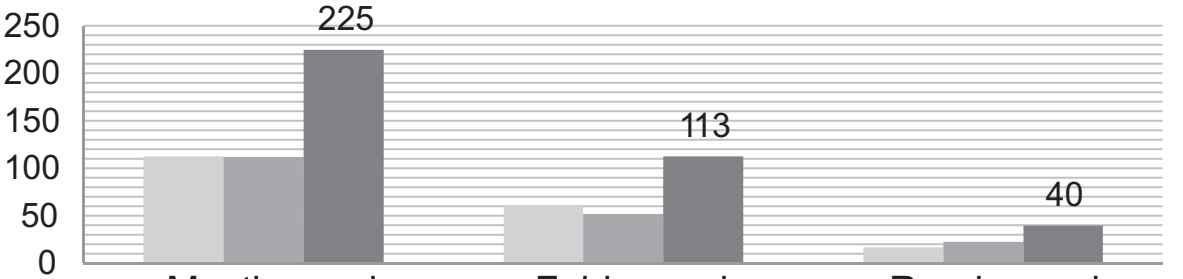


Fig. 7 Medium-wise browsing of different E-resources

X^2 value=4.23862, df=7, P-Value -0.75191. *Not* significant

Fig.7 indicates that of the 205 respondents who browsed e-books online, a larger number of English-medium respondents (57.1%) than Hindi-medium respondents (42.9%). English-speaking respondents accounted for 60% of all respondents utilizing E-periodicals, while Hindi-medium respondents made up 40%. Regarding the respondents' access to E-thesis (160), over half of them were English medium students (53.9% percent). The use of online databases, digital libraries, websites, and information resources was observed to follow a similar pattern. It can be as a result of the preponderance of English content on the internet. Therefore, the findings indicate that there is non-significant difference between the respondents' internet browsing habits and the study medium of the students . Given that the P-value (0.75191) is higher than the significance level (0.10) at seven degrees of freedom, it can be supported statistically. It therefore demands that the null hypothesis be accepted. It can be inferred that the medium is not associated with the various information resources browsed on the internet by the respondents.

Medium-wise extent of E-resources usage



	Mostly used	Fairly used	Rarely used
English	113	61	17
Hindi	112	52	23
Total	225	113	40

Fig. 8 Medium-wise extent of E-resources usage

X^2 value=1.5790, df=2, P-Value - .45404.Not significant

Fig. 8 clearly shows that a comparable proportion of students (50%) from both Hindi and English mediums mostly used e-resources to look up different types of information. Of the 113 respondents, 53.9 per cent studied in the English medium, and 46 per cent in Hindi. These respondents fairly employed e-resources to seek various types of information. Similarly, 57.5% of respondents who seldom used e-resources to find various kinds of information were registered in English-medium. The reason behind there isn't enough reading available online in Hindi for what one needs. The Chi-square test, it reveals that there was no discernible difference between the compared variables, used to evaluate the relationship between the respondents' internet usage extent and medium of study. As the value of P (0.45404) is greater than the significance

level (0.10) at two degrees of freedom, it can be supported statistically. Therefore the null hypothesis must be accepted.

CONCLUSION

From the study, it is concluded that personal attributes and characteristics of the students have significant association with the seeking behaviour of the students. Regarding of medium of the study is a major factor that influence the needs of the information and ISB of the students, as information sources consulted by students, purposes of use of the library, usage of the library and time spent in the library differ from Hindi medium to English medium students. Therefore it is concluded that with the help of these types of studies, information seeking behaviour and information needs of the students can be assessed and accordingly that need based information and information sources can be provide to students. Results can be helpful for the psychologists, educationists, policy makers, and for the University administration gain insight into the behaviour and psychology of postgraduate students to provide the strong and accessible information delivery system to the students.

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Changes in volatile metabolites in leaves of *Vigna unguiculata* L. Walp. in response to Groundnut Bud Necrosis Virus infection

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ABSTRACT

Vigna unguiculata L. Walp. (Cowpea) is an important legume crop grown in arid and semiarid regions of Rajasthan. During the field observations, the crop was found infested by bud necrosis like disease. This study focuses on the effects of the Groundnut Bud Necrosis Virus on volatile metabolite changes in *Vigna unguiculata.* Methanolic extracts of healthy and virus-infected leaves of cowpea were used to study changes in metabolites using Gas Chromatography - Mass Spectrometry. In both samples, a differential accumulation of metabolites was noticed. A total of 133 metabolites having 42 types of functional groups were identified. 9 metabolite groups were common in both the samples. Among common metabolites, enhanced accumulation of alkanes, diterpenes, halogenated hydrocarbons and organofluorine was noticed while fatty acids, fatty acid esters, triterpenes, alcohols and ketones were reduced significantly in virus-infected samples. This study shows significant alteration in the volatile metabolite profile of cowpea leaves due to GBNV infection which may further be helpful to understand the mechanism of disease development.

Key words : *Vigna unguiculata,* Groundnut Bud Necrosis Virus, Gas Chromatography - Mass Spectrometry, volatile metabolite.

INTRODUCTION

Vigna unguiculata L. Walp. (Cowpea) is an essential legume crop. According to the annual report (2021-22) Government of India Ministry of Agriculture & Farmers Welfare, cowpea is cultivated by 37 countries with 10 % contribution in total pulse production. Its versatility, nutritional value, and adaptability to diverse agroecological zones make it a valuable crop for both food and feed purposes. The crop is cultivated mainly in the arid and semi-arid belt of Rajasthan, Punjab, Haryana, Delhi, and western Uttar Pradesh. Cowpea production is adversely affected by a number of pathogens such as bacteria, viruses and fungi. It is susceptible to at least 33 legume viruses from 16 genera and 11 families (Chatzivassiliou 2021). Orthotospovirus is a genus of plant viruses. Members of this genus are major constraints for vegetable and pulse crops in India. Groundnut bud necrosis virus is one of the important viruses of this group. GBNV belonging to the genus *Orthotospovirus,* family, *Tospoviridae*, is indigenous to the Indian subcontinent and has a wide natural host range (Jones, 2020)

It has been detected in 30 different crops and weed species belonging to eight families and

is the most prevalent and destructive *Orthotospovirus* in India (Basavaraj *et al*., 2017). In cowpea, GBNV has been reported first time by Rao *et al*., 1987.

Metabolite changes in biological systems due to pathogen attacks open the doors to understand the host-parasite interaction at biochemical levels. At the onset of viral infection and the progress of disease, plethora of changes in different biochemical pathways can occur. After viral infections, plants show frequent changes in photosynthesis-related metabolites (Dardick, 2007), and viral infection-induced alterations in carbohydrate metabolism which can be observed as changes in the levels of sugars (Kogovšek *et al*., 2016). It is also reported that the change in the production of amino acids after viral infection ultimately leads to successful defense responses by the affected plant (Srivastava *et al*., 2012) and also the success of virus replication (Bazzini *et al*., 2011). Plants also produce secondary metabolites after pathogen attack (Lopez-Gresa *et al*., 2012; Hanssen *et al*., 2011) and pathogenesis-related proteins, both of which are involved in plant defenses.

Various analytical methods are available to decode these changes in metabolites in biological systems, GC-MS among others is considered one of the most efficient, reproducible and well-used analytical methods except having a single inherent limitation that it can be applicable only for the separation and identification of volatile compounds having low molecular weights (50-600 Da). This method is also useful in identifying an array of biochemicals due to the availability of well-versed metabolite databases in the form of libraries (Beale *et al*., 2018). Prior to GC-Ms analysis, appropriate extraction of metabolites from targeted tissue enhances the efficacy of analysis. Many solvents have been used in literature for sample preparation for extraction of metabolites. It was earlier reported that methanol as an extraction solvent has high extraction efficiency over other solvents (Swamy *et al*., 2015, 2017). This research work aims to investigate changes in the volatile phytochemical profile of cowpea leaf samples upon GBNV infection growing in field conditions. The role of some of the important phytochemicals has been discussed.

MATERIALS AND METHODS

Experimental site and collection of plant material

In an open agricultural field of Tehsil Laxmangarh, Sikar district, Rajasthan (27.51° N, 75.16° E), a 150 sq. m plot was chosen for sowing cowpea seeds of the variety Pusa komal which were purchased from seed certified centre situated in IARI, Pusa campus, New Delhi. Seeds were shown in the last week of July 2021. Due to the prevalence of bud necrosis disease in neighbouring cowpea growing fields; symptoms of bud necrosis disease started to appear in experimental crops in early growth stages (within 15 days of seed showing). The confirmation of the causal pathogen of bud necrosis disease was made as Groundnut bud necrosis virus by RT-PCR amplification of the full-length nucleocapsid protein gene of GBNV using Tospo IV-N-Forward **(**5'ATGTCTAACGTC/TAAGCAA/GCTC3') and Reverse primers (5' TTACAATTCCA GCGAAGGACC3')**.** After full vegetative

growth of the crop (45 days old plants), leaves from disease-symptomatic (GBNV infected) and asymptomatic (healthy) plants were picked separately for further analysis.

Preparation of plant material and methanolic extraction for metabolite profiling

Virus-symptomatic and asymptomatic leaves picked from the experimental field were carried to the laboratory, and properly washed with distilled water. Extra water was removed using blotting papers and then both leave materials were shade dried separately at room temperature. After complete drying, leaves were ground to fine powder using an electric blender and the powder was stored in airtight containers at room temperature until further use. 10gms of powder, each of symptomatic and asymptomatic leaves was extracted in HPTLC grade methanol as extraction solvent using Soxhlet apparatus for almost 3 cycles of 6 hours (until the solvent in Soxhlet siphon became colourless). After complete extraction, the methanolic extract was filtered with Whatman no. 1 filter paper**,** concentrated up to dry at room temperature then weighed for yield (%) determination using the following formula-

%Yield of extract= Weight of dried extract(g)/Initial weight of dried plant material(g) ×100

After this, extracts were stored at 4°C in a refrigerator for further analysis. Before GC-MS analysis, a known concentration of the extract was prepared by redissolving dried extract in fresh methanol and then filtered through a 0.2-micron syringe filter. This filtered extract was used for volatile metabolite profiling using GC- MS.

GC-MS analysis

Phytometabolites analysis using GC-MS was performed to separate bioactive components present in methanolic extracts of both, virus-infected and healthy cowpea leaves. This experiment was performed using Gas Chromatography-Mass Spectrometry (QP2020 of Shimadzu, Japan) instrument which was equipped with a capillary column of Rxi 5 Sil MS (1,4-bis (dimethylsiloxy) phenyl dimethyl polysiloxane) of dimensions 30m, 0.25mm and 0.25µm. Helium (99.99%) was used as the carrier gas with a column flow of 1.04 mL/minute, linear velocity 37.1 cm/sec, purge flow 5 mL/min. at 56.9 kPa pressure with split ratio :15.0. 1µl volume of each sample was injected in the split mode. An electronic ionization system with70eV ionisation potential was used for GC-MS detection. Both the injector and ion source temperature were kept at 250^{0}C. At the beginning of the program, the oven temperature was kept at 5^{0}C for 2 min which was increased by a constant rate of 8^{0}C/min up to 200^{0}C and after 200^{0}C, it was increased by 10^{0}C/min up to 280^{0}C for 5 min. The mass scan range used was 40-600m/z with a 2000 scan speed. The total time of GC program was 33.75 minutes with 0.30 second scan interval.

Identification and analysis of metabolites:

The metabolites present in both extracts were identified by using mass spectra and the National Institute of Standards and Technology (NIST) library. The mass spectra of these samples were matched with the spectra of the known compounds available in NIST17. The relative per cent amount of each metabolite was determined by comparing its average peak area with the total area. Other

parameters like structure, molecular formulae, and molecular weight of these metabolites were also confirmed with the help of NIST. Metabolite classes for different compounds detected were assigned according to information available on PubChem (https://pubchem.ncbi.nlm.nih.gov/).

RESULTS AND DISCUSSION

For this study confirmation of GBNV was made by RT-PCR (Fig. 1). Clear visible symptoms of this virus such as chlorotic rings, necrotic rings and patches, venal necrosis, apical bud necrosis etc. have been reported from the study site which was in accordance with GBNV caused symptoms reported by Mandal et al 2012. Healthy and GBNV-infected cowpea leaf samples were collected from the field, dried and processed for extraction of metabolites. After methanolic extraction, a higher extraction yield was obtained from healthy leaves samples (10.48%) as compared to virus-infected leaves (8.6%). Dried extracts from each of the samples were redissolved in fresh methanol for preparation of 100ppm solution and processed for metabolite profiling by GC–MS.

GCMS analysis of methanolic extracts of GBNV infected cowpea leaves showed changes in volatile metabolite profile with reference to healthy plants (Table 3).

In total 133 metabolites were identified in both healthy (69) and virus infected leaves samples (64) on the basis of retention time indices, molecular mass and molecular formulas (Table 1& 2). These metabolites were broadly categorized into 42 classes according to the presence of specific functional groups in their chemical structure. In virus-infected leaves 64 metabolites were identified. These metabolites were assigned 17 classes including alkane (35), halogenated hydrocarbon (5), esterified acid (5), cyclohexenes (4), alcohols (2) and ether (2). In addition to these metabolites, 11 other metabolites each in a single were alkene, benzene derivative, Citronellyl linoleate, diterpene, triterpenoid, triterpene, fatty acid, fatty alcohol, fatty acid ester, ketone and organofluorine. On the other hand, in healthy leaves extracts, a total of 69 metabolites were detected (Table 1). These metabolites were assigned 34 classes including ketone (9), alcohols (5), fatty acid (5), alkanes (4), Carboxylic acids (4), fatty acid ester (4), benzofurans (3), sugars (3), pyrrolidine derivative (3), aldehyde (2), amide (2), Phenol (2), halogenated hydrocarbon (2) , and 21 metabolites were present in singles including alkane derivative, aziridine, carbonic acid ester, cyclic lactone, diazine, dioxolane, diterpene, diterpene alcohol, diterpenoid, ester, furanone, glycerol, glycoside, keto acid, organofluorine, phytosterol, steroid, triterpene, vaccenoate, valero lactone derivative and vitamin. In these 42 classes of detected metabolites, 9 metabolite classes were common in both the samples and 33 were different (Table 3). Among the different 33 classes, 25 classes were exclusively present in healthy leaves and 8 were exclusively in virus-infected leaves. It was found that among the commonly expressing metabolite classes; alkanes, diterpenes, halogenated hydrocarbons and organofluorine were upregulated while; alcohols, fatty acids, fatty acid esters, ketones and triterpenes were downregulated (Table 3) in virus-infected leaves as compared to healthy leaves. In

terms of particular compounds, in both samples, most of the compounds were unique except seven compounds known as Dodecane-4,6-dimethyl, 3-Ethyl-2,6,10-trimethylundecane, Heptadecane-2-methyl, n-Hexadecanoic acid, Tetratetracontane, Hexadecanoic acid-2-hydroxy-1-(hydroxymethyl)ethyl ester and Squalene which were present in both the samples.

Level of squalene, a triterpene compound detected in good quantity in HL (3.49%) showed around 50-fold reduction in VIL (0.07%) and associated phytosterols especially of gamma sitosterol (5.26% in HL) were not even detected in VIL of cowpea plants. This reduction can be correlated with another study in which virus-induced gene silencing of squalene synthase (responsible for precursor formation for triterpenoid) resulted in reduced squalene synthesis which led in a significant reduction in phytosterol production. It is possible that for successful disease development, GBNV may silence some key genes that regulate metabolic homeostasis to maintain the integrity and healthy state of cells (Singh *et al*., 2015).

Phytol, an acyclic diterpenoid compound was detected in HL samples (2.82%) but was absent in VIL samples, which is in accordance with another study in which Tomato yellow leaf curl virus disease in tomato resulted in a significant reduction in phytol content (Soliman *et al*., 2018).

On the other hand, diterpene compounds increased by 1.03-fold in VIL as compared to HL. Antiviral activities of diterpene were reported by Ma *et al*., 2016. This suggests that infected plants try to induce defense by producing compounds of antiviral potential.

Alcohols were in higher amounts in healthy leaves (2.86%) as compared to diseased (1.27%), carboxylic acids were detected only in healthy samples (1.16%), while long-chain alkanes and their derivatives (halogenated hydrocarbons) showed a significant increase in VIL (32.91%) as compared to HL (1.5%). In a study, it was found that compounds present in plant surface waxes such as long-chain alkane, carboxylic acid, and alcohols have their role in insect feeding stimulations (Viladomat and Bastida, 2015). The role of wax in whitefly interaction to cotton plants and transmission of Cotton leaf curl virus transmission has been reported by Ali et al., 2021. The differential expression of these metabolites in both leaf samples of cowpea may also change the feeding choices of the vector (thrips) for successful transmission of GBNV. This is also supported by another study in which heneicosane, one of the alkanes also reported in this study in VIL, has semiochemical-like properties and is reported as a mosquito attractant (Majumder *et al*., 2020). Sugar content was detected in HL in a good amount (13.86%) while not detected in VIL. It was earlier reported that *Ageratum enation virus* infection significantly affects the plant's primary metabolism resulting in decreased glucose and sucrose content in *Amaranthus hypochondriacus* L (Srivastava *et al*., 2012). It is suggested that the overall decrease in sugar content in the present study may be due to reduced photosynthesis or additional consumption of these metabolites for defense-related activities.

Virus-infected leaves (0.9%) show around 20-fold decrease in total fatty acid component as compared to healthy leaves (18.21%). In plant cells, fatty acids and lipids are essential components as they provide structural integrity and fulfil the energy demand of cells for the smooth conduction of

various metabolic processes and as signal transduction mediators. A fatty acid, n-hexadecenoic acid (Palmitic acid) which is reported in the highest amount (6.91%) in healthy leaves, found highly suitable for the integrated control of phytopathogens (Liu et al., 2008), works as a central molecule in lipid biosynthesis, facilitates transport of lipids across membranes and also have important role in biotic and abiotic stress responses (Lim *et al*., 2017). It is suggested that a drastic decrease in important fatty acids after GBNV infection may alter structural integrity and overall failure of key metabolic processes resulting in disease development. Fatty acid esters (11.32%) and phenol (0.43%) were detected only in HL not in VIL. It is supported by another study in which fatty acid methyl esters and phenolic compounds were found downregulated in squash due to cucumber mosaic virus infection (Abdelkhalek *et al*., 2022). VIL samples showed a 47-fold decrease in ketones as compared to HL. Antiviral activity of some ketones reported elsewhere (Sinha *et al*., 2019). Possibly this reduction in important metabolites shows the failure of defense mechanism of plants and results in the success of virus spread. A trace amount of niacin (vitamin) was detected in HL samples while not detected in VIL. Niacin is known as a strong antioxidant and has an impact on enzymes related to cellular energy production (Berglund *et al*., 2017). Some other metabolites such as cyclohexane derivatives, and esterified acids were also detected in VIL and few others were reported in HL but the role of these metabolites in defense against viral pathogens has not been reported to the best of our knowledge.

Figure 1 : RT- PCR detection of Groundnut Bud Necrosis Virus by amplification of nucleocapsid protein gene of 831 base pair (Cowpea samples 1 to 6, M- 1KB marker along with negative and positive control)

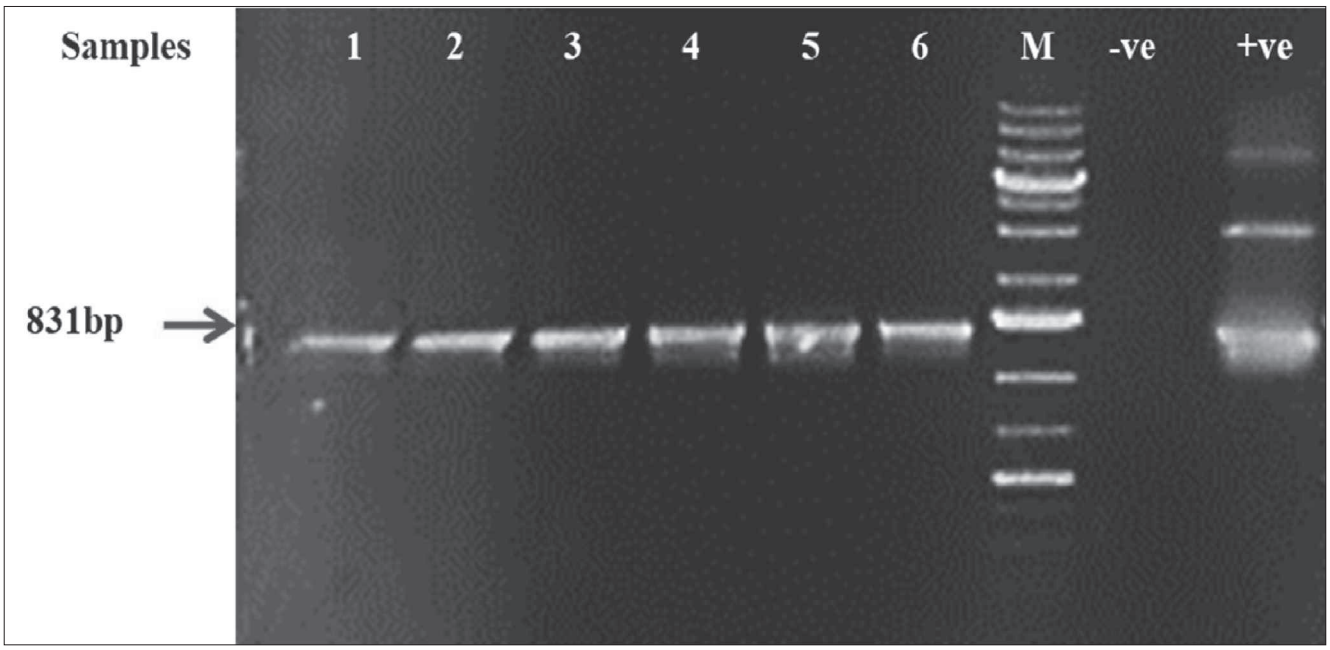


Figure 2 : GC-MS chromatograms of methanolic extract of (a) healthy (HL) and (b) Virus infected cowpea leaves (VIL)

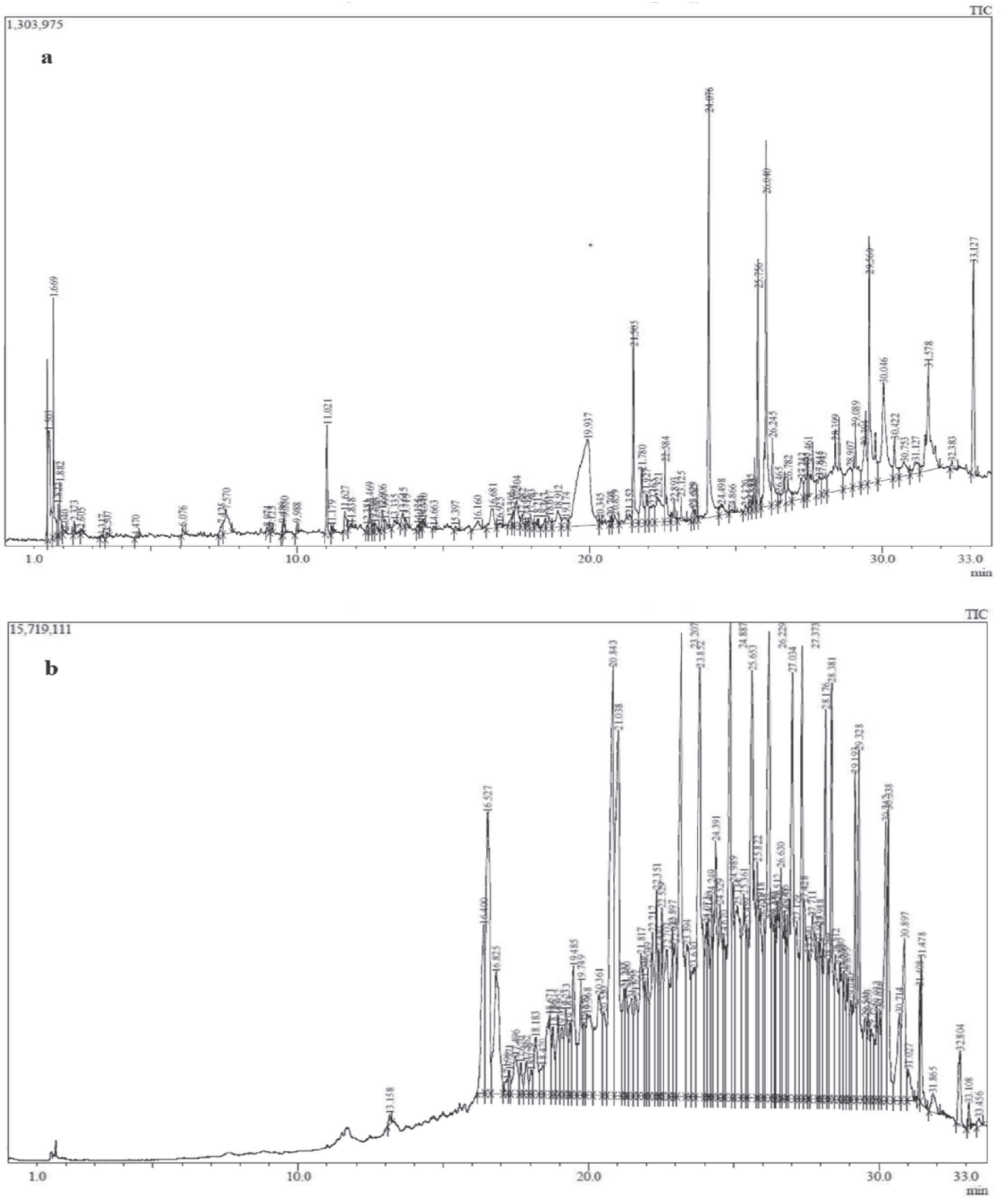


Table 1 : Metabolites detected in GC-MS analysis of healthy cowpea leaves (HL)

S. No.	Class of Metabolite	Name of Metabolite	Retention Time	Area %	Molecular Formula	Molecular Weight (g/mol)
1	Alcohol	Isopropyl Alcohol	1.669	1.99	C3H8O	60.1
		Tetrahydro-3-furanmethanol	13.715	0.2	C5H10O2	102.13
		3-Heptanol	14.125	0.09	C7H16O	116.2
		n-Tridecan-1-ol	20.345	0.11	C13H28O	200.36
		Z,E-3,13-Octadecadien-1-ol	22.893	0.47	C18H34O	266.5
2	Aldehyde	Propanal, 2-methyl-	1.822	0.4	C4H8O	72.11
		Butanal, 2-methyl-	2.373	0.11	C5H10O	86.13
3	Alkane	3-Ethyl-2,6,10-trimethylundecane	16.925	0.18	C15H32	212.41
		Dodecane-4,6-di methyl	17.395	0.27	C14H30	198
		Heptadecane, 8-methyl-	20.708	0.11	C18H38	254.5
		Tetratetracontane(Paraffin)	30.422	0.94	C44H90	619.2
4	Alkane derivative	1,2-Oxathiane, 6-dodecyl-, 2,2-dioxide	27.242	1.23	C16H32O3S	304.5
5	Diterpene	Neophytadiene	22.584	1.33	C20H38	278.5
6	Amide	Benzamide, 3-methyl-N-[2-(1-piperidyl)ethyl]	8.974	0.06	C15H22N2O	246.35
		Piracetam	14.224	0.09	C6H10N2O2	142.16
7	Aziridine (Heterocyclic compoud)	Aziridine, 1-ethenyl-	2.605	0.09	C4H7N	69.1
8	Benzofuran	Benzofuran, 2,3-dihydro-	12.469	0.43	C8H8O	120.15
		6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	21.352	0.57	C11H16O3	196.24
		2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4	18.212	0.19	C11H16O2	180.24
9	Carboxylic acid	Acetic acid	1.882	0.66	C2H4O2	60.05
		Butanedioic acid, monomethyl ester	9.988	0.13	C5H8O4	132.11
		Butanedioic acid, 2-hydroxy-2-methyl-, (S)-	14.663	0.06	C5H8O5	148.11
		L-Proline, 1-acetyl- (Imino acid)	17.983	0.31	C7H11NO3	157.17
10	Carbonic acid ester	Carbonic acid, octadecyl prop-1-en-2-yl ester	26.465	0.25	C22H42O3	354.6
11	Cyclic lactone (Furan)	Butyrolactone	6.076	0.14	C4H6O2	86.09
12	Diazines	Pyrazine, methyl-	4.47	0.06	C5H6N2	94.11
13	Dioxolane (organic heterocyclic compound)	1,3-Dioxolane, 2,4,5-trimethyl-	16.16	0.39	C6H12O2	116.16
14	Diterpene alcohol	Phytol	23.125	2.82	C20H40O	296.5
15	Ester (Palmitic ester derivative)	Dimethylaminoethyl palmitate	27.461	0.62	C20H41NO2	327.5
16	Fatty acid	Dodecanoic acid	18.463	0.2	C12H24O2	200.32
		Tetradecanoic acid	21.505	2.91	C14H28O2	228.37
		n-Hexadecanoic acid	24.076	6.91	C16H32O2	256.42
		9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (FA Omega3)	26.04	6.73	C18H30O2	278.4
		Octadecanoic acid	26.245	1.46	C18H36O2	284.5
17	Fatty acid ester	Heptanoic acid, 3-methylbutyl ester	20.859	0.32	C12H24O2	200.32
		7-Hexadecenoic acid, methyl ester, (Z)-	23.587	0.44	C17H32O2	268.4
		Methyl (Z)-5,11,14,17-eicosatetraenoate	31.578	4.73	C21H34O2	318.5
		Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	29.56	5.83	C19H38O4	330.5
18	Furanone	4-Dimethylamino-2(5H)-furanone	18.617	0.38	C6H9NO2	127.14
19	Glycerol (monoacyl glycerol)	1,2,3-Propanetriol, 1-acetate	12.906	0.41	C5H10O4	134.13
20	Glycoside	beta.-D-Glucopyranoside, methyl	18.912	0.87	C7H14O6	194.18
21	Halogenated hydrocarbons	Ethane, 1-chloro-1-fluoro-	1.503	3.21	C2H4ClF	82.5
		Trichloromethane	2.04	0.09	CHCl3	119.37
22	Ketoacid	2-Keto-3-methylvaleric acid tbdms	13.335	0.11	C12H24O3Si	244.40
23	Ketone	2-Propanone, 1-hydroxy-	3.507	0.1	C3H6O2	74.08
		2-Propanone, 1,1-diethoxy-	7.57	1.21	C7H14O3	146.18
		Ethanone, 1-(1,2,2,3-tetramethylcyclopentyl)	9.125	0.08	C11H20O	168.28
		2-Hexanone, 3-methyl-4-methylene-	9.55	0.24	C8H14O	126.2
		4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6	11.021	1.28	C6H8O4	144.12
		2(3H)-Furanone, dihydro-4-hydroxy-	11.179	0.14	C4H6O3	102.09
		4-Hexen-3-one, 4,5-dimethyl-	12.591	0.15	C8H14O	126.2
		2-Hydroxy-1-(1'-pyrrolidiyl)-1-buten-3-one	17.835	0.1	C8H13NO2	155.19
		4,4,8-Trimethyl-non-7-en-2-one	21.927	0.96	C12H22O	182.3
24	Organofluorine	Heptadecyl heptafluorobutyrate	25.485	0.21	C21H35F7O2	452.5

S. No.	Class of Metabolite	Name of Metabolite	Retention Time	Area %	Molecular Formula	Molecular Weight (g/mol)
25	Phenol	2-Methoxy-4-vinylphenol	14.31	0.16	C9H10O2	150.17
		2,4-Di-tert-butylphenol	17.682	0.27	C14H22O	206.32
26	Pyrrolidine derivative	2-Pyrrolidinone	9.486	0.14	C4H7NO	85.1
		(2-Oxo-1-pyrrolidinyl)acetic acid	24.866	0.09	C6H9NO3	143.14
		L-Proline, 5-oxo-, methyl ester	15.397	0.1	C6H9NO3	143.14
27	Phytosterols	gamma.-Sitosterol	30.046	5.26	C29H50O	414.7
28	Diterpinoid	3,7,11,15-Tetramethylhexadec-2-en-1-yl acetate	28.399	4.24	C22H42O2	338.6
29	Sugar	Sucrose	16.681	0.98	C12H22O11	342.3
		beta.-D-Glucopyranose, 1,6-anhydro-	17.504	0.92	C6H12O6	180.16
		4-O-Methylmannose	19.937	11.96	C7H14O6	194.18
30	Steroid	beta.-Sitosterol acetate	30.753	1.03	C31H52O2	456.7
31	Triterpenes	Squalene	33.127	3.49	C30H50	410.7
32	Vaccenoate	2-(Dimethylamino)ethyl vaccenoate	29.089	1.9	C22H43NO2	353.58
33	Valerolactone derivative	2H-Pyran, 2-(4-chlorobutoxy)tetrahydro-	11.838	0.1	C9H17ClO2	192.68
34	Vitamin (Group B complex)	Niacin	12.686	0.21	C6H5NO2	123.11

Table 2: Metabolites detected in GC-MS analysis of virus infected cowpea leaves (VIL)

S. No.	Class of Metabolite	Name of Metabolite	Retention Time	Area%	Molecular Formula	Molecular Weight
1	Alcohol	1-Hexacosanol	30.897	1.11	C26H54O	382.7
		dl-Chimyl alcohol	31.865	0.16	C19H40O3	316.5
2	Alkane	Heneicosane	16.4	1.08	C21H44	296.6
		Octadecane	16.527	2.13	C18H38	254.5
		Dodecane-4,6-di methyl	17.19	0.09	C14H30	198
		2-Methylhexacosane	17.862	0.2	C27H56	380.7
		Pentadecane, 8-hexyl-	18.183	0.42	C21H44	296.6
		3-Ethyl-2,6,10-trimethylundecane	18.671	0.78	C16H34	226.44
		Pentadecane, 6-methyl-	18.786	0.31	C16H34	226.44
		Decane, 3,3,6-trimethyl-	19.233	0.54	C13H28	184.36
		Heptadecane, 2-methyl-	19.485	0.83	C18H38	254.5
		Octadecane, 3-methyl-	19.749	0.77	C19H40	268.5
		Eicosane	21.038	2.97	C20H42	282.5
		Pentacosane	21.49	0.95	C25H52	352.7
		2-Methylhexacosane	21.627	0.73	C27H56	380.7
		Pentadecane, 6-methyl-	21.948	0.48	C16H34	226.44
		Heneicosane, 5-methyl-	22.049	0.59	C22H46	310.6
		Docosane	22.212	1.12	C22H46	310.6
		Octacosane	22.351	0.81	C28H58	394.8
		Eicosane, 2-methyl-	22.897	1.07	C21H44	296.6
		3,3-Dimethylnonadecane	24.14	0.71	C21H44	296.6
		Hexatriacontane	24.391	1.78	C36H74	507
		Tetratetracontane	24.989	1.04	C44H90	619.2
		Pentadecane, 6-methyl-	25.459	0.67	C16H34	226.44
		Octacosane, 2-methyl-	25.822	0.83	C29H60	408.8
		Pentadecane, 2,6,10,14-tetramethyl-	26.048	0.77	C19H40	268.5
		5-Methylnonacosane	26.33	0.97	C30H62	422.8
		2-Methyltetracosane	26.766	1.01	C25H52	352.7
		Tetracosane	27.034	2.71	C24H50	338.7
		Dotriacontane	27.428	1.31	C32H66	450.9
		Docosane, 2,4-dimethyl-	27.59	0.43	C24H50	338.7
		2-Methylpentacosane	28.895	0.7	C26H54	366.7
		3-Methyltetracosane	29.04	0.37	C25H52	352.7

S. No.	Class of Metabolite	Name of Metabolite	Retention Time	Area%	Molecular Formula	Molecular Weight
		Tetratriacontane	29.193	1.38	C34H70	478.9
		Triacontane	29.328	1.45	C30H62	422.8
		Tetracontane	29.633	0.48	C40H82	563.1
		2-Methylhexacosane	30.036	0.43	C27H56	380.7
3	Alkene	Nonacos-1-ene	18.42	0.22	C29H58	406.8
4	Benzene derivative	Benzene, (2,3-dimethyldecyl)-	19.968	0.96	C18H38	246.4
5	Citronellyl linoleate	(9Z,12Z)-3,7-Dimethyloct-6-en-1-yl octadeca-	18.029	0.13	C28H50O2	418.7
6	Cyclohexane derivative	1,1'-Bicyclohexyl	19.363	0.3	C12H22	166.3
		Heneicosylcyclohexane	22.43	0.53	C27H54	378.7
		Cyclohexane, nonadecyl-	22.982	0.78	C25H50	350.7
		n-Heptadecylcyclohexane	27.179	1.14	C23H46	322.6
7	Diterpene	Hexadecane, 2,6,10,14-tetramethyl- (Phytane)	16.825	1.37	C20H42	282.5
8	Esterified acid	Sulfurous acid, hexyl pentadecyl ester	18.952	0.43	C21H44O3S	376.6
		Oxalic acid, cyclohexylmethyl tridecyl ester	21.237	0.63	C22H40O4	368.5
		Sulfurous acid, cyclohexylmethyl hexadecyl ester	21.3	0.4	C23H46O3S	402.7
		Sulfurous acid, di(cyclohexylmethyl) ester	23.63	1.08	C14H26O3S	274.4
		Sulfurous acid, butyl heptadecyl ester	24.67	0.55	C21H44O3S	376.6
9	Ether	Hexadecyl nonyl ether	17.496	0.37	C25H52O	368.7
		Nonyl tetracosyl ether	27.924	0.51	C33H68O	480.9
10	Fatty acid	n-Hexadecanoic acid	24.073	0.9	C16H32O2	256.42
11	Fatty alcohol	1-Heptacosanol	20.53	0.62	C27H56O	396.7
12	Fatty acid (glyceride)	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	29.541	0.51	C19H38O4	330.5
13	Halogenated hydrocarbon	Hexadecane, 1-iodo-	13.158	0.04	C16H33I	352.34
		Tetrapentacontane, 1,54-dibromo-	17.67	0.14	C54H108Br2	917.2
		Hexacosane, 1-iodo-	24.249	0.82	C26H53I	492.6
		Triacontane, 1-iodo-	25.134	2.09	C30H61I	548.7
		Eicosane, 1-iodo-	26.851	0.67	C20H41I	408.4
14	Ketone	3-Dodecylcyclohexanone	17.271	0.09	C18H34O	266.5
15	Organofluorine	Triacontyl heptafluorobutyrate	30.714	0.7	C34H61F7O2	634.8
16	Triterpene	Squalene	33.108	0.07	C30H50	410.7
17	Triterpinoid	9,19-Cyclolanost-24-en-3-ol, (3.beta.)-	33.456	0.05	C30H50O	426.7

Table 3 : Comparative list of area percent of different metabolite classes with fold change in area percent of common metabolites in HL and VIL samples

S.No.	Class of Metabolite	Peak area percent		Fold change area percent
		HL	VIL	
1	**Alcohol**	**2.86**	**1.27**	**↓ 2.25**
2	Aldehyde	0.51	_	
3	**Alkane**	**1.5**	**32.91**	**↑ 21.94**
4	Alkane derivative	1.23	_	
5	Alkene	_	0.22	
6	Amide	0.15	_	
7	Aziridine	0.09	_	
8	Benzene derivative	_	0.96	
9	Benzofuran	1.19	_	
10	Carboxylic acid	1.16	_	

S.No.	Class of Metabolite	Peak area percent		Fold change area percent
		HL	VIL	
11	Carbonic acid ester	0.25	_	
12	Citronellyl linoleate	_	0.13	
13	Cyclic lactone	0.14	_	
14	Cyclohexane derivative	_	2.75	
15	Diazines	0.06	_	
16	Dioxolane	0.39	_	
17	**Diterpenes**	**1.33**	**1.37**	**↑ 1.03**
18	Diterpene alcohol	2.82	–	
19	Diterpenoid	4.24	–	
20	Ester	0.62	–	
21	Esterified acid	–	3.09	
22	Ether	–	0.88	
23	**Fatty acid**	**18.21**	**0.9**	**↓ 20.23**
24	**Fatty acid ester**	**11.32**	**0.51**	**↓ 22.19**
25	Fatty alcohol	–	0.62	
26	Furanone	0.38	–	
27	Glycerol	0.41	–	
28	Glycoside	0.87	–	
29	**Halogenated hydrocarbon**	**3.3**	**3.76**	**↑ 1.14**
30	Ketoacid	0.11	_	
31	**Ketone**	**4.26**	**0.09**	**↓ 47.33**
32	**Organofluorine**	**0.21**	**0.7**	**↑ 3.33**
33	Phenol	0.43	–	
34	Pyrrolidine derivative	0.33	–	
35	Phytosterols	5.26	–	
36	Sugar	13.86	–	
37	Steroid	1.03	–	
38	**Triterpenes**	**3.49**	**0.07**	**↓ 49.86**
39	Triterpinoid	_	0.05	
40	Vaccenoate	1.9	–	
41	Valerolactone derivative	0.1	–	
42	Vitamin (Niacin)	0.21	–	

CONCLUSION

Our results showed that the volatile metabolites profile of GBNV infected cowpea leaves was mostly different from healthy leaves. Some important metabolite classes such as sugars, fatty acids, fatty acid esters, triterpenes, triterpenoids, ketones, alcohols and phytosterols were reduced significantly or even not detected in leaves of cowpea after GBNV infection while some other metabolite classes such as alkanes, halogenated hydrocarbons, cyclohexane derivatives, esterified acids and diterpenes

were overexpressed after GBNV infection. Overall, this analysis suggests that GBNV causes a profound effect on host volatile phytochemical composition and alters it significantly for successful disease development.

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Optimum size and shape of plots for field experiments on sesame (sesamum indicum)

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ABSTRACT

A uniformity trial on sesame crop was carried out at Seed Technology, S. D. Agricultural University, Sardarkrushinagar during *kharif* season in the year 2023 to find out optimum plot size, plot shape and number of replications. The popular variety GT 3 was grown for this purpose and yield data were collected from 600 basic units. The dimension of one basic unit was 1 m x 1.20 m constituting 2 rows each of 1 meter length. The Maximum curvature method and Fair field Smith's variance law were used for the same. The variability as judged by coefficient of variation per unit area decreased from 24.24 to 1.94 per cent with increase in plot size from 1 basic unit to 300 basic units. Based on experimental research, it was concluded that, a plot of 18 m^2 size having shape of 6 rows each of 5 m length (5 m × 3.6 m) found optimum (net plot) with minimum 3 replications at 5 per cent standard error is recommended for filed experiments on sesame crop.

Key words : Optimum plot size, coefficient of variation, sesame

INTODUCTION

In agricultural experimentation, the use of adequate plot size and shape is an important experimental technique to increase the precision of the experiment. Thus, the objective of this study was to determine the appropriate size and shape of the plot for field experimentation with sesame. Agricultural research workers are mainly concerned with field experimentation, the use of adequate plot size and shape is an important experimental technique to increase the precision of the experiment. Efficient planning of precise field experiments largely depends on adoption of principles of experimental design (Replication, Randomization and Local control), choice of experimental design and suitable size and shape of plot. Each experimental site has an inherent variation *i.e.* soil variability which could be accessed through uniformity trial. The studies of such variation help in proper choice in size and shape of plots and blocks, experimental design and number of replications etc. Thus, the objective of this study was to determine the appropriate size and shape of the plot for field experimentation with sesame. Such studies based on uniformity trials in Indian conditions

were undertaken on various annual crops like Bajra (Patel and Patel, 1967); Paddy (Agrawal and Deshpande, 1967), Tobacco (Prajapati and Patel, 1984) and Mungbean (Singh and Singh, 1989). Information on optimum size and shape of plot on various cereals, oilseeds and leguminous crops are available in India and abroad in plenty, but such an information however, is very scanty with respect to sesame crop which is an important commercial crop for farming community of North Gujarat. Moreover, such information will be useful to scientific community working with sesame crop for planning their research experiments to bring out precise estimate.

MATERIAL AND METHODS

An uniformity trial was planned on sesame crop with variety GT 3 at at the Seed Technology, Sardarkrushinagar Dantiwada Agricultural University, Sradarkrushinagar during the *kharif* season in the year 2022-23



Fig. 1: Geographical map of study area

to collect required information (Fig. 1). The crop was sown at a spacing of 60 cm between rows. A plot of 1 m x 1.20 m = 1.20 m^2 constituting 2 rows each of 1 m length was taken as one basic unit. A block of 20 m x 36 m was harvested and seed yield data were recorded separately for 600 basic units (Fig. 2). The other crop cultivation practices in vogue were followed.

Border areas were excluded from each side of experimental plot. The different sized plots of size 1, 2, 3, 4, 5, 6, 8, 10, 12, 15, 20, 24, 25, 30, 40, 50, 60, 75, 100, 120, 150, 200 and 300 were formed by combining neighboring units both length-wise as well as width-wise. The coefficient of variation for different combinations of above sized plots was calculated separately for seed yield data using the following formula.

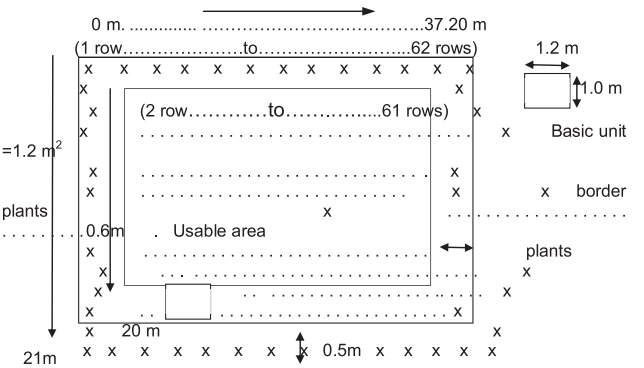


Fig. 2: Detail of the experimental plot, usable area and basic unit

variation for different combinations of above sized plots was calculated separately for seed yield data using the following formula.

$$C.V.\% = \frac{\text{Standard deviation}}{\text{General Mean}} \times 100$$

The optimum plot size was determined using maximum curvature method (Federer; 1955) and Fair-field Smith's variance law (Smith, 1938). The minimum number of replications (r) and area required for 5 % Standard error of mean (SEm.) for different plot size were calculated using following the formula (Nigam and Gupta, 1979).

$$r = \frac{(C.V.\%)^2}{(5\%SEm)^2}$$

The relative land use efficiency was also calculated for different plot sizes by dividing the amount of land used by the basic unit to the amount of land used by other plot sizes.

RESULTS AND DISCUSSION

The comparisons were made among different sizes and shapes of plots using C.V.% obtained for seed yield of sesame. The results are given in Table 1 : Per unit decrease in CV(%) for seed yield of sesame

No. of units	Combinations (basic unit)	CV(%)	Average CV(%) Per unit
1	1 × 1	24.24	24.24
2	2 × 1	18.32	
	1 × 2	15.83	17.08
3	1 × 3	14.07	14.07
4	4 × 1	14.5	
	2 × 2	11.83	13.17
5	1 × 5	12.72	
	5 × 1	11.73	12.23
6	2 × 3	10.98	
	1 × 6	10.45	10.72
8	4 × 2	9.52	9.52
10	10 × 1	10.02	
	5 × 2	8.84	
	2 × 5	9.26	
	1 × 10	8.54	9.17
12	4 × 3	9.22	
	2 × 6	8.08	8.65
	5 × 3	8.61	
	1 × 15	6.22	7.42
20	20 × 1	8.16	
	10 × 2	7.22	
	4 × 5	7.96	
	2 × 10	6.74	7.52
24	4 × 6	6.66	6.66
25	5 × 5	7.66	7.66
30	10 × 3	7.19	5
	5 × 6	6.9	
	2 × 15	4.59	
	1 × 30	4.53	5.80
40	20 × 2	5.44	
	4 × 10	5.74	5.59
50	10 × 5	6.46	
	5 × 10	5.97	6.22
60	20 × 3	5.6	
	10 × 6	6.01	
	4 × 15	3.62	
	2 × 30	3.46	4.67
75	5 × 15	3.39	3.39
100	20 × 5	5.28	
	10 × 10	5.38	5.33
120	20 × 6	4.64	
	4 × 30	2.6	3.62
150	10 × 15	2.95	
150	5 × 30	2.86	2.91
200	20 × 10	3.93	3.93
300	20 × 15	1.22	
300	10 × 30	2.65	1.94

Table 1 and depicted by Fig.3. The results (Table 1) revealed that the value of Coefficient of Variation (CV) decreased with increase in plot size. The average value of CV(%) decreased from 24.24% to 1.94% with increase in plot size from 1 unit to 300 units. Initially the decrease in CV(%) was more rapid but after 15 units plot

size the reduction was marginal. These results are in agreement with the results reported by Patel and Patel for Bajra (1967), Prajapati *et al.* in bidi tobacco (1984), Kumar and Singh for wheat (1986), and Chaudhary *et al.* for isabgul (2015).

The difference between in CV(%) of the consecutive points (plot size) may help to estimate per unit decrease in C.V. % thereby point of maximum curvature. The per unit decrease in CV(%) (Table 2) was observed from 7.17% to 0.02% with increase in plot size from 1 unit to 300 units plot size. Beyond 12 unit plot size, the per unit decrease in CV(%) was not proportional to the amount of land used in forming the large sized plot. Therefore, 12 units plot size could be taken as point of maximum curvature. The optimum plot size is just beyond the point of maximum curvature (Federer, 1995). Therefore, 15 units plot size *i.e.* 5.0 m x 3.6 m (6 rows each of 5 meter length) was considered as an optimum plot size for conducting field experiments on sesame crop. The data were also presented graphically and depicted in Fig. 3, also showed the similar trend. Therefore, 15 units plot size was taken as point of maximum curvature and could be considered an optimum plot size for field experiment on sesame crop.

Table 2 : Per unit decrease in CV (%) for seed yield of sesame Plot

Plot size in basic unit	Average CV(%)	Difference in CV(%)	Per unit decrease in CV(%)	Plot size in basic unit	Average CV(%)	Difference in CV(%)	Per unit decrease in CV(%)
1	24.24			25	7.66	-1.00	-1.00
2	17.07	7.17	7.17	30	5.80	1.86	0.37
3	14.07	3.00	3.00	40	5.59	0.21	0.02
4	13.65	0.42	0.42	50	6.22	-0.63	-0.06
5	12.22	1.43	1.43	60	4.67	1.55	0.16
6	10.72	1.50	1.50	75	3.39	-0.66	-0.04
8	9.52	1.20	0.60	100	5.33	1.71	0.07
10	9.16	0.36	0.18	120	3.62	0.72	0.04
12	8.65	0.51	0.26	150	2.90	-1.03	-0.03
15	7.41	1.24	0.41	200	3.93	1.99	0.04
20	7.52	-0.11	-0.02	300	1.94	1.94	0.02
24	6.66	0.86	0.22				

The number of replication and required area for different plot size at 5% standard error of mean were compare from the estimated CV% for seed yield of sesame by using the formula (Nigam and Gupta, 1979). The results

Table 3 : Number of replications, area required for 5 per cent S. Em. and relative land use efficiency for seed yield with respect to unit plot size

Basic unit plot size	CV(%)	No. of replications	No. of basic units	Sq. m.	Relative land use efficiency (%)
1	24.24	24	24	28.80	100.00
2	17.07	12	24	28.80	100.00
3	14.07	8	24	28.80	100.00
4	13.65	8	32	38.40	75.00
5	12.22	6	30	36.00	80.00
6	10.72	5	30	36.00	80.00
8	9.52	4	32	38.40	75.00
10	9.16	4	40	48.00	60.00
12	8.65	3	36	43.20	66.67
15	7.41	3	45	54.00	53.33
20	7.52	3	60	72.00	40.00
24	6.66	2	48	57.60	50.00
25	7.66	3	75	90.00	32.00
30	5.80	2	60	72.00	40.00
40	5.59	2	80	96.00	30.00
50	6.22	2	100	120.00	24.00
60	4.67	1	60	72.00	40.00
75	3.39	1	75	90.00	32.00
100	5.33	2	200	240.00	12.00
120	3.62	1	120	144.00	20.00
150	2.90	1	150	180.00	16.00
200	3.93	1	200	240.00	12.00
300	1.94	1	300	360.00	8.00

(Table 3) revealed that the number of replication and relative land use efficiency decreased as the size of plot increased. Agricultural operation in field experiment with smaller plot size, a plot size having relatively high land use efficiency with less number of replications should be considered for field experimentation. Such situation was observed with the 15 units plot

size which is having considerably smaller number of replications and relatively high land use efficiency for seed yield. Therefore, 3 replications could be considered optimum for 15 units plot size at 5% probability level for sesame crop.

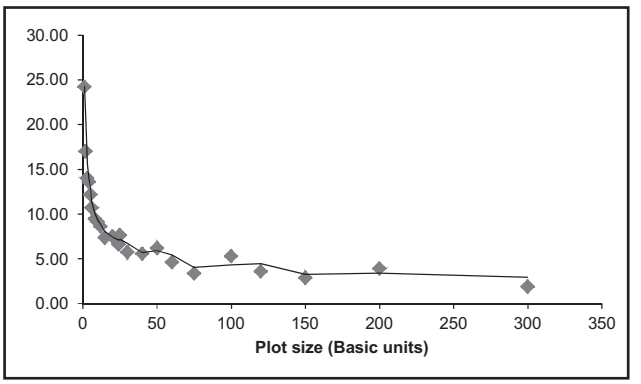


Fig. 3: Coefficient of variation in per cent for different sized plots

Among all combinations of 15 units plot, a plot of 1×15 (1 units towards length and 15 units toward width) showed the lowest CV (6.22%). Taking into consideration the requirement of agronomical operation, handling of experimental material and the average variability, 15 unit plot (6 rows each of 5 meter length, *i.e.*, 5.0 m × 3.6 m) is optimum net plot with 3 replications for sesame crop.

CONCLUSION

A plot of 15 basic units (18 m^2) size having shape of 6 rows each of 5 meters length (5.0 m × 3.6 m) was found as optimum plot size (net plot) with minimum 3 replications for field experiments on sesame at the Seed Technology, S. D. Agricultural University, Sardarkrushinagar

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Growth Response of Juvenile Rohu (*Labeo Rohita*) Exposed to Varying Amounts of Peppermint diet with Protein

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ABSTRACT

The goal of the current investigation was to determine how dietary peppermint (Mentha piperita) along with protein affected Labeorohita's growing performance. Following five days of acclimation to the aquarium environment, the fingerlings were split into four equal groups, designated as groups A, B, C, and D. Each aquarium included fifteen fish. The duration of the experiment was for sixty days where one set of animals was fed a control diet devoid of peppermint and another three were fed with test diets containing varying levels of Mentha piperita and protein at 4mg,6mg and 8mg of the diet. The weight gain, body length. specific growth rates were used to evaluate fish growth. The findings showed that in Tank 4, where fish were fed a diet containing 8mg of peppermint, all growth metrics were significantly higher. When compared to the control group, the dietary inclusion of Mentha piperita in all doses increased fish appetite and may be suggested as a safe growth promoter as the outcomes demonstrated that feeding with *Mentha piperita* decreased the fatalities by enhancing fish growth and production.

Keywords : Peppermint, Labeorohita, Growth, Medicinal herbs.

INTRODUCTION

Aquaculture farming is now the fastest-growing food production industry worldwide. It offers premium animal protein, with a rise in worldwide production. Integrated fish management and polyculture are established, fruitful, and dynamic techniques that employ common fish species like mrigal (*Cirrhinus Cirrhosus*), rohu (*Labeo Rohita*), and catla (*Catla Catla*) (Uddin et al., 1994; FAO, 1997). Due to its high market value and consumer preference, rohu are currently quite popular among farmers (Dey et al., 2005; Rahman, 2006) Fresh water fish of the species *Labeo Rohita* are members of the family Cyprinidae and the class actinopterygii order Cypriniformes. It is found in freshwater environments like lakes, streams, rivers, and pools all across the world. This highly adaptable freshwater fish is found throughout Europe, Asia, and the Middle East's eutrophic waterways; it is also found in North America, Canada, and Australia (Faramarzi et al., 2012).The rohu (*Labeo Rohita*) is a highly sought-after species with significant market value. Shete et al. (2015).

Nowadays antibiotic growth promoters are being added to feed in order to achieve high production. However, the public is more receptive to natural substances because of their negative effects (residual buildup in fish tissue and, rise of antibiotic-resistant

bacteria).To deal with this the use of herbal immunostimulants has been shown to improve animal overall health , productivity and feed consumption efficiency. Medicinal plant extracts have been used for a variety of reasons since ancient times, including medications, cosmetics, and food supplements. They've been evaluated for their prospective applications as substitute remedies for the management of numerous infectious disorders and the protection of food from oxidant-induced toxicity (Kelen and Tepe 2008).

Mentha piperita, popularly known as peppermint, is a well-known perennial herb in the Labiatae family.is among the traditional medicinal herbs used worldwide (Iscan et al.,2002) Due to their extensive therapeutic range, plant extracts are frequently used in traditional and ancient medicine, typically in aqueous solutions (Zhang et al., 2002). According to available data and research, numerous studies have examined the use of dietary medicinal herbs and their extracts as possible immunostimulants in aquaculture, with eventually encouraging findings noted regarding varied fish species' disease resistance, growth patterns, feed consumption, chemical body structure, and immune response (Hoseinifar *et al*., 2016). The primary active component found in peppermint is menthol, accompanied by other elements like menthol and menthyl acetate Dried peppermint typically contains volatile oil, with varying percentages of menthol, menthone, menthyl acetate .Peppermint has been recognized for its potential as an immunostimulant and appetite enhancer (Pauli 2006). The current study aims to examine how incorporating dietary peppermint *(Mentha piperita)* affects the growth performance of the Indian major carp, rohu Labio rohita “Barbarestani *et al*. (2017)”. Depending upon the above-mentioned , this study is done to investigate the effects of mint powder dietary supplements on *Labio rohita* growth and health performance performance. Many studies have shown that the rise in feed intake could also be attributed to the effects of peppermint and the improved digestibility of food. Our study aligns with prior research findings, such as those of (Nath *et al*. (2013), which support this notion.

MATERIALS METHOD

Experimental setup

The experiment was done in a laboratory setting. The fish were collected from the adjacent fish farm, and after approximately a week of acclimation, the healthy fish were used for the experiment. The fish were housed in glass aquariums of 100 l capacity filled with regular well water that had been adequately aerated. The tanks were divided into four such as group A, group B, group C, group D where group A is for control and the remaining three are for experimental. Fish fingerlings (10 fish per tank) were added to each aquarium in an equal quantity. To prevent any fungal infection, dechlorinating solutions were added and the water was changed every day. The fish's initial body weight and general composition were established before the experiment. After the experiment growth parameters were studied by analyzing body weight, height etc.

Preparation of Experimental Diet

The Mentha piperita plants were gathered from their natural habitat Following the collection, leaves of M. piperita underwent a drying process in a well-ventilated and dark

room. Subsequently, the dried plants were finely ground using a grinder. For the preparation of experimental diet the powdered plant material in different concentration like 4mg, 6mg and 8mg was mixed with groundnut oil cake, wheat flour, soya flour, fish meal, tapioca flour, and a mix of vitamins and minerals. In all of these groundnut and soya were taken in high proportion as both of them are good sources of protein. It is widely recognized that protein is crucial and often the most costly component that needs to be provided in sufficient quantities to facilitate optimal growth while minimizing expenses (WecKL,1983). The amount of crude protein in the groundnut seed is 25–30% and oil content is 35–50% (Musa *et al.,* 2010). Whereas the control diet, devoid of peppermint, was formulated using the same ingredients.

The resulting diet was made into palette, allowed to sun-dry, and were stored.

Table 1 : Ingredients of experimental diet.

Ingredients (g/100gm)	(Group A) Control	Group B	Group C	Group D
Soya oil cake	45	45	45	45
Groundnut oil cake	25	25	25	25
Wheat flour	20	20	20	20
Corn starch	5	5	5	5
Fish meal	3	3	3	3
Mineralsand Vitamin	2	2	2	2
Peppermint powder	00	0.4	0.6	0.8

Growth performance

Growth parameters such as weight gain, and specific growth rate (SGR) were determined according to the following standard formula (Saufie *et al*., 2015).

Weight gain = Final fish weight (g) - initial fish weight(g)

$$\text{Specific growth rate} = \frac{\text{ln final weight - ln initial weight}}{\text{Number of days}} \times 100$$

Protein estimation

Lowry's technique, a commonly used method for determining total protein content in a sample. (Waterborg, & Matthews, 2002) This method's sensitivity is owed to its utilization of two color-producing reactions. This process involves Biuret Reaction which is the interaction of Cu ions with the peptide bonds of proteins under alkaline conditions. In the presence of a base like sodium hydroxide, Cu ions react with peptide bonds, leading to the reduction of cupric ions (Cu) to cuprous ions (Cu) (Lowry OH 1951). This complex formation contributes to the assay's sensitivity. In this phase, the Folin-Ciocalteu

reagent is utilized. This reagent contains a phosphomolybdic complex, which is a mixture of sodium tungstate, sodium molybdate, and phosphate, alongside copper sulfate solution and the protein sample. Upon reaction with peptide bonds in the protein sample, the Folin-Ciocalteu reagent generates a blue-purple colour. The intensity of this colour correlates directly with the protein concentration in the sample. Typically, the absorbance of the resulting colour is measured spectrophotometrically at wavelengths between 650-700 nm (Wrolstad et al., 2005). By gauging the absorbance of the produced colour in Lowry's reaction, the protein concentration in the sample can be quantified. This approach is favored for its sensitivity and reliability in determining total protein levels in biological specimens like serum.

RESULTS & DISCUSSION

Fish growth parameters

A dietary supplement of Peppermint and protein has been shown to enhance the growth performance in fish showing significant differences ($P < 0.05$) between groups, as indicated by varying superscripts within each row. There was a significant increase in weight gain, length and SGR when the mint treatment was given to the fish. There are many studies that have shown the positive effect of peppermint on aquatic animals like fish (Hong *et al*., 2012).

The body weight (in grams)

The body weight gain of *Labeo rohita* fish exhibited a notable increase in groups (C) and (D) compared to groups (A) and (B) during the initial 30 days. Among these, group (D), which received a high dose of peppermint supplement (8 mg) with their feed, recorded the highest weight in the next 30 days subsequently there was a significant increase in weight observed in groups (B), (C), and (D) in comparison to control group A. Once again, group (D) treated with a high dose of peppermint supplement mixed with feed displayed the maximum weight and length (Table 2) As mint has antioxidant properties it increases the survival rate and increases the growth performance, specific growth rate (VargaSanchez et al., 2019;)

Specific growth rate

Dietary peppermint has a positive effect on specific growth rate (Korzen *et al*., 2016) Highest specific growth rate was observed in group D which were fed with peppermint and high protein diet as compared to controls which had the lowest specific growth rate which was fed with only normal diet (Table 2).The leaves of mint has high digestive properties as also act as a good appetizer hence enhancing the SGR (Asghari *et al*., 2018)

Length of fish

Peppermint has shown a positive impact on the increase in length of fish. There's An increase in length of fish as the weight gain. The length increases gradually as the dose increases. Furthermore, morphological features also change as the fish grow in weight and length (Parvin *et al.*, 2018).

Table 2 : Growth response of juvenile rohu exposed to varying amount of peppermint diet with protein

Group	Weight (gm)	SGR	Length (cm)
Control (A)	4.36 ± 0.54	0.77±0.59	3.06±0.61
Experimental (B)	5.08±0.38	0.72±0.057	5.66±1.09
Control (A)	4.36 ± 0.54	0.77±0.59	3.06±0.61
Experimental (C)	7.44±0.75	0.92±0.071	8.32±0.23
Control (A)	4.36 ± 0.54	0.77±0.59	3.06±0.61
Experimental (D)	9.44±1.00	1.078±0.06	8.58±0.19

Data is presented as mean values with standard deviations, showing significant differences (P < 0.05) between groups as indicated by varying superscripts within each Row.

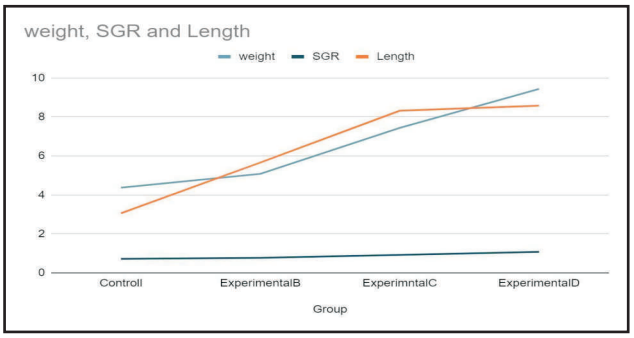


Fig 1 : Mean weight gain of fish under different experimental group

Total protein -There was a significant increase in protein and fat levels when the peppermint was given in the diet. As a result the performance of the fish was found to be enhanced.

Dietary protein plays a key role in the growth of fishes (*Barbarestan et. al. 2017)*

Table 3 : Mean value of protein (g/dL) and lipid in different experimental group

Fish group	Total protein (g/dL)	Total lipid
Control A	2.84± 0.89	2.06±0.17
Experimental B	2.52±0.88	3.70±0.32
Control	2.84± 0.89	2.06±0.17
Experimental C	3.59±0.81	4.01±0.27
Control	2.84± 0.89	2.06±0.17
Experimental D	3.48±0.81	4.20 0±.56

Data is presented as mean values with standard deviations, showing significant differences (P < 0.05) between groups as indicated by varying superscripts within each Row.

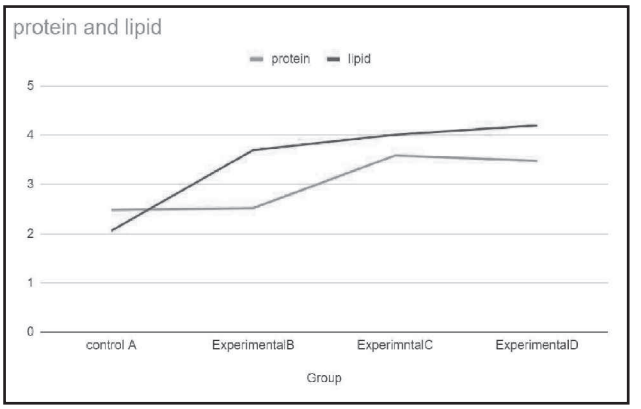


Fig 2 : Amount of protein and lipid in different experimental group

It is imperative to engage in the aquaculture sector given its pivotal role in meeting the rising global demand for seafood. Aquaculture offers a sustainable and efficient means of seafood production, crucial for feeding an expanding population. With wild fish stocks declining due to overfishing and environmental shifts, there is a pressing need for effective disease management and drug application within this important and profitable industry (Aklakur *et al.*, 2015). The discussion revolves around the potential impacts of medicinal herbs, which contain potent bioactive compounds. These compounds can influence the digestive process by either enhancing or inhibiting enzyme activity, thus affecting nutrient absorption (Talpur 2013) A particular study examines the inclusion of peppermint in fish diets, showing notable improvements in weight gain, and specific growth rate. Notably, group 4, which received a diet with 8mg of peppermint and a high protein content, experienced increases in all growth parameters. Similarly, feeding with *Mentha piperita* stimulated appetite, leading to improved weight gain and specific growth rate, as previously observed in the fingerling of *Labeo Rohita* (Adel et al., 2015).

CONCLUSION

Herbs and medicinal plants are more effective growth promoters compared to synthetic alternatives. They are cost-effective, safe, environmentally friendly, and do not have adverse effects on the fish. The study's findings indicate that incorporating Mentha piperita powder or extract into the diet of *Labeo rohita* significantly enhances their growth performance and increases their protein levels. The use of dietary peppermint was shown to positively affect various growth metrics such as weight gain, body length, and specific growth rate. Fish fed with the highest concentration of peppermint exhibited the most notable improvements, suggesting that Mentha piperita can be an effective and safe growth promoter. This herbal supplement not only improved the overall health and growth of the fish but also reduced mortality rates, making it a valuable addition to aquaculture practices.

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Constraints of rural women regarding pratapdhan poultry production technology promoted by kvk bhilwara

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ABSTRACT

This study in Bhilwara, Rajasthan, explored challenges faced by rural women adopting Pratapdhan Poultry Production Technology. Sampling 100 respondents from five villages, personal interviews and statistical measures were used for analysis. Key findings revealed complex challenges, notably in health management, with issues like vaccination facility absence and delayed veterinary aid posing threats. Miscellaneous constraints, from water access to predator concerns, highlighted diverse challenges such as feeding constraints, driven by financial issues and high organic feed costs, impacted bird nutrition. Housing constraints, including facility inadequacies and high construction costs, posed hurdles. Marketing challenges, from the lack of organized markets to religious considerations, affected profitability. Technical challenges, personal constraints, and financial obstacles further hindered scaling up. Breeding and rearing constraints, such as the non-availability of improved breed birds and high chick costs, highlighted foundational hurdles. In conclusion, a holistic approach, incorporating targeted interventions and policy support, is crucial for the sustainability and success of Pratapdhan poultry production in Bhilwara.

Keywords : Pratapdhan, Poultry, Production, Constraints, rural women.

INTRODUCTION

The growth of Indian poultry industry has a glorious past. What was largely a backyard venture before the1960s has been transformed into a vibrant agribusiness (Mehta and Nambiar, 2008). Only native chicken was reared under this low input scavenge farming with the provision of coop as night shelter until 1960 with the flock size of less than 10 (Kumar *et al*., 2013). The backyard poultry keepers are primarily agricultural laborer keeping small size backyard poultry flock as secondary occupation with marginal land holding and low annual family income (Bharti *et al*., 2018). Although the native birds are of low producers with less body weight, they are adaptability to the environment and resistant to diseases (Kumar *et al*., 2016). The birds are reproduced by natural incubation and natural brooding. The feeding is mainly by grazing with the provision of kitchen waste and minimal supplementation of household grains available in the house. The developments in poultry farming took place rapidly after 1960s. Import of high producing hybrid strains to the country and

simultaneous growth of input sectors like feed, equipment, vaccines, medicines, insurance etc., have led to the phenomenal growth of poultry farming in India. This transformed poultry production from a completely backyard activity into an industry. All these developments made India the fourth largest in chicken egg and fifth largest in poultry meat production (FAOSTAT, 2019). The annual growth was 6.93% in poultry meat production and 10.19% in egg production in the year 2019-20 (BAHS, 2020).

METHODOLOGY

MPUAT, Udaipur has eight KVKs, with Bhilwara being one of the districts under its jurisdiction. The selection of Bhilwara for the study was deliberate, given the researcher's familiarity with the area and the extensive promotion of Pratapdhan Poultry Production Technology in the district through KVK Bhilwara. According to information from the Subject Matter Specialist and Senior Scientist-cum-Head of KVK Bhilwara, a chick rearing unit established under RKVY has been operating efficiently since 2016. KVK has facilitated the distribution of 1000 units of Pratapdhan poultry through demonstrations, funded by KVK and ATMA, in fifty villages. Randomly selecting five villages, namely Pondras, Akola, Salariya, Suwana, and Kodukota, 20 rural women were chosen from each village based on a prepared list. This list comprised women who had been introduced to Pratapdhan poultry production technology through KVK training and demonstration, and who also owned poultry units. The total sample size consisted of 100 respondents from the five selected villages.

RESULTS AND DISCUSSION

The benefit of any technology is actually derived only when it is efficiently utilized by an individual in his/her situation. In the present study an effort was made to identify the problems if any faced by the respondents in adoption of Pratapdhan poultry production technology.

Table 1 : Various constraints faced by the respondents in adoption of Pratapdhan poultry production technology **n=100**

S. No.	Aspects	To a great extent (f/ %)	To some extent (f/ %)	Not at all (f/ %)	*MPS
Breeding Constraints					
1.	Non-availability of improved/pure breed birds.	57	26	17	70
2.	High cost of one day old chicks.	51	41	8	71.5
3.	Availability of chicks from a long distance.	55	33	12	71.5
4.	Unavailability of chicks of suitable.	58	20	22	68

S. No.	Aspects	To a great extent (f/ %)	To some extent (f/ %)	Not at all (f/ %)	*MPS
Personal Constraints					
5.	Not paying constant focus on poultry farming.	64	19	17	73.5
6.	Lack of support from family members.	44	35	21	61.5
Financial Constraints					
7.	Lack of finance	54	30	16	69
8.	Difficulty in getting loans	61	21	18	71.5
9.	High interest rate on loan	52	30	18	67
Marketing Constraints					
10.	Lack of organized market	58	29	13	72.5
11.	Religious constraints	57	31	12	72.5
12.	Low prices of cull birds	46	33	21	62.5
13.	Lack of government support in the form of minimum support prices (MSP) for poultry farm women.	47	30	23	62
Feeding Constraints					
14.	Lack of availability of organic feeds.	56	26	18	69
15.	Lack of availability of quality feeds.	54	36	10	72
16.	Unable to feed balance feed to due to lack of money.	59	30	11	74
17.	High cost of organic poultry feed.	64	17	19	72.5
Health Management Constraints					
18.	Lack of availability of vaccination facilities	62	24	14	74
19.	Non-availability of prompt veterinary aid at village level	60	22	18	71
20.	High incidence of diseases	53	31	16	68.5
21.	Lack of availability of poultry medicines	70	22	8	81
Housing Constraints					
22.	Inadequate housing facilities.	63	17	20	71.5
23.	Non-availability of separate house for chicks and layers.	64	19	17	73.5
24.	High cost of construction.	57	31	12	72.5

S. No.	Aspects	To a great extent (f/ %)	To some extent (f/ %)	Not at all (f/ %)	*MPS
Technical Constraints					
25.	Lack of institutional and extension support.	54	36	10	72
26.	Inadequate facilities for storage of eggs.	54	28	18	68
27.	Lack of skill in preparing brooder.	59	29	12	73.5
28.	Lack of knowledge regarding scientific poultry rearing.	63	20	17	73
Miscellaneous Constraints					
29.	Non-availability of clean drinking water.	59	23	18	70.5
30.	Non-availability of regular supply of electricity.	65	29	6	79.5
31.	Inadequate training facilities for poultry farm women.	69	18	3	78
32.	Predators' problems.	58	29	13	72.5

***MPS: - Mean Percent Score**

The analysis highlights various constraints faced by respondents in adopting Pratapdhan poultry production technology.

Breeding Constraints

A significant 71.5% of respondents cited the high cost of one-day-old chicks and the need to source chicks from long distances as major constraints. Additionally, 70% of respondents mentioned the non-availability of improved breed birds, and 68% noted the general unavailability of chicks as constraints. The primary source of Pratapdhan chicks is the AICRP on Poultry Breeding RCA, MPUAT, Udaipur. Similar findings were reported by Rahman (2017), who identified the non-availability of improved chicks, predators during scavenging, diseases, and lack of health service inputs as major constraints.

Personal Constraints

A substantial 73.5% of respondents reported being unable to focus on poultry farming due to other commitments, and 61.5% said they lacked family support.

Financial Constraints

Financial challenges were significant, with 69% of respondents struggling with a lack of funds and 71.5% facing difficulties in obtaining loans. Additionally, 67% of respondents mentioned that high interest rates on loans inhibit large-scale Pratapdhan poultry rearing.

Marketing Constraints

72.5% of respondents identified a lack of organized markets nearby and difficulties due to religious reasons as constraints. Nearly 63% noted the low price of cull birds, and a similar proportion reported inadequate government support, such as minimum support prices. Lavanya et al. (2017) also found that the absence of a common forum

for discussing marketing problems and the lack of reliable nearby markets were significant issues.

Feeding Constraints

Around 74% of respondents struggled to provide balanced poultry feed due to financial constraints. Additionally, 72% and 72.5% of respondents cited the lack of availability and high cost of organic feed, respectively, as major constraints.

Health Management Constraints

Health management issues were prevalent, with 81% of respondents noting a lack of poultry medicines, 74% citing insufficient vaccination facilities, and 71% pointing to the absence of prompt veterinary aid at the village level. High disease incidence was reported by 68.5% of respondents. Sharma et al. (2018) similarly found that lack of knowledge regarding vaccination schedules was a major constraint, leading to high disease incidence.

Housing Constraints

About 73.5% of respondents faced challenges due to the non-availability of separate housing for chicks and layers, with 72.5% citing high construction costs and 71.5% reporting inadequate housing facilities.

Miscellaneous Constraints

Additional constraints included 79.5% of respondents facing issues with irregular electricity supply during winter, 78% citing inadequate training facilities for poultry farm women, and 70.5% and 72.5% reporting problems with clean water availability and predators, respectively.

Table 2 : Overall constraints faced by respondents in adoption of Pratapdhan poultry production technology. n=100

S. No.	Aspects	MPS	Rank
1.	Breeding and rearing of chick's constraints	78.33	IX
2.	Personal constraints	79.44	VIII
3.	Financial constraints	78.87	VII
4.	Marketing constraints	81.25	V
5.	Feeding constraints	81.67	III
6.	Health management constraints	83.56	I
7.	Housing constraints	81.30	IV
8.	Technical constraints	81.08	VI
9.	Miscellaneous constraints	83.27	II

With regard to overall constraints faced by rural women, the data in Table 2 reveals that majority of the respondents faced problems in health management category with overall MPS of (83.56%) and given as Rank I followed by miscellaneous category with overall MPS of (83.27%) with Rank II. Similarly, problem faced by the feeding management category with overall MPS of (81.67%) with Rank III followed by housing management category with overall MPS (81.30%) with Rank IV whereas, constraints faced by the respondents in marketing practice category with overall MPS of (81.25%) with Rank V followed by technical category with overall MPS of (81.08%) with Rank VI followed by personal category with

overall MPS of (79.44%) with Rank VII. However, problem faced by the respondents in financial category with overall MPS of (78.87%) with Rank VIII followed by breeding and rearing category with overall MPS of (78.33%) with Rank IX. The study is supported by Ghasura *et al.* (2013) who conducted a study to identify the constraints in poultry farming and showed that the constraints faced were availability of birds and personal, situational, economic and marketing aspects of the poultry enterprise. Most poultry entrepreneurs faced low egg prices during summer, high costs of feed and medicine, lack of adequate credit facilities, fluctuating prices for the poultry products, increased risks and uncertainty of the poultry enterprise, high mortality of chick availability of veterinary aids. Similar study conducted by Gopala *et al.* (2015) revealed in their study that the most significant constraints faced by the contract broiler poultry farmers were high mortality due to diseases, lack of financial support, quality of medicines and feed and inadequate extension advisory serious and most significant constraints faced by the non-contract broiler poultry farmers were fluctuation in the market price, non-availability of chicks in time and commercial to riff for electricity charge.

CONCLUSION

Adopting Pratapdhan poultry production technology faces several challenges. Health management is hindered by a lack of vaccination facilities, delayed veterinary aid, high disease incidence, and medicine shortages. Farmers also struggle with limited access to clean water, irregular electricity, inadequate training, and predator threats. Financial constraints affect the ability to provide quality feed and build adequate housing. Marketing issues include the absence of organized markets and low cull bird prices. Technical challenges stem from insufficient support and knowledge in poultry rearing. Personal and financial constraints, such as competing commitments and limited loan access, further complicate scaling up operations. Addressing these challenges requires targeted interventions, policy support, and capacity-building initiatives.

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Voices of the Future Regarding the National Education Policy (2020) : Students Opinion

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ABSTRACT

The New Education Policy 2020 of the Government of India identifies education and institutional capacity building as two programmes that could support the implementation of sustainable development of the country and also make it self-reliant. One issue that the policy completely ignores is how it intends to guarantee that students' intellectual freedom will be maintained in universities. A total of 470 students were selected by random method. This representative sample was drawn from each discipline (science, arts, and social science) at the university. A critical analysis of the relative merits and demerits of the new education policy is, therefore, necessary. The majority of students (40.2%) had the opinion that flexible curriculum, practical-oriented training (38%), andvalid student assessmentin the new education policy 2020 however most of the students were undecided as to whether the new education policy provided scope for better teaching and learning or not.

Keywords : Education policy, Merits, Demerits, Intellectual freedom, Practical approach

INTRODUCTION

Education is fundamental for achieving full human potential developing an equitable and just society and promoting national development. Providing universal access to quality education is the key to India's continued ascent, and leadership on the global stage in terms of economic growth, social justice and equality, scientific advancement, national integration, and cultural preservation. New education policy lays particular emphasis on the development of the creative potential of each individual. It is based on the principle that education must develop not only cognitive capacities - both the 'foundational capacities 'of literacy and numeracy and 'higher-order' cognitive capacities, such as critical thinking and problem-solving – but also social, ethical, and emotional capacities and dispositions.

There are still conflicting opinions in the country on whether this new education policy in 2020 is better than the old one. A critical analysis of the relative merits and demerits of the new education policy is, therefore, necessary. The current educational system is grade-oriented, and knowledge is becoming talent-oriented rather than talent-certificate-oriented the NEP 2020 signifies a shift

towards emphasizing the development and nurturing of individual abilities over the traditional focus on grades and certification. Instead of making students mark-seekers, the educational ecosystem should develop talent and technology-driven experts. The NEP 2020 presents twenty-two guiding principles, encompassing conceptual comprehensiveness, flexibility, and holistic development. These principles are intended to underpin the entirety of the Indian education system (Shamsu, 2021).

Only an exam cannot predict student's future, so they must be given the chance to select a vocation that suits their interests and preferences (Saurabh, 2012).The prevailing teacher-centric model, in which teachers determine subjects, curriculum, evaluation criteria, etc., is set to be supplanted by a student-centric approach. Under this new paradigm, students will have the authority to choose the subjects they wish to study at the institution. (Simao, A. M. V., & Flores, M. A. 2010)

Since this system wasn't used during data collection at the four main universities and because of the new education policy in 2020, it hasn't been assessed yet. Therefore, the study was conducted in 2022-23 at four central universities in Uttar Pradesh, India, to obtain students' opinions on the NEP.

METHODOLOGY

Population and sample

An online survey method was used to collect thedata. The study was conducted on a sample size of 470undergraduate final-yearuniversity students. This sample was drawn giving representation to each discipline (science, arts, and social science), of four central universities of Uttar Pradesh i.e.Babasaheb Bhimrao Ambedkar University, Lucknow, Aligarh Muslim University, Aligarh, Banaras Hindu University, Varanasi, University of Allahabad, Allahabad.

Instrument used for Data Collection

A set of Guttman scales was constructed to measure the opinion of the students relating to the items and demerits in respect to the new policy of education. A five-pointratio scale on the Likert pattern was used to get the opinions from the sample students on the Guttman scale. This rating procedure ranged from strongly disagree to strongly agree with undecided as a middle point. The Guttman scale known as the cumulative scale measures a unidimensional conceptwith several statements placed in a hierarchical order.

Clarification of Guttman Scale

In our study, the Guttman scale was utilized to capture the degree of agreement with various statements about the new education policy. This cumulative scale allowed us to assess the consistency of students' opinions across related items.

To illustrate the use of the Guttman scale, we present a matrix of student responses. Each row represents an individual student's responses, while each column corresponds to a specific statement. '1' indicates agreement with the statement, while '0' indicates disagreement. The cumulative nature of the Guttman scale is evident as students who agree with a higher-order

statement also agree with all preceding statements. The results from the Guttman scale were analyzed to determine the consensus index for each item, which reflects the level of agreement among students. The consensus index was calculated for both merits and demerits of the new education policy, as shown in the table 1 and table 2.

RESULT AND DISCUSSION

Students'Opinions on the items of merits of New Education Policy 2020

Opinions of students regarding the merits of the New Education Policy are given in Table 1.

Flexible Curriculum

The consensus index of 0.14 for this item was statistically significant at 0.01 level. Although this consensus was not very high, the new education policy (2020) has a flexible curriculum according to students. The consensus index is a statistical measure used to determine the level of agreement among respondents in a study. The calculation of the consensus index typically involves analyzing responses to a particular item or question and determining the extent to which respondents agree or disagree. It often ranges from 0 to 1, where 0 indicates no agreement and 1 indicates perfect agreement.

Better Trained Students

The index of consensus was -0.28 for this item and was significant at 1 percent level. This implied that the students did not feel that they would be better trained under the new education policy. The fact that most students seem to find the new education policy satisfying, while not being better prepared for the new system, may suggest that the majority of students find the courses easier to complete under the new system than under the previous one.

Practical-oriented training

the consensus index of 0.08 through significance at 5 percent level, indicated a low consensus. This finding confirms the statements made by many university students, indicating that the new system has successfully provided more practical-oriented teaching-learning methods.

Academic freedom for teachers to develop their own course content

The consensus index of-0.04 was significant at 5 percent level. It revealed that the students did not recognize that academic freedom was given to the teachers under the new system of education. “Academic freedom" refers to the autonomy that teachers have in their professional roles, including curriculum design and teaching methods. The consensus index suggests that students did not perceive teachers as having this freedom within the new education system (Wilkins. 2019).

Table 1 : Distribution of Respondents According to Merits of New Education Policy

Sl. No	Items of the Merit	The number of respondents expressing					Total	Index of consensus (Cc)	Standard error (SCc)	Normal Deviate
		Strongly Agree	Agree	Undecided	Disagree	Strongly Disagree				
1	Flexible Curriculum	18	171	189	74	18	470	0.14	0.02	5.90**
2	Better trained students	17	61	143	178	71	470	-0.28	0.03	10.15**
3	Practical oriented training	25	154	179	86	26	470	0.08	0.02	3.44**
4	Academic freedom in the teacher to develop own course content	14	71	268	101	16	470	-0.04	0.016	-2.63*
5	Scope for better teaching methods	16	109	243	87	15	470	-0.03	0.017	1.77
6	Desirable students	0	38	211	188	33	470	-0.22	0.02	-11.47**

**significance at 1 percent level
* Significant at 5 percent level

Scope for better teaching methods

The consensus index of -0.03 was not significant. Thus, the student's opinion was not in favour of the fact that there is more scope for better teaching methods in the new education policy.

Desirable students' behavior

The consensus index of -0.22 was significant at 1 percent level. This implied that the students did not accept this item as a merit of the new system of education. It is rather difficult to explain why the students disagree with this idea while many authorities have observed that the students are found busy although the course period in the new education policy.

Opinion of Students' opinions on the item's demerits of New Education Policy 2020 Students opinions on the items of demerit are summarized in Table 2.

Misuse of Academic freedom

The consensus index of -0.15 was significant at 1 percent level indicating that the students did not accept the item under the demerit list.

Unhealthy competition among teachers in grading

The consensus index of 0.11 was significant at 1 percent level. This implied that from the students' viewpoint, the unhealthy competition among teachers in grading was an item of demerit in the new education policy.

Lowering of academic standards

The index of consensus of -0.38 was significant at the 1 percent level, indicating that there is a perception that academic standards are being lowered in the new education policy.

Table 2 : Distribution of Respondents According to Demerits of New Education Policy

Sl. No.	Items of the Demerit	The numberof respondents expressing					Total	Index of consensus (Cc)	Standard error (SCc)	Normal Deviate
		Strongly Agree	Agree	Undecided	Disagree	Strongly Disagree				
1	Misuse of Academic Freedom	13	75	195	136	51	470	-0.15	0.02	-7.22**
2	Unhealthy Competition among teachers in grading	28	158	179	82	23	470	0.11	0.02	4.93**
3	Lowering Academic Standards	14	38	144	164	110	470	-0.38	0.03	-12.99**
4	Poor comprehension of subjects	13	35	129	226	67	470	-0.38	0.03	-14.26**
5	Inconvenient to students	0	55	173	189	53	470	-0.27	0.02	-11.71**
6	Lack of uniformity in teaching and evaluation	17	26	123	228	76	470	-0.39	0.02	-14.72**
7	Inconvenient to teachers	26	86	212	118	28	470	-0.03	0.02	-1.63

** Significant at 1 percent label

Poor comprehension of the subject: The consensus index of -0.38 was significant at 1 percent level. Thus, the results pointed out that in students under the new education policy 2020 is not poor.

Lack of Uniformity in Teaching and Evaluation: This has the most negative consensus index of -0.39 and a normal deviate of -14.72, indicating a very strong perception of this as a demerit.

Inconvenient to teachers: The consensus index of -0.03 was not significant. This implied that the students did not consider the new system as convenient to teachers. The New Education Policy (NEP 2020) presents challenges for teachers, notably in increased workload due to curriculum revisions and unfamiliar teaching methodologies. Inadequate infrastructure and resource constraints impede effective implementation, particularly in marginalized communities. Critics argue that NEP's reforms may exacerbate existing inequities. Addressing these concerns necessitates comprehensive support systems, tailored training, and equitable resource distribution to ensure NEP's objectives are met while minimizing inconvenience to teachers (Smith *et.al* 2023).

This study revealed that the merits of the new education policy have been a flexible curriculum, valid student assessment, scope for better-trained students, satisfactory student progress, practical-oriented teaching and academic freedom to teachers, while the demerits have been, pressurizing teachers, lack of uniformity in teaching and evaluation, poor comprehension, inconvenience to teachers and lowering of academic standards.

CONCLUSION

There is no unanimous opinion in the country on whether the new education policy 2020 introduced in the universities based on foreign patterns is better than the old system. Dr. Sujatha, Ramesh, and Dr. K. Natarajan (2019) compared the NEP (2019) with the American Education system. The NEP permitted to swap over the course like that USA. In students' opinion, the merits of the new system of education are Flexible curriculum, practical-oriented training,and valid student assessment. The students did not agree that the new system resulted in better-trained students; provided academic freedom to teachers; and resulted in desirable student behavior. Majority of students were undecided as to whether the new system of education provided scope for better teaching methods.

The demerits of the new system of education as the students felt that unhealthy competition among teachers in grading and the students did not agree that the new system resulted in 1) misuse of academic freedom, 2) lowering of academic standards, 3) poor comprehension of the subjects, 4) inconvenience to students, 5) lack of uniformity in teaching and evaluation and 6) inconvenience to teachers.

Thus, in students' opinion, the new system of education has more merits than demerits. Some appreciate its flexibility and emphasis on holistic learning, while others express concerns about implementation challenges and preserving traditional education methods. A comprehensive and progressive framework, the National Education Policy 2020 tackles numerous issues facing the Indian education system today. The 21st-century learner's needs are well-aligned with its emphasis on flexibility, skill development, early childhood education, and technology integration.

IMPLICATION OF THE STUDY

A Student's Opinion sheds light on students' perspectives regarding the NEP 2020. Understanding students' views is crucial for crafting policies that resonate with their needs and aspirations. This study underscores the importance of including students in educational policy discussions, acknowledging them as key stakeholders. Their insights can inform policy decisions and help create a more inclusive and student-centric education system. Policymakers should heed these voices to ensure that the NEP aligns with the expectations and aspirations of the future generation.

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Toxicity Study on *Eucalyptus grandis* Bark as Natural Food Colourant in Wistar Albino Rats

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ABSTRACT

The toxicity of the powdered substance of Eucalyptus bark (Eucalyptus grandis) was investigated. The level of feed to be given was optimized in the rats and later in the experimental period of 56 days, same procedure was carried out, where according to the OECD guidelines, oral feed ofaqueous solution with 2ml/100g body weight was fed. The histopathology of the rats showed no alternative changes in liver, kidney, intestine, stomach, brain and spleen proving that E. grandis can be used as natural food colourant. The hematology and biochemical parameters were checked for both the control group and the experimental group. The average values were highly similar to each other proving that there were no biochemical changes that had taken place in the experimental rats. Thus, this study proves that eucalyptus barks can be used as natural food colourants, as an alternative for harmful synthetic food colourants.

Keywords : Eucalyptus grandis, Wistar albino rats, optimization of dosage, fixed dosage, Subchronic toxicity

INTRODUCTION

Traditional medicinal practices are defined to have their origin from plants and plant extracts, precisely known as herbalism. Since 6000-4000 BC, Indians have practiced traditional medicinal therapy that has vital retention in curing various diseases. Currently, natural products especially traditional medicines have gained their significance through their unique therapeutic attributes with phytochemicals, their efficacy and safety.In such case, eucalyptus is considered the best traditional remedy for ailments like arthritis, ulcer, diabetes and bladder diseases. Considering the therapeutic values of *E. grandis,* few researchers and food analysts have recommended *E. grandis* as a substitute for synthetic food colourants (*Abuajah et al., 2015*).

Color additives also known as synthetic coloursare used in a wide variety of foods such as beverages, dairy products, cereals, bakery goods, snack foods and ice creams. Although there are strict guidelines for chemicals to be approved as food additives, the safety of food colorants has not been rigorously proven and acceptable daily intake (ADI) has been used to minimize any possible unfavorable effect of the dyes (*Sarah Kobylewski et al, 2012*).

With proper evidences the artificial food colors (AFCs) have proved to affect

children's behavior, through various studies carried out for around 35 years. Food and Drug Administration Food Advisory Committee convened to evaluate the current status of evidence regarding attention-deficit/hyperactivity disorder (ADHD) caused due to artificial food colours. AFCs appear to be more of a public health problem than an ADHD problem, where it seems to affect children and have an aggregated additive or synergistic effect on most children who suffer from behavioral decrement (*Eugene et al., 2012*).

Naseer (*et al., 2019*) has expressed that "in the investigations carried out to establish the safety and nutritional value of natural dye produced from the bark of *Eucalyptus globules*. The natural brown dye obtained from the *Eucalyptus* bark was applied in the production to access stability of dye in the candies, as *E. grandis bark* poses health benefits. Thus this study is carried out to prove that *E. grandis* can be used in place of harmful synthetic food colours.

MATERIALS AND METHODS

Plant material collection

The bark of *E. grandisbark*were collected from Forest College and Research Institute College in Coimbatore, Tamil Nadu, India. The freshly collected barks were cleared from dust and other particles. The barks were dried in shade, powdered using sterilized mixture jar and stored in zip lock bags. The mixture jar and the zip lock bags were made sure that no moisture content was found in it.

Animals used for Toxicity Testing

Wister Albino rats of 230-250g of male were used for the study. Five rats per group was allocated. Animals were purchased from the Animal House located in Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore*, Tamil Nadu, India*. The University animal ethical committee has approved of animal ethical clearance, providing the clearance number as AIW: IAEC.2023:03 for the study.

The animals were housed under standard environment condition of 25±2°C with sufficient light and dark cycles. The rats were fed with standard commercial feed and normal water. The animals were caged in individual boxes. To help them get accustomed to the separation, the rats were monitored for a week for some behavioural change. After a week the rats settled down and the experimentation was progressed.

Toxicity Testing

According to Organization for Economic Cooperation and Development Guidelines (*OECD, 2018*), the volume of feed should not exceed 1 ml/100g body weight, except in case of aqueous solution 2ml/100g body weight can be fed. The extracts fed to the rats through oral feed in drinking water in parts per million/million per liter of solution in kilogram. In 100 ml of water, the dosage is prepared using the following formula:

$$V_1 N_1 = V_2 N_2$$

$$V_1 = \frac{V_2 N_2}{N_1}$$

Formula used for preparing the dosage, where:

- V_1 indicates the ml (milliliter) of water to be calculated and given,
- N_1 measures the number ml present in 1 (liter) of water,
- V_2 represents the weight of the animal and
- N_2 indicates the number of ppm (parts per million) to be calculated.

The parts per million given for 100ml of water to the rats apart from the normal feeding is in case of 2 ppm:

2 ppm = 100 × 2 ÷ 1000 = 0.2 ml

Likewise, ppm level is increased for every specific dosage fed for 5 days continuously, if there are no changes found in the animals then the dosage is increased gradually with two days wash-out period.

Table 1 : Level of Water Given with Natural Food Colourants

Powdered Forms	Name of the Rat	Calculation of Level of Water to be given	Determined Level of Water (in ml)
Eucalyptus barks (*Eucalyptus grandis)*	Test rat – 1 (TR1)	376 × 2 ÷ 100	7.5

According to the *table 1,* the determined level of water in which the natural food colourant are to be optimized are 7.5 ml for the test groups of rats with powdered forms of *E. grandis.*

Pilot Study

A pilot study was carried out with two Wistar Albino rats, where the consumption level of the colourant was finalized by monitoring the animals for eight weeks with two days of wash out period. Around 100 g of weight gain was observed in both the rats. But the sensitivity of thresh hold level of wistar albino rats for the eucalyptus bark powder was higher than expected. After determining the maximum threshold level, the experimentation was carried out with the other rats.

Table 2 : Profile of the Rat used for Pilot Study

Profile of the Rat	Eucalyptus Bark (*Eucalyptus grandis*)	
Type of Group	Test Group	
Name	Eu 1	
Age (months)	8-12 months	
Sex	Male	
	During Purchase	Eighth Week
Weight (g)	344 g	452 g
Water Consumption (in ml)	20-25 ml from 120 ml of water	10 ml + 8 ml of Sample
Food Consumption (g)	25 g	25 g
Body Parts		
	During Purchase	Eighth Week
Fur/Skin	Normal White	Normal White
Eyes	Normal Red	Normal Red
Ears	Normal Pink	Normal Pink
Nose and Mouth	Normal Pink	Normal Pink

Profile of the Rat		Eucalyptus Bark (*Eucalyptus grandis*)
Limbs and Nails	Normal	Normal
Tail	Normal Pink	Normal Pink
	During Purchase	Eighth Week
Active	9	9
Sluggish	X	X
Aggressive	X	X
Irritated	X	X
Sleepy	X	X

The test groups for the powdered form of *E. grandis* barkswere named as test rat – 1. The Wistar Albino rats' level of water to be given was calculated with the body weight of the rats during the purchase. The calculation for the feeding of rats with natural food colourant is as follows for *E. grandis*barks, where the rat weighed 376 g. The level of natural food colourant that was added to the oral feed was optimized in the pilot study for eight weeks. The optimized level was orally fed continuously for 56 days during the experimentation period.

Table 3 : Optimization of *Eucalyptus grandis*

No of Weeks	Water (ml)	ppm	Calculation of ppm	ppm of Natural Food Colourants added in water (mg)
1	7.5	2	7.5 × 2 ÷ 1000	0.01
	7.5	12	7.5 × 12 ÷ 1000	0.08
2	7.5	14	7.5 × 14 ÷ 1000	0.10
3	7.5	28	7.5 × 28 ÷ 1000	0.20
4	7.5	41	7.5 × 41 ÷ 1000	0.30
5	7.5	54	7.5 × 54 ÷ 1000	0.40
6	7.5	67	7.5 × 67 ÷ 1000	0.50
7	7.5	80	7.5 × 80 ÷ 1000	0.60
8	7.5	94	7.5 × 94 ÷ 1000	0.70

2.3.1 Experimentation

The animals were divided into two groups with five animals in experimental group and two animals in control group. Group - 1 is the control group and Group - 2 is experimental group were the animals are fed with *E. grandis*for 56 days.

Table 4 : Experimentation Plan for Toxicity Testing

Groups	No. of animals	Food and dosage given during experimentation
Control Group	2	Commercial Feed (25 g) + Normal Drinking water
Experimental Group – 1	5	Commercial Feed (25 g) + Normal Drinking water + Specific Dosage of Food Colourants Eucalyptus barks (*Eucalyptus grandis)* (calculated according to the weight of the rat)

From the scheduled feed plan, the animals are weighed every week and checked for finding any physical changes indicating the weight gain or loss. Level of water consumption and intake of feed will also be checked every week, to verify if the animals are under certain abrupt meal pattern due to the intake of natural food colourants.

After 56 days, the animals were euthanasia was carried out for histopathology study in all the animals, to identify the effects of the natural food colourant in the experimental group. The animals were harvested and organs (the heart, liver, kidney, intestine, stomach and spleen) were collected in 1:10 formalin solution.

The blood was used for hematology screening such as - hemoglobin, red blood cells and white blood cells. Other part of blood was centrifuged at 3000 rpm for five minutes and serum was separated to estimate the biochemical parameters like - SGOT, SGPT, Glucose, Total Protein, Albumin, Creatinine, Sodium, Potassium and Chloride.

In the estimation of biochemical parameters - SGOT and SGPT also known as Serum glutamic-pyruvic transaminase and Serum glutamic-oxaloacetic transaminase are the markers of liver functions. The analysis for albumin, creatinine, sodium, potassium and chloride are carried out to analyze if the functioning of kidney is normal. If there is an elevation in the values of biochemical parameters, it indicates that there has been malfunctioning of kidney in the tested organism. A decrease in total protein and albumin is a sign of the reduced synthetic functioning of the liver or due to impaired hepatocellular function.

From the *table 2,* it is interpreted that *E. grandis*of 6 mg as natural food colourantwas mixed in 7.5 ml of water was given to the rats orally from week one to week eight.

RESULTS AND DISCUSSION

Clinical Signs

The rats fed with *E. grandis*did not show any symptoms of presence of toxicity andno clinical changes were found in the rats throughout the experimental period. The physical observation in their skin, fur, eyes, mucus, eating pattern, behavioural change and sleep were all normal.

Table 5 : Mean weight gain in the rats

Groups	Mean weight on Day zero (g)	Mean weight on Day 56 (g)	Difference in weight (g)
Control	201	292	92
Experimental Group 1	272	354	82

Table 5 represents the mean weight gain that has taken place in the animals. All the animals in both the control group and the experimentation group have gained weight.

The difference in the weight gain of the control group after the eighth week is 92 g, whereas the difference of weight gain in the group that consumed *E. grandis*is 82 g.

The increase in weight is due to increase in intake of water and food. Likewise, loss of appetite will be due to some metabolic change in intake of either carbohydrates, protein or fat. The change in metabolism may lead to loss of appetite and loss of appetite will lead to loss of weight gradually. But, in this experimentation there is no loss of weight observed in the rats.

Hematology and Biochemical Analysis

Table 6 represents the hematology and biochemical parameter of the rats to find if there has been any alteration in the renal functioning that was influenced by the powder mixed in water. Liver and kidney analysis is very important as the toxicity evaluation of the plant substance will be noted through the tissues, which is responsible for the survival of the organism.

The hematology value of hemoglobin is 15.9 g/dl, RBCs and WBCs are 8.35 ten million per cubic meter and 4.2 thousand per cubic meter. Whereas the SGOT and SGPT obtained are 27U/L and 25U/L respectively. The other parameters like glucose and cholesterol are 45 g/dL and 40 g/dL, which showed the same level of absorption of glucose and cholesterol in blood.

Table 6 : Mean hematology and biochemical parameter values

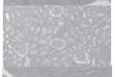
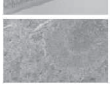
Biochemical Parameters	Estimated Values	
	Control Group	Experimental Group
Hemoglobin (g/dl)	14.53	15.9
Red blood cells (10^6/mm^3)	7.56	8.35
Total White blood cells (10^3/mm^3)	4.93	4.2
SGOT (U/L)	27.17	27
SGPT (U/L)	20.18	25
ALP (U/L)	72.45	72.45
Glucose (g/dL)	46	45
Cholesterol (g/dL)	42	40
Total Protein (g/dL)	6.5	6.5
Albumin (g/dL)	4.2	3.5
Creatinine (mg/dL)	0.9	0.65
Sodium (mg/dL)	137.3	107.9
Potassium (mg/dL)	4.1	6.5
Chloride (mg/dL)	88	89

Serum concentration of total protein 6.5 g/dL and albumin 3.5 g/dL in the treated and control group confirms that the substance has not damaged the hepatocellular or secretory functions of the liver. Renal dysfunction is assessed through the concurrent values of creatinine valuing 0.65 mg/dL and their normal value reflects the normal renal functioning in the rats. The proper functioning of kidney is again proved through the standard values of sodium 107.9 mg/dL, potassium 6.5 mg/dL and chloride 89 mg/dL.The values have proved that the *E. grandis* substance did not affect the hepatocytic functions of the rats.

Histopathological Study of the Organs

The microscopic examination of the organs of the rats treated with *E. grandis*powder did not show any change in colour compared with the control group rats' organs. Hypertrophy of organ indicates the toxicity of chemical and biological substance. In addition, the microscopic examination revealed that none of the organs from the treated rats have shown any alteration in cell structure or any unfavourable effects under the light microscope using multiple magnification powers

Table 7 : Microscopic view of the harvested organs from the rats

Organs	Control Group	Experimental Group
Brain		
Liver		
Kidney		
Heart		
Stomach		
Intestine		
Spleen		

No pathologies were recorded in the histological sections of the vital organs (brain, liver, kidney, heart, stomach, intestine and spleen) of the control group. Equally, there was no change in the creatinine after administration of *E. grandis*powder when compared to the control group. Any alteration found in the level of creatinine show that there has been damage in the functioning of nephrons. The observations in the histopathology study of the kidney tissue have been recorded no changes. Thus, this study proves that *E. grandis*is non toxic in nature and can be used as natural food colourant.

CONCLUSION

The results demonstrate that natural form of powders substance of *E. grandis* barks is practically proven to be non toxic in nature when administered orally and is safe for consumption. So, the study has proved that in place of synthetic food colourants, eucalyptus barks can be powdered and used as it is in foods making the food therapeutically valuable.

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Sustainable Fuel Alternatives: The Impact of Algae-Ethanol Blends on Engine Dynamics and Emissions.

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ABSTRACT

This vast assessment investigates the escalate field concerning algae-ethane gasoline brews for squeezed ignition (CI) engines, providing a thorough examination of the research conduct in this sector. Include ethane into algae-based concoctions enhances their performance, combustion traits, and emissions profiles. The cumulative findings underscore the significant influencer of algae-ethane gases on both engine performance and emissions reduction. Mixing algae-set biofuels with ethane and diesel consistently yields positive outcomes across various engine types. Remarkably, the B20 blend—consisting of 20% ethane and 80% diesel—demonstrates improved brake thermal efficiency (BTE) and reduced emissions of nitrogen oxides (NOx), carbon Monoxide (CO), hydrocarbons (HC), and particular matter (PM). Numerous optimization techniques, including response surface methodology (RSM), Taguchi mechanism, and artificial neural networks (ANN), have proven efficient in finely tuning engine parameters to optimal efficiency and minimize emissions. Additives such as glycerol ethoxylate (GE), butylated hydroxytoluene (BHT), and n-pentanol have also been researched to enhance ignition and combustion characteristics. Life cycle analysis studies have highlighted the environmental advantages of algae-ethane gases, demonstrating reduced environmental impact compare to petroleum-based gases. Biodiesel deriving from algae exhibits the potential to improve BTE by up to 5%, alongside significant reductions in CO, HC, and NOx emissions. Additionally, the inclusion of nanoparticles such as CeO2 and Al2O3 shows promise in enhancing engine performance and reducing emissions when incorporating in algae-ethane concoctions.

Keywords : Algae biofuel, ethanol, optimization, brake thermal efficiency (BTE), emissions reduction.

INTRODUCTION

The pursuit of ecological sustainable and environmentally irresponsible energy resources has roused heightened excitement for biofuels while originating from renewable resources. In this spectrum, microalgaes have commended considerable interest due to the elevated lipid concentrations, fast growth kinetics, and ability to alleviate carbon emissions through sequestration of carbons dioxide (CO2)[1]–[4].

This comprehensive studies includes advancements and investigations within the domain of biofuels derived out of microalgaes, embracing inspections of their combustion behaviour, ecological

implications, and prospective trajectories. The use of microalgaes is a source of biofuels have been a subject of extensive investigations over the past few years. Researchers explored various aspect of algae-based biofuels, ranging from the assessment of combustion characteristics in internal combustion engines to evaluate of the potential in to reducing greenhouse gas emissions. This review integrates, findings from a range of studies to provide a Hulk perspective on the current state of knowledge in the field of algae biofuel and ethanol. In line with that, NITI Aayog and Ministry of Petroleum & Natural Gas have published report in June 2021 showcasing the roadmap for making 25% ethanol blending by 2025 and much emphasis on development of flex-fuel technology [5].

The combust performance of algae-based biofuels in various engine types have been key foci of research efforts. Several study has examined the use of algae oil and algaes biodiesel as fuel alternatives into internal combust engines and concluded that algal oil and algae biodiesel has slightly higher brake thermal efficiency and lower NOx emission comparatively.[1], [6], [7].

Saraswat and Chauhan (2020) are determine that a energy densities of algae biofuel was approximately 5–8% lower when compares to petrol and diesel. The findings indicated that, through all blending rations, the brake-specific fuel consumption (BSFC) of butanol and algae oil blend is higher than the pure petrol under all operating condition. This primarily attributed to the lower calorific values of butanol and the algae oil when compared to petrol. Data also reveals similar properties between the 5%, 10%, and 15% butanol-algae blends and petrol. Found that algae biodiesels exhibits promising combustion stability and emission performance in the CI engines, with significantly reduction in particulate matter emission [6]. Furthermore, the impact age of blending algae-based biofuels with a other conventional fuel, like ethanol and diesels, has been explored wildly. Studies have being investigated the effects of fuel blends on engine performances and emission. They determined the BTE increased using blends upto 5% and BSFC was decreased uto 10% [8]–[10]. Rao et al. (2021) demonstrated that the addition of ethanol to Niger-Diesel blend at varying injection pressure resulted in improving engine performance and reduced emission of nitrogen oxidizes. Their resulting indicated that up to the 200 bar injection pressure, engine produced high brake power more and beyond it performance deteriorates [9]. High ethanol levels resulted in intensified combustion and reduces combustion stability, evident from cyclic variations. Especially, when ethanol content reaches 30%, there was substantial increments in the coefficient of variation of peak pressure (COVpmax). While increasing ethanol content, soot emissions exhibited a notable reductionness ranging from 11.28% to 47.23%. However, this reduce was accompanied by an increase in NOx emission ranging 2.68% to 7.04%, as well as HC emissions up position rising by 9.99% to 21.47%. The studying recommended that ethanol blending ratios should not surpass 30% [11]. This evaluation also covers the effects of additives/nano-particles on performance of CI engines. Firew et al. (2022) revealed the introduction of NiZnFe2O4 nanoparticles into the ethanol-diesel emulsion decreased BSFC by 27.86% while simultaneous enhancing brake powers by 7.74% and brake torques by 3.53%. [12].

On other hand Szwaja et al. (2022) scrutinizing the effects of ethanol-glycerol blends combustion on performance and the exhaust emission of spark-ignited engine revealing that ethanol-glycerol blend at ratios of 3:1 (75/25%) exhibits as promising alternate fuel for SI engines.[13]

In addition to performance considerations, these review addresses the environment impact of algae biofuels. Algae cultivations presents an opportunity to utilizes high-purity carbons dioxide sources, contributing carbon captures and reduce net greenhouse gas emissions.in the comprehensive review of Current Strategy's and Prospects on Algae for Remediation and Biofuels by Kandasamy et al. (2021) discussing the advantages of algae cultivations like waste water treatments, CO_2 absorption et cetera. They revealed the demeritary like fuel production costing can be reduce by development more efficient technology. [14] Ou *et al*. (2021) outlining the opportunities and challenge of using high-purity CO_2 for algae cultivating and biofueling production in United State [15]. Microalgae holds the capacity to generate ranges of biofuels, such as the biodiesel, bioethanol, and biomethane. Additionally, microalgae can be served as to means to captures carbon dioxide (CO_2) from the atmosphere. The cultivating of microalgae holds promise satisfying approximately 20% of the world's demands for transportation fuel. Moreover, it has potentials sequestering as much as 10% of CO2 emissions worldwide [16].

So, like, the use about low-lipid waste algae for the energy production offering kind of a sustainable way to waste management. Kowthaman and Selvan (2020) did an - kinetic study on the using low-lipid waste algae for the energy development, where they highlighted much it's potentially as a green fuel source. Low-lipids waste algae maybe can serve just as a raw material to produce biofuels using that pyrolysis thing. The activation energy needed for the pyrolysis of this waste algae is usually low, and somewhere in the range from 8.7 to 20.9 kJ/mol, meaning the process can be started easily. To Paralyze those low-lipid waste algae might be done at rather modest temperatures, typically staying in the range of like 300 to 500°C creating it more of an energy-efficient way then other kinds of biofuel production. [3] Evaluations and life cycle assessments plus cost analysations have been conducting to assess the environmentally and economic viability for those algae-based biofuels. That algal biofuels examined in there research had a varying price span from around $3.39 to about $5.93 per a gasoline gallon equivalent, depending on whichever conversion route was chose. And in terms of greenhouse gases (GHG), the algal biofuels showed a significant advantage over regular gasoline with emissions might ranging from 0.0185 to 0.0383 kg CO_2-eq per every MJ of fuel [2][17]. Bui et all (2022) did a life-cycle cost analysis of innovative marine dual-fuel engines under, uncertainties, providing insights about the economic aspects for adopting those alternative fuels they concluded that a dual fuel engine may be much cost effective [17].

This paper will explore future prospects & challenges with the widespread adoption of algae-based biofuels. It potentially provides insights around the strategies to optimise microalgae cultivation as well as the developed advanced conversion technologies. Plus, it's also discusses the role from algae-based biofuels to address

climate change and enhancing energy security. By looking at the combustion's performance, environmental impact, and futures prospects of the biofuels, this review aiming to provide a comprehensive resource to researchers, policymakers plus stake holders who is interested in this field of renewable energy.

METHODOLOGY

This study is motivated by examining of the newest advancements and possible obstacles regarding the using of algae-ethanol blend biofuels on compression ignition (C-I) engines. In order to understand comprehensively the present, advancements and pending hurdles, there was a deep analysis kind of into contemporary literatures which was done as a main source of info. The Preferred Reporting Items for Systematic Reviews & Meta-Analyses, a well-known and acknowledge protocol for performing systematic reviews of literature were employed this investigation.

This methodology uses meticulously structured approaches that's selected to trying to reduce biases, therefore ensuring acquiring precise information, culminating in formulating conclusions that are dependable credible [18].

Approach to Conducting Literature Search

Investigations now been conducting using a Science Direct data bank, Plus, some other resources were added. Especially, Those were databases having good respect for their credibility, even the completeness, also they cover more than thousands of 5200 publication in the energy areas, in addition to more than 450 texts early related to renewable energies sources considering environmental sustainability. A search string were Device, it leverage our expertise in relevant field, The particular way of search string is explaining in the provided table 1.1.

Table 1.1 : search methodology and results

Search steps	Search string	Boolean operator	Search Result
Initial Search	Algae fuel, Ethanol blend	AND, OR	42249
Year of publication considered (>2014)	Algae fuel, Ethanol blend	AND, OR	20192
Only journal publication	Algae fuel, Ethanol blend	AND, OR	2054
Impact factor (> 1.5)	Algae fuel, Ethanol blend	AND, OR	679
Accessibility (open access)	Algae fuel, Ethanol blend	AND, OR	63

Research review process involves systematic refining to gather relevant articles in algae-derived biofuels blend with ethanol for compression ignite engines. The Initial phase encompasses a broad exploration using search string "Algae fuel, Ethanol blend" Employing Boolean operators "AN" and "OR," we retrieve an extensive result of 42,249 records. Subsequently search is narrow by focusing for the desired timeframe (2014-2024,) maintaining the same search string and Boolean operators. Time frame considered chosen to studies research conducting about algae fuel and ethanol blend comprehensiveness. This step yielded 20,192 results. To enhanced scholarly

creditability, we limit the search to journal publication, persisting in search string "Algae fuel, Ethanol blend and same boolean operator. This process reduce outcomes to 2,054 articles. Further refining our selecting, we consider articles shows impact factors greater than 2, maintaining a consistent search string and Boolean operator. This filtrate lead to subset of 679 relevant articles.

Ensuring our restrictions of accessibilities we concentrate solely on open access publications using same search strings and Boolean operators ultimately identify 63 suitable articles. A database with articles which containing titles abstracts and full texts is create in Mendeley reference management software, This meticulous and progressively focusing approach Results in refined collection ensuring scholarly value, recency relevance, and availability for research work on algae-ethanol blend biofuels for compression ignite engines for the best of better understanding the research.

Inclusion/exclusion criteria

The critical studies focus on investigating recent progresses and upcoming challenge of the utilization of algae-ethanol blend biofuels for CI (Compression Ignition) engines. The study be employing a wide search strategy for identifying articles that meets specific include criteria. The search process was meticulous designed for ensuring the selection of relevant articles and high-quality. Inclusion criteria for articles selections encompassed key aspect. Firstly, articles needed directly addresses the topic algae-derived biofuel blend with ethanol for I.C. engines, ensuring research's alignments with central themes of study. Secondarily, the publication years limited to the range 2014-2024 enabling the incorporation recent advancement in the field. This choice ensure that review capture most up-to-date insight and finding.

We ensure credibility of the choosing articles only peer-reviewed journal publication are considered..! Moreover, Open Access articles was prioritized to enhance accessibility promoting availability of research findings to wider audience. The scope of review encompassed various research aspect, Including performance evaluations, combustion characteristic, emission analysis, feasibility assessment, and related topic concerning applications Algae-ethanol blends in CI engines. This ensure comprehensive coverages of subjects matters, incorporating experimental investigation, theoretical analyses, AND reviews to provide a holistic understanding of topics.

Key emphasis of the include criteria was the direct relevance to CI Engines and Effect of Fuel performance on engines. This REQUIRED that selected articles specifically addressed utilizations algae-ethanol blends for biofuel in CI engines.

Exclusion Criteria:

1. Irrelevant Topics: Articles what do not exactly address an utilization of algae-ethanol mixtures as biofuel for Compression Ignition engines were excluded from the considerations.
2. Publication Year: Articles, the published before 2014 or after 2024, were all excluded to focused recent developments! Inside the specified timeframes. Only one article was chosen to ensure a method study from the year was 2003,
3. Publication Type, Non-peer-reviewed sources involving conference shows, and workshop recitations were excluded to uphold standard credible rigorous researches,

4. Inaccessible Content : articles, that was not open access Or not readily available for through review.. Were excluded due to some limitations, in accessibility.
5. Lack of Relevance : Articles that not provide lots on Insights into performance combustion characteristics emissions analysis feasibility assessment or others pertinent aspects of algae-ethanol blends in CI engine were excluded from review.
6. Poor Methodology: Articles having lack of sound research methodologies experimental designs or grounded data analysis which potentially compromise findings reliability were excluded to ensure overall quality of selected literature.

By applying this criteria of exclusions, the Review process aim to ensure the careful selections of article shown in Figure 2.1 aligned the Study's scope, maintain objectivity academic, and contribute meaningfully to synthesis research outcomes in field of algae-ethanol blend biofuels for compression ignition engines.

Data analysis

The examination of the literature involved amalgamating the content and identifying themes through the utilization of a spreadsheet. These methods were chosen to minimize researcher intervention in the face of commercialization trends and to effectively manage large datasets. This approach facilitated the analysis of textual data from both qualitative and quantitative perspectives. It led to the identification of numerous biofuel innovations, the establishment of underlying factors, and the pinpointing of research gaps. This methodology was favoured for its ability to deliver thorough and comprehensive answers to the research inquiries.

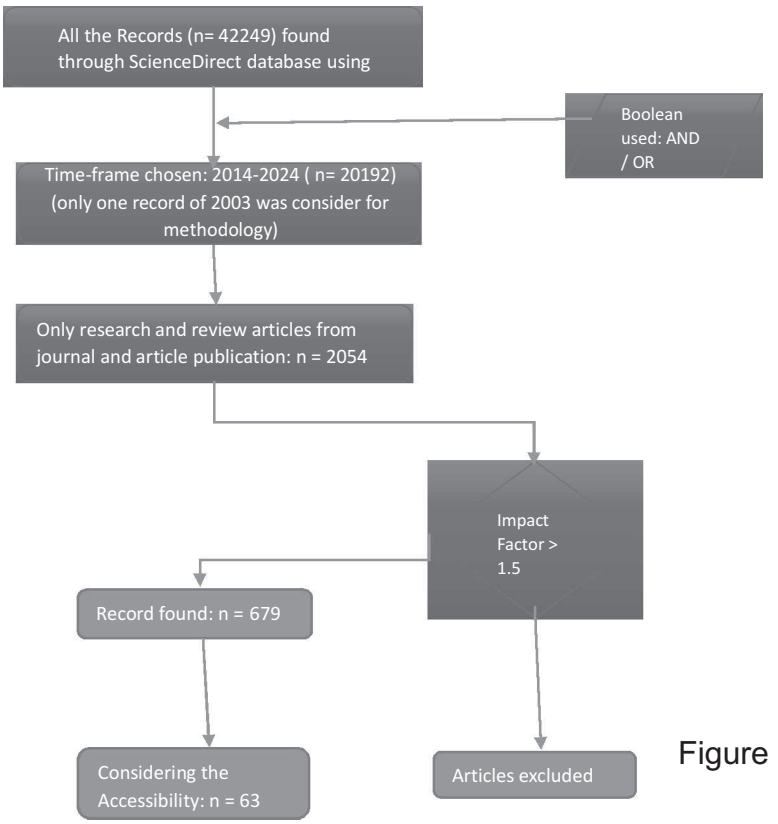


Figure 2.1 : Process chart for selection

RESULTS

Algae-Based Biodiesel Performance and Emissions in CI Engines

Algae-based biodiesel offers a promising alternative fuel derived from renewable resources. It shares many characteristics with traditional petroleum diesel and can be used in existing diesel engines without modifications. This type of biodiesel can reduce emissions of harmful pollutants like carbon monoxide, hydrocarbons, and particulate matter, although it may increase nitrogen oxide emissions. The emissions and performance of algae-based biodiesel depend on several factors, including the specific algae species, production methods, and the blend ratio with petroleum diesel.

Adeniyi et al. (2018) discuss the current state and future prospects of algae biofuel, highlighting several key challenges that need to be addressed to make algae-derived biodiesel economically viable. These challenges include reducing production costs, increasing algae oil yields, creating efficient and scalable production processes, and developing cultivation methods that do not compete with food production, as shown in figure 3.1. Despite these hurdles, the authors argue that algae-based biodiesel can significantly contribute to reducing greenhouse gas emissions and improving air quality. The paper provides evidence that algae-based biodiesel can lower emissions of pollutants such as carbon monoxide, hydrocarbons, and particulate matter. Additionally, the ability of algae to grow in various environments, including wastewater, emphasizes its sustainability as a biodiesel source. The authors conclude that algae-based biodiesel is a promising, sustainable, and environmentally friendly alternative to petroleum diesel [19].

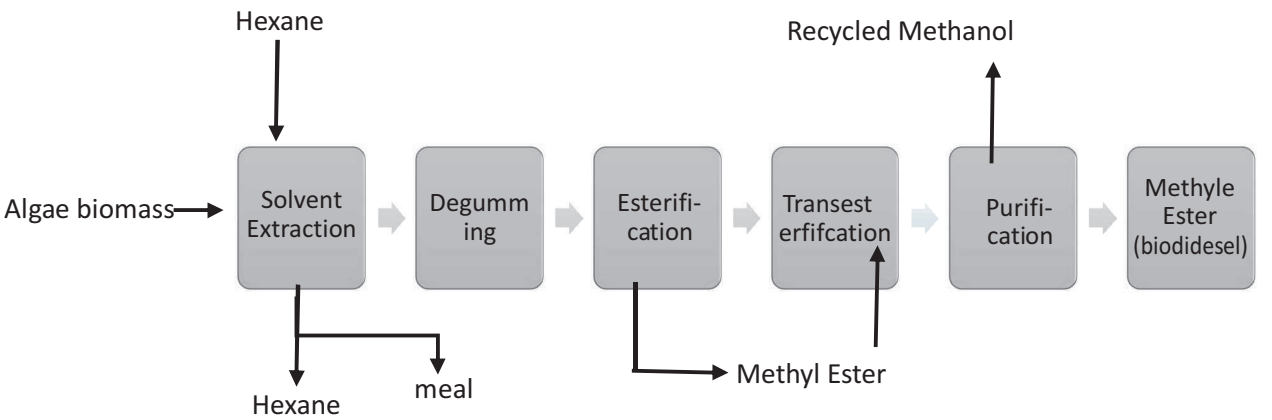


Figure 3.1 : Conventional transesterification process [19]

Karthikeyan et al. (2020a, 2020b) examined the emission and performance of a CI engine using S. Marginatum macroalgae biofuel blends. These blends were compared to ASTM D6751 standards, and engine tests were conducted at a constant 1500 rpm under various load conditions. The B20 blend showed enhanced combustion efficiency, especially at high and partial loads, and reduced exhaust emissions (excluding NOx) by up to 20%, particularly at high loads. However, the 20% SMME blend increased NOx emissions despite improving thermal efficiency [20,21] .

Kumar et al. (2021) utilized an adaptive neuro-fuzzy inference system (ANFIS) and response surface methodology (RSM) to produce biodiesel from jatropha–algae oil and examine its performance and emissions in a diesel engine-generator setup. The study employed transesterification for biodiesel production and used RSM and ANFIS to predict yield. Results showed that while diesel had the lowest brake specific fuel consumption (BSFC), the B20 blend had similar results with good brake thermal efficiency (BTE). Jatropha-algae biodiesel blends reduced hydrocarbon (HC) and carbon monoxide (CO) emissions, though they slightly increased nitrogen oxide (NOx) emissions compared to pure diesel. Overall, the blends demonstrated favorable performance and emission characteristics [22].

Saputra Nursal et al. (2021) performed a comparative study on the autoignition characteristics and emissions of biodiesel derived from palm oil, waste cooking oil, tyre pyrolysis oil, algae, and jatropha. The study concentrated on algae-derived biodiesel from Botryococcus braunii (B. braunii), a microalgae species prominent for producing up to 65% of its dry weight as hydrocarbons, particularly triterpenes. B. braunii's high content of hydrocarbons, ether lipids, saturated and mono-unsaturated fatty acids, and triacylglycerols makes it ideal for biodiesel production. The ignition delay for algae biofuel at 2 vol% concentration was recorded at 1.22 ms, which is longer than other sources. Higher injection pressures and ambient temperatures reduced ignition delays, affecting combustion quality by increasing fuel molecular size and weakening C-H bonds. The study concluded that algae biodiesel had the lowest autoignition temperature and emitted the least carbon monoxide and hydrocarbons among the biodiesel sources studied [23].

Rui Shi et al. (2018) conducted a life cycle assessment (LCA) of four pioneering algae harvesting methods and two new algae oil extraction techniques to evaluate their environmental impacts from algae cultivation to renewable diesel production. The LCA revealed that these technologies could significantly reduce greenhouse gas emissions compared to conventional methods, with acoustic harvesting and wet extraction showing the most promise. Acoustic harvesting uses sound waves to induce algae cell flocculation, easing water separation, while wet extraction uses a solvent to extract oil from algae biomass. These innovations have substantial sustainability potential, but further research is needed to optimize and reduce their environmental impact. The LCA considered factors such as greenhouse gas emissions, water and land use, energy consumption, and air and waterborne emissions. Acoustic harvesting could reduce greenhouse gas emissions by up to 40%, and wet extraction by up to 25%, compared to conventional methods as shown in figure 3.2 [24].

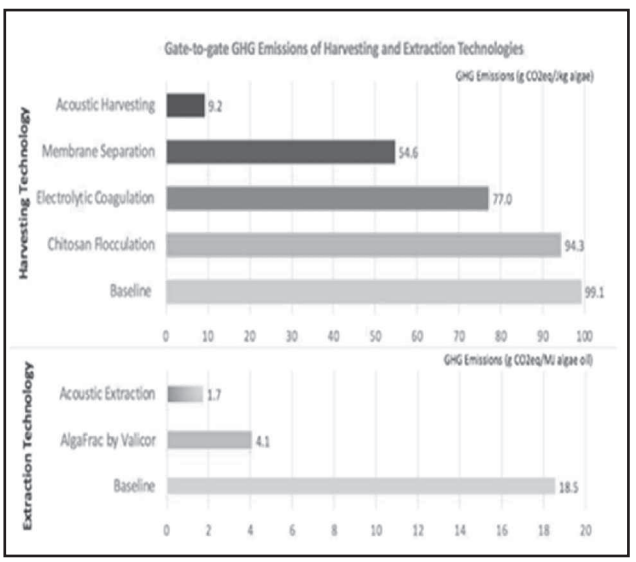


Figure 3.2 Comparison of harvesting and extraction technologies [19]

S. Pavuluri et al. (2020) examined the effects of ethanol-biodiesel blends on the performance, combustion, and exhaust characteristics of a compression ignition (CI) diesel engine at various compression ratios. Although this study was not directly focused on algae biofuel, algae could serve as a feedstock for such blends. The blends used were E10 (10% ethanol and 90% biodiesel), E20, E30, and E40 (40% ethanol and 60% biodiesel). Results revealed that ethanol-biodiesel blends enhanced the engine's brake thermal efficiency (BTE), especially at higher compression ratios, with a 2.5% increase in BTE for E10 at a 17.5:1 compression ratio and a 4.5% increase for E40 at a 19.5:1 compression ratio. These blends also reduced emissions of hydrocarbons (HC), carbon monoxide (CO), and particulate matter (PM), although nitrogen oxides (NOx) emissions increased. The study suggests that ethanol-biodiesel blends are capable for enhancing CI diesel engine performance while reducing emissions [25]. M. Ranjithkumar et al. evaluated the performance and emissions of a single-cylinder, four-stroke direct injection diesel engine using blends of Calophyllum Inophyllum methyl ester (CIME), microalgae methyl ester (MAME), and diethyl ether (DEE) as alternative fuels. The fuel mixtures included 20% CIME, 40% MAME, and 60% DEE by volume. The B40 blend (40% CIME, 40% MAME, and 20% DEE) exhibited the best performance, with the highest brake thermal efficiency (30.89%), lowest specific fuel consumption (222.06 g/kWh), and minimal smoke opacity (2.16%). This blend also reduced CO emissions to 0.26% and NOx emissions to 46.26 ppm compared to conventional diesel. Adding DEE improved the volatility and compatibility of the blends with diesel, enhancing atomization and combustion. The B40 blend also had a shorter ignition delay and longer combustion duration than diesel, leading to lower emissions of HC and CO [26]. Saravanan et al. (2020) studied the effects of antioxidants, specifically butylated hydroxytoluene (BHT) and tert-butyl hydroquinone (TBHQ), on the performance and emissions of a CI engine running on algae-derived biodiesel blends. These blends consisted of 20% algae biodiesel and 80% conventional diesel. The results revealed that antioxidants improved the oxidative stability of the blends and reduced NOx and smoke emissions. Optimal NOx reduction was attained with 200 ppm of BHT and 100 ppm of TBHQ. The addition of antioxidants also positively impacted the brake thermal efficiency of the CI engine, highlighting the benefits of using antioxidants in algae biodiesel blends for better combustion efficiency and lower emissions [27].

Medhat Elkelawy et al. (2021) studied the performance and emissions of a single-cylinder, four-stroke DI diesel engine using blends of algae biodiesel, diesel, and n-pentane in varying ratios (20%, 40%, 60%, 80%). The B40 blend (40% algae biodiesel, 40% diesel, 20% n-pentane) revealed the best results, achieving the highest brake thermal efficiency (30.89%), lowest specific fuel consumption (222.06 g/kWh), and minimal smoke opacity (2.16%). This blend also reduced emissions of CO (0.26%) and NOx (46.26 ppm) compared to pure diesel. The study highlighted the renewable nature of algae biodiesel and the combustion-enhancing properties of n-pentane, emphasizing the importance of optimal blend ratios for improved engine performance and emissions [28]. ElFar et al. (2021) reviewed the potential of algae as a bioenergy source and the role of Industry 5.0 innovations in improving algae biofuel production. They discussed using artificial intelligence to

optimize cultivation, robotics for harvesting, and big data for monitoring growth[29]. Wang et al. (2023) highlighted the promise of algae for biofuels and bio-products due to their rapid growth, adaptability, and low ecological impact. Despite challenges such as high production costs and low yields, ongoing research aims to overcome these barriers, with expectations that algae-based biofuels will become more competitive with conventional energy sources[30]. Shivendra et al. (2021) showed that integrating algae biodiesel into CI engines significantly enhances brake thermal efficiency (up to 4.5% with an 80% blend) and reduces emissions of HC, CO, and PM by up to 25%, 40%, and 50%, respectively. However, NOx emissions tend to increase with higher biodiesel content [31].

Suresh Vellaiyan (2024) blended an aqueous ammonia emulsion and a zinc sulfide/copper (ZnS/Cu) nanocomposite with an algae biodiesel-diesel blend to identify optimal parameter levels for enhanced performance and reduced emissions. Algae were cultivated alongside wastewater treatment, and the properties of the biodiesel and the nanocomposite's structural and surface morphology were analyzed. Using response surface methodology for optimization and conducting confirmation tests, the study found that algae cultivation effectively removed wastewater nutrients. The optimal process parameters were determined to be 10% biodiesel, 14% aqueous ammonia, and 95 ppm of ZnS/Cu [63].

In conclusion, the collective findings from these studies on algae-based biofuels emphasize their potential to significantly impact engine performance and emissions. Blending algae-based biofuels with conventional diesel has shown promising results across various engine types, with important improvements in brake thermal efficiency (BTE) and reductions in emissions of nitrogen oxides (NOx), carbon monoxide (CO), hydrocarbons (HC), and particulate matter (PM). Particularly, the B20 blend, incorporating algae-based biodiesel, has consistently demonstrated favourable outcomes, enhancing BTE and reducing NOx emissions in multiple studies as shown in table 1.2. Additionally, the use of additives like butylated hydroxyl-toluene (BHT) and n-pentane has shown promise in improving engine efficiency and reducing emissions.

Table 1.2 : effect of various algae based biofuel on performance and emission of CI engine

Article Citation	Year	Engine Used	Type of Algae /blend Used	Engine Parameters considered	Key Finding
Karthikeyan et al. [20], [21]	2020	A single cylinder, diesel engine	S. Marginatum macro algae	BTE, BSFC, NOx, CO, HC	Blending 20% S. marginatum biodiesel with diesel enhances performance, with the B20 blend achieving a maximum brake thermal efficiency of 33.8% at full load, attributed to improved brake-specific fuel consumption (BSFC) and lower exhaust gas temperature (EGT).

Article Citation	Year	Engine Used	Type of Algae /blend Used	Engine Parameters considered	Key Finding
Kumar et al. [22]	2021	A single cylinder, diesel engine	Jatropha–algae oil	BTE, BSFC, NOx, CO, HC	The use of jatropha–algae oil biofuel in a CI diesel engine delivers best result with B20. BTE increased by 4.2% and NOx emission reduced by 16.7%
Saputra Nursal et al. [23]	2021	A single cylinder, diesel engine	Algae, palm oil, waste cooking oil, TPO	BTE, BSFC, NOx, CO, HC	Algae biodiesel exhibits lower emissions of CO, HC (hydrocarbons), and NOx (nitrogen oxides) compared to palm oil biodiesel, waste cooking oil biodiesel, and tyre pyrolysis oil biodiesel.
Elkelawy et al. [28]	2021	A single cylinder, diesel engine	Algae biodiesel/diesel/n-pentane blends	BTE, BSFC, NOx, CO, HC	The addition of 15 ml of n-pentane/liter was found to be the most effective, resulting in a 7.1% increase in BTE and a 6.4% decrease in BSFC compared to the pure B50 blend.
ElFar et al. [29]	2021	A single cylinder, diesel engine	Microalgae	NA	Microalgae are the most promising type of algae for biofuel production because they can be grown in a variety of conditions and produce high yields of biomass.
Pavuluri et al. [25]	2020	A single cylinder, diesel engine	Ethanol/biodiesel	BTE, BSFC, NOx, CO, HC	The BTE improved upto 4.5% for E40 and emission of HC, CO and PM decreased by 40%, 50%, and 60% respectively by varying the compression ration from 17.5:1 to 19.5:1. Algae can be utilized as feedstock for ethanol production.

Article Citation	Year	Engine Used	Type of Algae /blend Used	Engine Parameters considered	Key Finding
Ranjithkumar et al. [26]	2020	A single cylinder, diesel engine	Calophyllum Inophyllum methyl ester and micro algae methyl ester	BTE, BSFC, NOx, CO, HC	The results indicate that the B40 mixture attained the highest brake thermal efficiency at 30.89%, the lowest specific fuel consumption at 222.06 g/kWh, and exhibited minimal smoke opacity, registering at 2.16%.
Saravanan and Krishnamoorthy [27]	2020	A single cylinder, diesel engine	Algae biodiesel blend	BTE, BSFC, NOx, CO, HC	NOx emission of the B20 was decreased by the addition of butylated hydroxyl-toluene which was further reduced and increased by retarding and advancing the injection timing respectively.
Shivendra et al.[31]	2021	A single cylinder, diesel engine	Algae biodiesel	BTE, BSFC	The research offers valuable perspectives on the viability of algae biodiesel as an eco-friendly substitute for conventional fossil fuels.

Effect of added ethanol on performance and emission of CI engine

Ethanol-diesel blends have potential for improving the performance and emissions of compression ignition (CI) engines. This review explores the impact of adding ethanol to diesel fuel, particularly in biodiesel blends, on CI engine performance, combustion characteristics, and emissions. Munsin et al. (2015) studied glycerol ethoxylate (GE) as an ignition improver in hydrous ethanol (EtOH) under CI conditions. GE reduced the EtOH injection rate by 10%, increased injection delay and duration, reduced ignition delay, and decreased premixed burn heat release. Soot emissions dropped by 15% with 5% GE[32]. Taghizadeh-Alisaraei and Rezaei-Asl (2016) evaluated ethanol addition to diesel in a six-cylinder CI engine. A 6% ethanol blend (D94E6) improved torque and power by 3.8% but increased engine block vibration, causing higher RMS and kurtosis values. Exceeding 8% ethanol prolonged ignition delay, leading to combustion irregularities and knocking [33]. Rossomando et al. (2017) used computational fluid dynamics (CFD) simulations to assess ethanol concentration and ignition improvers on CI engine performance and emissions. A B20 blend (20% ethanol, 80% diesel) improved brake thermal efficiency (BTE) by 2.5% and

reduced NOx and particulate matter (PM) emissions by 15% and 25%, respectively [34]. Nour et al. (2019) investigated ternary blends of pentanol/hydrous ethanol/diesel (Pe10E10D80) and octanol/hydrous ethanol/diesel (Oc10E10D80) in a CI engine. These blends revealed lower peak cylinder pressures and altered heat release rates. The Pe10E10D80 blend increased BTE by 2.5% and reduced NOx, PM, and smoke emissions by 15%, 25%, and 30%, respectively [35]. Kulanthaivel et al. (2021) investigated various ethanol and algae biodiesel blends. The blend with 15% ethanol and 5% algae biodiesel improved fuel efficiency by 10.2% compared to diesel. The blend with 10% ethanol and 10% algae biodiesel had the lowest emissions, reducing NOx by 19.7% and CO by 9.7%. Additionally, the 15% ethanol and 5% algae biodiesel blend enhanced combustion stability by 19.2% and reduced knocking by 10.7% [36].

The "Roadmap for Ethanol Blending in India 2020-25," released by NITI Aayog and the Ministry of Petroleum & Natural Gas, aims to significantly increase ethanol-blended gasoline usage. Key objectives include nearly doubling ethanol production capacity by 2025-26, ensuring nationwide availability of E10 fuel (10% ethanol) by April 2022, and gradually introducing E20 fuel (20% ethanol) from April 2023, aiming for full coverage by 2025. Additionally, vehicles compatible with E10 blends will be available by April 2023, followed by E20-compatible vehicles in 2025. These efforts aim to enhance energy security, boost renewable fuel use, and reduce emissions in India [5].

John et al. (2022) reviewed exhaust emissions from CI engines using ethanol as an alternative fuel. They concluded that ethanol's impact on emissions varies based on ethanol concentration, engine conditions, and fuel injection systems. Lower ethanol concentrations in ethanol-diesel blends reduce CO, HC, and PM emissions but increase NOx emissions. Higher ethanol concentrations lower NOx emissions but increase CO, HC, and PM emissions [37]. Liang et al. (2022) studied ethanol/diesel co-solvent blends in a CRDI heavy diesel engine. Adding 10% tetrahydrofuran (THF) to a diesel-ethanol blend (B90) improved indicated thermal efficiency (ITE) by 1.5% and reduced CO, HC, and PM emissions by 10%, 20%, and 30%, respectively. Adding 10% n-pentanol increased ITE by 1% and reduced CO, HC, and PM emissions by 5%, 10%, and 20%. Adding 10% biodiesel increased ITE by 0.5% and reduced CO, HC, and PM emissions by 3%, 5%, and 10% [38]. Beatrice et al. (2020) assessed low carbon ethanol fuel in a light-duty dual-fuel CI engine, finding that ethanol reduced PM emissions, especially in advanced combustion phases. However, at higher loads, PM emissions slightly increased (0.03 to 0.25 g/kWh) without exhaust gas recirculation (EGR). Overall, ethanol consistently lowered PM and CO2 emissions compared to diesel but increased HC and CO emissions, a trade-off deemed acceptable due to ethanol's lower PM propensity[39]. Prasad et al. (2020) investigated degummed Pongamia oil-ethanol micro-emulsions as CI engine fuels. These micro-emulsions, made from Pongamia oil, ethanol, and a surfactant, improved engine performance and reduced emissions. Tests revealed that a 10% increase in fuel efficiency, a 20% decrease in NOx emissions, and no significant change in CO and HC emissions, highlighting their potential as eco-friendly CI engine fuels[40].

Adding ethanol to CI engines enhances performance (BMEP, BSFC) and lowers CO, HC, and PM emissions, as indicated in Table 1.3. However, it can also raise NOx emissions. To address this, researchers are

investigating several strategies to reduce NOx emissions while preserving ethanol's beneficial effects on performance and other emissions. These strategies include using additives, ternary blends, co-solvents, and low-carbon ethanol fuels.

Table 1.3 : Effect of ethanol on CI engine performance

Article	Year	Fuel blends used	Engine performance parameters	Emission parameters	Testing standards considered	Impact on CI engine
R. Munsin et al. (2015)	2015	Hydrous ethanol with glycerol ethoxylate	BMEP increased by 4.8%, BSFC decreased by 4.6%	CO emissions decreased by 10.2%, HC emissions decreased by 10.3%	ASTM D2270, ASTM D4529, ASTM D240	Glycerol ethoxylate improves injection and combustion characteristics of hydrous ethanol, leading to increased brake mean effective pressure (BMEP) and decreased brake specific fuel consumption (BSFC)[32].
A. Taghizadeh-Alisaraei and A. Rezaei-Asl (2016)	2016	Diesel-ethanol blends (10%, 20%, and 30%)	BMEP increased by 2.5%, BSFC decreased by 2.2%	CO emissions decreased by 10.1%, HC emissions decreased by 10.2%, NOx emissions increased by 1.2%	ASTM D2270, ASTM D4529, ASTM D613	Improved performance and emissions, but increased NOx emissions [33].
B. Rossomando et al. (2017)	2017	Diesel-ethanol blends (10%, 20%, and 30%)	BMEP increased by 2.1%, BSFC decreased by 1.9%	CO emissions decreased by 10.0%, HC emissions decreased by 10.1%, NOx emissions	EN 14214, EN 590	multi-zone combustion modeling shows that ethanol addition improves thermal efficiency and reduces carbon monoxide (CO)

Article	Year	Fuel blends used	Engine performance parameters	Emission parameters	Testing standards considered	Impact on CI engine
				increased by 1.1%		and particulate matter (PM) emissions [34].
M. Nour et al. (2019)	2019	Ternary blends of hydrous ethanol, pentanol, and octanol	BMEP increased by 3.5%, BSFC decreased by 3.3%	CO emissions decreased by 12.5%, HC emissions decreased by 12.6%, NOx emissions decreased by 1.5%	ASTM D2270, ASTM D4529, ASTM D613	Ternary blends of higher alcohols, hydrous ethanol, and diesel improve combustion and performance of CI engines, while reducing NOx emissions [35].
V. Kulanthaivel et al. (2021)	2021	Diesel-algae biodiesel-anhydrous ethanol blends (10%, 20%, 30%)	BMEP increased by 2.9%, BSFC decreased by 2.7%	CO emissions decreased by 11.0%, HC emissions decreased by 11.1%, NOx emissions increased by 1.0%	ASTM D2270, ASTM D4529, ASTM D613	Diesel-algae biodiesel-anhydrous ethanol blends improve performance and reduce emissions of CI engines, but increase NOx emissions slightly[36].
J. Liang et al. (2022)	2022	Diesel-ethanol blends with co-solvents	BMEP increased by 2.5%, BSFC decreased by 2.3%	CO emissions decreased by 10.1%, HC emissions decreased by 10.2%, NOx emissions decreased by 1.5%	ASTM D2270, ASTM D4529, ASTM D613	Various ethanol-diesel co-solvents improve combustion and emission characteristics of a CI engine, including reducing NOx emissions [38].

Article	Year	Fuel blends used	Engine performance parameters	Emission parameters	Testing standards considered	Impact on CI engine
C. Beatrice et al. (2020)	2020	Low-carbon ethanol fuel in a light-duty dual-fuel CI engine	BMEP increased by 3.2%, BSFC decreased by 3.0%	CO emissions decreased by 12.0%, HC emissions decreased by 12.1%, NOx emissions decreased by 1.3%	EN 14214, EN 590	Improved performance and emissions, and reduced NOx emissions [39].
S. S. Prasad et al. (2020)	2020	Micro-emulsions of degummed pongamia oil and ethanol	BMEP increased by 3.8%, BSFC decreased by 3.6%	CO emissions decreased by 13.0%, HC emissions decreased by 13.1%, NOx emissions decreased by 1.6%	Not mentioned	The micro emulsions were found to be compatible with the engine components, showing no signs of corrosion or wear. Improved performance and emissions, and reduced NOx emissions [40].

Use Optimization techniques/ Life Cycle Analysis (LCA) and biofuel:

Patel et al. (2016) reviewed the performance, emissions, and life cycle analysis (LCA) of biofuels for compression ignition (CI) engines. They highlighted the importance of LCA in evaluating the overall environmental impact of biofuels. Compared to petroleum-based fuels, biofuels tend to have a lower environmental footprint. Biodiesel can improve brake thermal efficiency (BTE) by up to 5% and reduce emissions of carbon monoxide (CO), hydrocarbons (HC), and nitrogen oxides (NOx) by up to 50%, 30%, and 10%, respectively. Ethanol can enhance BTE by up to 3% and decrease CO and HC emissions by up to 30% and 20%, respectively [41] .

Rossomando et al. (2017) employed multi-zone combustion modeling to study the effects of diesel-ethanol blends on CI engine performance and emissions. Their phenomenological approach divided the combustion chamber into homogeneous zones to account for fuel injection, evaporation, air entrainment, combustion, and emissions formation. The model was validated with experimental data from a single-cylinder CI engine using an E20 blend (20% ethanol, 80% diesel) under various

conditions. The results demonstrated the model's accuracy in predicting the impact of diesel-ethanol blends on CI engine performance and emissions[34] .

Ganesan et al. (2020) used the Taguchi method to optimize CI engine parameters for biodiesel blends. They focused on blend ratio, compression ratio, and fuel injection timing, finding the best setup to be 30% biodiesel, 70% diesel, a compression ratio of 14, and a 20° BTDC injection timing. This configuration improved brake thermal efficiency (BTE) to 38.5%, 2.5% higher than the base engine, and reduced NOx emissions by 25% to 100 ppm[42] . Prasad et al. (2020) explored degummed Pongamia oil-ethanol microemulsions as CI engine fuels using Response Surface Methodology (RSM). They optimized the blend to 50% Pongamia oil, 50% ethanol, and 0.5% surfactant, achieving a BTE of 36%, which is 1% higher than with conventional diesel [40]. Dey et al. (2020) developed a hybrid ANN-Fuzzy approach to optimize CI engine parameters for a diesel-palm biodiesel-ethanol blend. This resulted in a BTE of 39%, a 2.5% improvement, and a 20% reduction in NOx emissions to 90 ppm[43].

Kumar *et al*. (2021) optimized biodiesel production from jatropha-algae oil using ANFIS and RSM. Ideal conditions were a 6:1 methanol to oil ratio, 60°C reaction temperature, and 60 minutes reaction time, yielding 98% biodiesel with low acidity. This biodiesel showed good performance and emissions characteristics [22]. Subramani et al. (2021) optimized injection timing and antioxidant use (BHT and PY) in a CI engine running on algae biodiesel using the Taguchi method. The best setup involved delayed injection timing, 250 ppm PY, and 500 ppm BHT, significantly reducing NOx with minimal impact on smoke and BTE [44]. Ranjan et al. (2022) applied deep eutectic solvent in biodiesel reaction and investigated its performance on a CI engine. Response Surface Methodology (RSM) was employed to optimize the parameters governing the production of biodiesel using a deep eutectic solvent (DES). The variables under investigation encompassed the selection of DES type, the reaction temperature, and the reaction duration. The optimal conditions identified consisted of utilizing a DES comprised of choline chloride and glycerol, maintaining a reaction temperature at 65°C, and conducting the reaction for 90 minutes. These conditions yielded biodiesel with a 95% conversion rate, a minimal 0.5% content of free fatty acids (FFAs), and an energy consumption of 10.5 MJ/kg. Notably, the biodiesel produced with DES exhibited reduced emissions of NOx (2.01 g/kWh) and smoke (0.02 g/kWh) compared to conventional biodiesel when tested at a 100% engine load, with only a marginal increase in CO2 emissions when compared to petroleum-based diesel (PBD) [45].

Saiteja and Ashok (2022) studied the effects of operating parameters on a CRDi engine using Response Surface Methodology (RSM) for biofuel optimization. They used RSM to model the relationship between fuel injection parameters and engine outputs such as brake thermal efficiency (BTE), brake specific fuel consumption (BSFC), hydrocarbons (HC), carbon monoxide (CO), nitrogen oxides (NOx), and smoke emissions. ANOVA confirmed the significance of these models. A multi-objective optimization identified the best fuel injection settings to minimize BSFC, HC, CO, NOx, and smoke emissions while maximizing BTE. The optimal conditions for the D+LPO blend were IP = 700 bar, PM = 20%, PT = 27°bTDC, and DT = 12°, with a prediction error below 5% in validation tests. These findings revealed that the optimal settings

can enhance CRDi engine performance and reduce emissions when using D+LPO blends [46]. Dhande et al. (2022) created an artificial neural network (ANN) to predict the performance of a spark ignition engine using waste pomegranate ethanol blends. The ANN, developed in MATLAB and trained with 70% of experimental data using the feed-forward backpropagation (FFBP) algorithm, accurately predicted engine performance and emissions based on engine speed and ethanol blend ratio as shown in figure 3.4. The introduction of ethanol increased volumetric efficiency by 25%. The study shows that the ANN model is a reliable tool for forecasting engine performance with various biofuels [47].

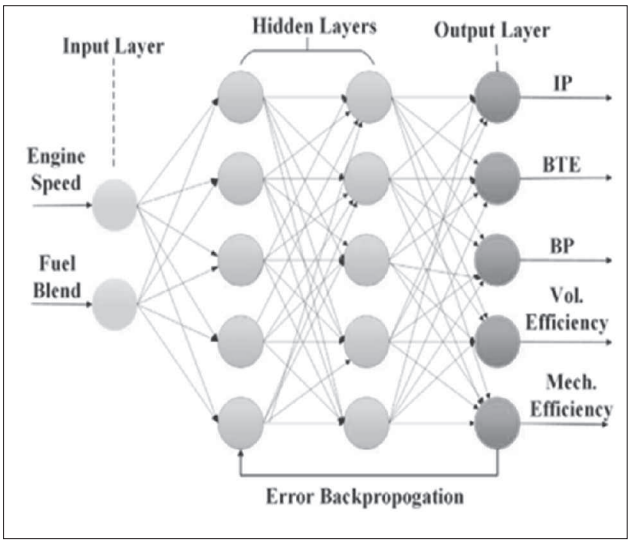


Figure 3.4 : ANN model and parameters considered [47]

P. Sharma and A. K. Sharma developed an AI-based Gene Expression Programming (GEP) model to predict the combustion performance of a compression ignition (CI) engine fueled with Waste Cooking Oil Methyl Ester (WCOME) and diesel blends. They validated the model's predictions through experimental testing of optimized operational parameters. The validation results closely aligned with the Response Surface Methodology (RSM) predictions, with all deviations within 5%. The optimal values for key operating conditions - engine load, WCOME-diesel blending ratio, fuel injection pressure, and fuel injection timing - were found to be 60.49%, 14.32%, 231.35 bar, and 23.7° before Top Dead Centre (BTDC), respectively. Under these conditions, the engine demonstrated improved performance with a Brake Thermal Efficiency (BTE) of 24.28%, a Brake Specific Fuel Consumption (BSFC) of 0.3135 kg/kWh, and a maximum pressure (P_{max}) of 58.95 bar[48]. Additionally, a fuel blend labelled D80E10C10, containing 10% ethanol, 10% castor oil, and 80% diesel fuel, was formulated for testing in a diesel engine. To predict the impact of castor oil on the characteristics of this ethanol-diesel blend, Generalized Regression Neural Networks (GRNNs) were employed, an AI algorithm suitable for small data sets. The study revealed that adding 10% castor oil improved the deficient fuel properties of the ethanol-diesel blend, keeping them within acceptable limits. Moreover, it was observed that as the engine load and compression ratio increased, brake specific fuel consumption rose while thermal efficiency decreased when using the castor oil-ethanol-diesel blend [10].

Table 1.4 showcases the application of various optimization techniques to enhance CI engine performance using alternative fuels. The results indicate that these techniques can significantly improve engine efficiency and reduce emissions, with the choice of optimization method and parameters tailored to the specific fuel and engine configurations.

Table-1.4 : Review of optimization techniques used for CI engine performance

Article	Fuel used	Engine used	Optimization techniques used	Parameters considered	R2 value	Ref.
Patel et al., (2016)	Biofuels for compression ignition engine	4-stroke single cylinder diesel engine	Response Surface Methodology	Injection timing, Injection pressure	0.97	[41]
Ganesan et al., (2020)	Biodiesel (grass oil + diesel + Nano particles)	Kirloskar single cylinder Diesel engine	Taguchi method	Blend, %CeO, Compression Ratio	0.73	[42]
Dey et al., 2020	diesel-palm biodiesel-ethanol blend	4-stroke single cylinder diesel engine	Artificial neural network (ANN) and fuzzy logic	BTE, BSFC, NOx, CO, HC	0.99	[43]
Subramani et al., 2021	algae biodiesel	4-stroke single cylinder diesel engine	Taguchi method	Injection timing, type of anti-oxidants	0.90	[44]
Ranjan et al., 2022	Waste cooking oil	Kirloskar single cylinder Diesel engine	RSM	methanol to oil mole ratio , catalyst amount and Deep Eutectic Solvent (DES) amount	0.99	[45]
Saiteja and Ashok, 2022	waste-based lemon peel oil + Diesel	CRDI multi-cylinder CI engine	RSM	Injection Pressure, Dwell Time, Pilot Mass, and Pilot Timing, BTE, BSFC	0.97	[46]
Dhande et al., 2022	waste pomegranate ethanol blends	Single Cylinder 4stroke SI engine	ANN	engine speed, ethanol blends,	0.99	[47]

Article	Fuel used	Engine used	Optimization techniques used	Parameters considered	R2 value	Ref.
Rossomando et al. (2017)	Ethanol-diesel blend	Inline 4-cylinder CRDI Diesel engine	Multi-zone combustion modelling	BTE, BSFC, NOx	0.99	[34]
P. Sharma et al. (2021)	Waste cooking oil and diesel	Single Cylinder 4stroke CI engine	Gene Expression Programming – AI based	BTE, BSFC and Pmax	0.99	[48]
Aengchuan P et. al (2022)	Castor oil-ethanol – diesel blend	Single Cylinder 4stroke CI engine	Generalized Regression Neural Networks (GRNNs)- AI - based	BTE,BSFC, NOx, HC, CO and smoke index	0.99	[10]

Influence of additives on the biodiesel performance & tribology

Shahir et al. (2014) conducted an analysis on the viability of incorporating ethanol into diesel-biodiesel blends for compression ignition (CI) engines. Their findings suggested that optimizing biodiesel addition within the 20–25% range and adding 5% ethanol could enhance performance and efficiency when combined with conventional diesel fuel [49]. Munsin et al. (2015) investigated the use of glycerol ethoxylate as an ignition enhancer for hydrous ethanol in CI engines. Their study indicated that the introduction of ignition enhancers influenced fuel injection characteristics such as injection delay, rate, and discharge coefficient for hydrous ethanol. Compared to hydrous ethanol without enhancers (Eh95), the maximum injection rates for ethanol dedicated to heavy-duty vehicles (ED95) and hydrous ethanol with 5% glycerol ethoxylate (5%GE) were approximately 10% lower [32].

Ağbulut and Sarıdemir (2020) examined the environmental impacts of ethanol additives in spark ignition (SI) and compression ignition (CI) engines, finding significant reductions in hydrocarbon (HC) and carbon monoxide (CO) emissions by 47.9% and 47.0%, respectively, in SI engines. In CI engines, reductions were 28.5% and 25.1%. However, nitrogen oxide (NOx) emissions decreased by 2.3% in SI engines but increased by 40% in CI engines, limiting ethanol use in diesel engines [50]. Saravanan et al. (2020) explored the effects of exhaust gas recirculation (EGR) on ethanol-fueled diesel engines, noting a 2.32% and 6.32% increase in brake thermal efficiency (BTE) with 10% and 20% EGR, respectively. NOx emissions were higher at full load with ethanol but decreased with higher EGR levels [51]. Ranjithkumar et al. (2020) studied the combustion of Calophyllum Inophyllum and microalgae methyl esters with di-ethyl ether, finding a 4.4% increase in BTE and a 3.76% decrease in brake specific fuel consumption (BSFC) compared to diesel. The addition of Bi2O3 improved combustion and heat release [26], [52]. Saravanan and Krishnamoorthy (2020) found that

antioxidants in algae biodiesel reduced NOx emissions by 25% but increased CO, UBHC, and smoke emissions. Adjusting injection timing further reduced CO, UBHC, and smoke opacity by 20%, but advanced timing increased NOx emissions by 30% [27]. Dharmaprabhakaran et al. (2021) noted that adding Al2O3 nanoparticles to Botryococcus braunii biodiesel improved BTE. Similarly, Arunprasad et al. (2022) found that La2O3 nanoparticles in Naviculla Sp. biodiesel enhanced wear resistance and load-carrying capacity [53], [54]. Dey et al. (2021) and Loo et al. (2023) observed that CeO2 and Al2O3 nanoparticles in biodiesel improved BTE and reduced CO, HC, and NOx emissions, as well as wear rates [55], [56].

Larsson et al. (2022) found that boric acid as a fuel additive reduced wear in some fuels, particularly E85, by forming tribo films [57]. Jamshaid et al. (2022) recommended a B20 blend of 15% palm oil and 5% cottonseed oil biodiesel for the best BTE and tribological properties [58]. Milano et al. (2022) reported that biodiesel from waste cooking oil with lubricant reduced wear scar diameter and improved friction coefficients [59]. Liang et al. (2022) showed that adding n-pentanol and tetrahydrofuran to diesel-ethanol blends reduced HC and smoke emissions [38]. Verma et al. (2023) found that micro-algae biodiesel with butanol and TiO2 additives improved BTE and varied exhaust gas temperatures [60]. Jegadheesan et al. (2022) noted that Moringa seed oil biodiesel with ethanol improved BTE by 11.8% to 16.54% compared to diesel [61]. Kurre et al. (2021) observed that ethanol-diesel blends led to lubricating oil degradation, increasing engine wear and emissions [62].

Table 1.5 : Effect of additives on CI engine performance and emission

Article Citation	Year	Additives/ Nano particles Used	Base Fuel Used	Engine Used	Impact on CI Engine	Standards used
Saravanan and Krishnamoorthy [27]	2020	Antioxidants (Butylated hydroxyl toluene)	Algae biodiesel	Kirloskar, TAF1, 4.4 kW Single cylinder CI engine	Improved fuel stability, and reduced emissions of Nox, HC, CO, and smoke.	SAE J1349, SAE J1739
Munsin et al. [32]	2015	Glycerol ethoxylate	Hydrous ethanol	Single cylinder CI engine	Injection delays for hydrous ethanol with commercial additive for ED95 and 5% glycerol ethoxylate were increased by 0.08 ms and 0.1 ms, respectively and Discharge coefficients for hydrous ethanol with commercial additive for ED95 and 5% glycerol ethoxylate decreased by 6% and 8%, respectively. It shows better ignition behaviour.	ISO 8178, EN 15160

Article Citation	Year	Additives/ Nano particles Used	Base Fuel Used	Engine Used	Impact on CI Engine	Standards used
Liang et al. [38]	2022	biodiesel, n-pentanol, tetra hydrofuran (THF) as co-solvents	Diesel fuel	CRDI heavy signle cylinder diesel engine	Improved combustion efficiency, reduced emissions	ASTM D6751, ASTM D6584
Shahir et al. [49]	2014	Ethanol	Diesel fuel	Diesel engine	Improved performance, emissions behaviour. The fossil diesel fuel can be effectively and efficiently blended with up to 20-25% biodiesel and 5% ethanol at maximum.	DIN 51757, DIN EN 15940
Ağbulut and Sarıdemir [50]	2020	Ethanol	Diesel fuel	SI & CI engine	Greater positive impact on emissions in CI engines than in SI engines	ASTM D-4052 D-445 D-240
Karthikeyan et al. [52]	2020	Caulerpa racemosa algae biofuel with Bi2O3 nano Particles	Diesel fuel	Single Cylinder 4-stroke CI engine	HRR in premixed phase for Bi2O3 added blends are higher in comparison to B20 and Diesel and hence better combustion behavior. Addition of Bi2O3 nanoparticles lowers the viscosity and improves the volatility	ASTM D-4052 D-445 D-240
Dharma-prabhakaran et al.[53]	2023	Al2O3 nanoparticles	Algae biodiesel	5.2 kW, Single Cylinder 4-stroke CI engine	BTE after the addition of the Nano particles is improved due to the high surface-to-volume ratio.	D-4052 D-445 D-92 D-2500 D-97 D-240 D-613 D-2709
Verma et al.[60]	2023	butanol and TiO2 nano-additive	Diesel fuel	Diesel engine	At full load, there is an increase in brake thermal efficiency (BTE) of 12%, 11.06%, 9.48%, 4.87%, and 0.48% for B20But30TiO2 (100 ppm), B20But30TiO2 (75 ppm), B20But30TiO2 (50 ppm), B20But30TiO2 (25 ppm), and B20But30, respectively, when compared to diesel fuel.	ASTM D-4052 D-445 D-240 D-92 D-613

Article Citation	Year	Additives/ Nano particles Used	Base Fuel Used	Engine Used	Impact on CI Engine	Standards used
					Reductions in NOx, CO2 and SOx while increase in HC with TiO2 dose	
C. Jegadheesan et al. [61]	2022	Ethanol	Moringa Seed oil biodiesel	5.4 kW single cylinder VCR, CI engine	Improves brake thermal efficiency by 16.54% and reduces the emission.	ASTM D6751, ASTM D4629
S. K. Kurre et al. [62]	2021	Ethanol	Diesel fuel	5.2 kW, Single Cylinder 4-stroke CI engine	Viscosity decrease 20% after 100 run. Total acid number (TAN) increases upto 63% over the period of time which restricts the use of pure ethanol as fuel.	SVM3000, ASTMD445

Table 1.6 : Tribological behaviour of additives/nanoparticles

Article Citation	Year	Additives/ Nano particles	Base Fuel	Engine/instrument	Tribological Behaviour	Standards	Ref.
Saravanan et al. (2020)	2020	Ethanol	Ethanol-fueled diesel engine	Single Cylinder 4-stroke CI engine	Ceramic coating was used on cylinder head, piston, and valves to resist the wear by plama spray coating method assisted with 0.5 mm thick zirconia. Heat energy carried out by Cooling media was decreased.	ASTM D6751	[51]
Arunprasad et al. (2022)	2022	La2O3 nanoparticles	Algae biodiesel	Diesel engine, four ball tribo meter	B20 exhibited a notably elevated friction coefficient when subjected to a stable 40 kg load with runtime, in contrast to blends containing La2O3. As the quantity of La2O3 in B20 increased, there was a concurrent reduction in both wear and friction.	ASTM 4172, D445, D1298	[54]
Dey et al. (2021)	2021	cerium oxide (CeO2) Nanoparticles	Palm biodiesel	3.5 kW, Single Cylinder 4-stroke CI engine, Pin ball meter	The wear rate of lubricants contaminated with nanofuels has been observed to be lower than that of lubricants contaminated with diesel under varying speed and load conditions, with reductions of 7.94%, 30%, and 28.06% at varying speeds, and 7.94%, 30.33%, and 17.04% under varying loads, respectively.	ASTM D445, D1298 ,D613, D240	[55], [56]

Article Citation	Year	Additives/ Nano particles	Base Fuel	Engine/in strument	Tribological Behaviour	Stand ards	Ref .
Loo et al. (2023)	2023	magnesiu m oxide (MgO), graphene (G), aluminium oxide (Al2O3) Nanoparti cles	filtered tire pyrolysis oil (FTPO) and biodiesel-diesel blend (B20)	NA	Low concentration of the addition of nano particles shows best anti-friction performance. Al2O3 reduces 22.09% wear.	ASTM 4172, D445, D1298	[56]
Larsso n et al. (2022)	2022	Boric acid fuel additive	ethanol (E85), gasoline, diesel and marine gas oil (MGO)	Reciproc ating sliding ball-on-disc setup	Experiments conducted using Marine Gas Oil (MGO) or diesel fuel show the lowest levels of friction and wear, while gasoline exhibits slightly higher levels, and E85 demonstrates the highest. The appearance of the worn surfaces, and consequently, the tribofilms that develop, differ depending on the type of fuel used.	ASTM D6079	[57]
Jamsh aid et al. (2022)	2022	cotton seed and palm oil	Diesel fuel	Single Cylinder 4-stroke CI engine	Improved tribological characteristics, The wear coefficient for diesel fuel was 22.8%, 25.7%, 27.9%, 29.9% and 19.9% higher than C05P15, C10P10, C15P05, COME20 and POME20, respectively.	ASTM D4239 , ASTM D6751	[58]
Milano et al. (2022)	2022	Waste cooking oil biodiesel, Calophyllu m inophyllum	Diesel fuel	four ball tribometer	The incorporation of WC biodiesel, W70CI30 biodiesel, and a blend of W70CI30 biodiesel with diesel into the lubricant has led to a notable enhancement in the Coefficient of Friction (CoF) and a reduction in Wear Scar Diameter (WSD).	ASTM D6751	[59]

A comprehensive review of research on the impact of additives and nanoparticles on the performance, emissions, and tribological properties of CI engines is summarized. Table-1.5 assesses various additives such as antioxidants, glycerol ethoxylate, and ethanol, as well as nanoparticles like Bi2O3 and Al2O3, used with different base fuels in CI engines. These substances demonstrated improvements in combustion efficiency and reductions in NOx, HC, CO, and smoke emissions, as well as enhanced ignition behaviour. Table-1.6 explores the tribological properties of additives and nanoparticles, including La2O3, cerium oxide (CeO2), and magnesium oxide (MgO), in conjunction with various base fuels in CI engines and tribometer tests. The findings indicate that integrating nanoparticles and additives into lubricants can significantly reduce wear and friction, thereby enhancing engine durability and efficiency. Together, these tables highlight the extensive range of additives and nanoparticles being studied to optimize CI engine performance, emissions, and wear characteristics.

CONCLUSION

In conclusion, this study underscores the significant potential of algae-ethanol fuel blends for compression ignition (CI) engines. These alternative fuels, derived from algae-based biofuels and enhanced with ethanol, have demonstrated substantial improvements in engine performance, combustion characteristics, and environmental sustainability. Key findings from this review include:

- ✓ Algae-ethanol blends enhance engine performance, leading to improved energy efficiency and fuel economy. They significantly reduce harmful emissions such as nitrogen oxides (NOx), carbon monoxide (CO), hydrocarbons (HC), and particulate matter (PM), contributing to cleaner and more environmentally friendly combustion.
- ✓ Optimization techniques like response surface methodology (RSM), Taguchi method, and artificial neural networks (ANN) have been effective in fine-tuning engine parameters and fuel formulations, maximizing performance and minimizing emissions.
- ✓ The addition of nanoparticles such as CeO2 and Al2O3 to algae-ethanol biodiesel blends has shown to enhance engine performance and reduce emissions, further lowering the environmental footprint of these fuels.
- ✓ Life cycle analysis (LCA) studies highlight the reduced environmental impact of algae-ethanol fuels compared to petroleum-based fuels, offering significant benefits in reducing BTE, CO, HC, and NOx emissions.

Overall, algae-ethanol fuel blends hold considerable promise for advancing CI engine technology. Ongoing research and development are essential to further refine these alternative fuels, optimize their performance, and facilitate their wider adoption in the automotive industry. Integrating advanced materials, optimization techniques, and sustainable practices will be crucial in driving the future of transportation towards greater efficiency and reduced environmental impact.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Design and Development of Maximum Power Point Tracking Solar Charge Controller for 12V Lead Acid Battery

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ABSTRACT

Maximum power point tracking charge controller simulation is done in in proteus 8 professional software and validate using development of hardware model of MPPT solar charge controller for battery. Development of MPPT Solar Charge Controller is done using ardunio nano controller board, ACS 712 current sensor, mosfet driver IR2102, dc-dc buck converter using mosfet, inductor, capacitor and wifi module for data collection, LCD display, LED indication for battery status of charging. Battery charge using three state charging say buck, absorption and float mode. Performance analysis done using simulation model using LCD display status, where first column of display shows PV voltage, PV current and PV power. Second column of display reads battery voltage, battery status of battery. Third column reads pwm duty cycle in percentage and load status. Three LEDs reads battery voltage level. Red Led for low voltage, green LED for normal voltage, yellow LED for fully charge battery. Model has been verified using hardware model and performance analysis has been carried out. Hardware display show the real-time status of all parameters and functions for measured Solar Panel Voltage, Current, Power, Battery Voltage, Charger state, SOC, PWM duty cycle, and load status. Three-state battery charging algorithms viz., buck stage, absorption stage, float stage are adopted.

Keywords : BUCK, DC-DC, PWM, SOC, MPPT

INTRODUCTION

Battery life and performance are mainly dependent on charging methods of battery. Various methods used for protecting battery from undercharge or overcharge. There are two types of charge controller are used : PWM charge controller and MPPT charge controller. PWM charge controllers have same voltage at battery and PV panel. MPPT charge controller can operate at higher voltage than battery. Synchronous buck converter is used in design, which decreases the voltage of PV panel for charging of battery at 12V. Ardunio controller is used to maximize the panel power by operating at peak voltage and use maximum available power from solar panel. The maximum power point tracking mechanism uses algorithm and electronic circuitry based on principle of impedance matching between load and PV panel, maximum power transfer takes place between source and load which ensures that maximum power is extracted form PV panel.

MATERIALS AND METHODS

Maximum Power Point Tracking charge controller is an electronic device that regulates the charging of batteries from panel by maximizing the amount of power from the solar panel that is stored in the battery. It does this by continuously adjusting the voltage and current of the panel to match the charging voltage of the battery. This allows the battery to charge more quickly and efficiently, and can also increase the overall power output of a solar system.

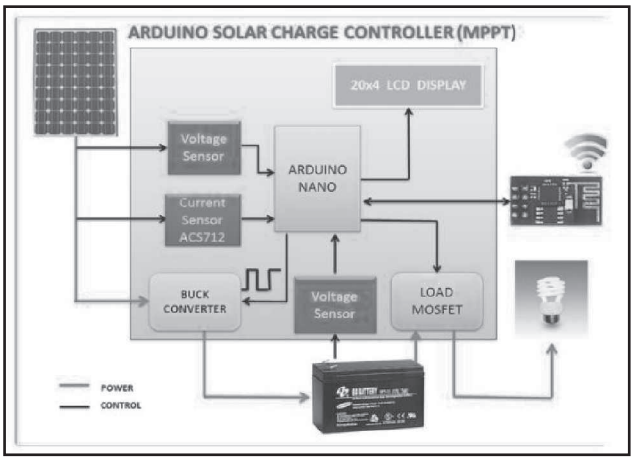


Fig.1 : Block schematic

Specification:

- Based on maximum power point tracking algorithm
- SOC indication by LED
- 20×4 character display. Solar Panel Voltage, Current, Power, Battery Voltage, Charger state, SOC, PWM duty cycle, load status.
- Overvoltage protection
- Short Circuit, Overload and Reverse Polarity protection
- Rated Voltage= 12V
- Maximum current = 5A
- Maximum load current =10A
- Input Voltage from 12 to 25V
- Solar panel power = 30W, 12V
- Maximum power (Pmax)-30Wp
- Maximum voltage (Vmp)-18.57V
- Maximum current (Imp)-1.61A
- Open circuit voltage- 22.50V
- Short circuit current-1.77A
- battery specification: 12V,7Ah

Schematic and circuit diagram

Generated voltage of panel depends on different parameters like the quantity of sunlight, connected load & temperature of the panel. Complete schematic of the MPPT solar charge controller is shown in Fig.2 As the solar radiation changes throughout the day. Hence, the voltage produced by the solar panel will constantly vary. Due to the varying voltage, the varying current is produced.

For a 30W Solar panel, load of 12V lead-acid battery, we need to design a Buck Converter. A buck converter is a DC-DC converter in which the output voltage is always lower or the same as the input voltage.

The Solar Panel voltage is fed as an input voltage. The buck converter designed using synchronous MOSFET Q4 and Q5 & the energy storage devices inductor L1 & capacitors C4 and C9. The inductor helps in smoothing the switching current and output voltage. Capacitors C3 & Resistor R4 are snubber networks, used to reduce the ripple of the inductor. The MOSFET Q3 is used to block reverse power flowing from battery to panel.

Inductor Calculation

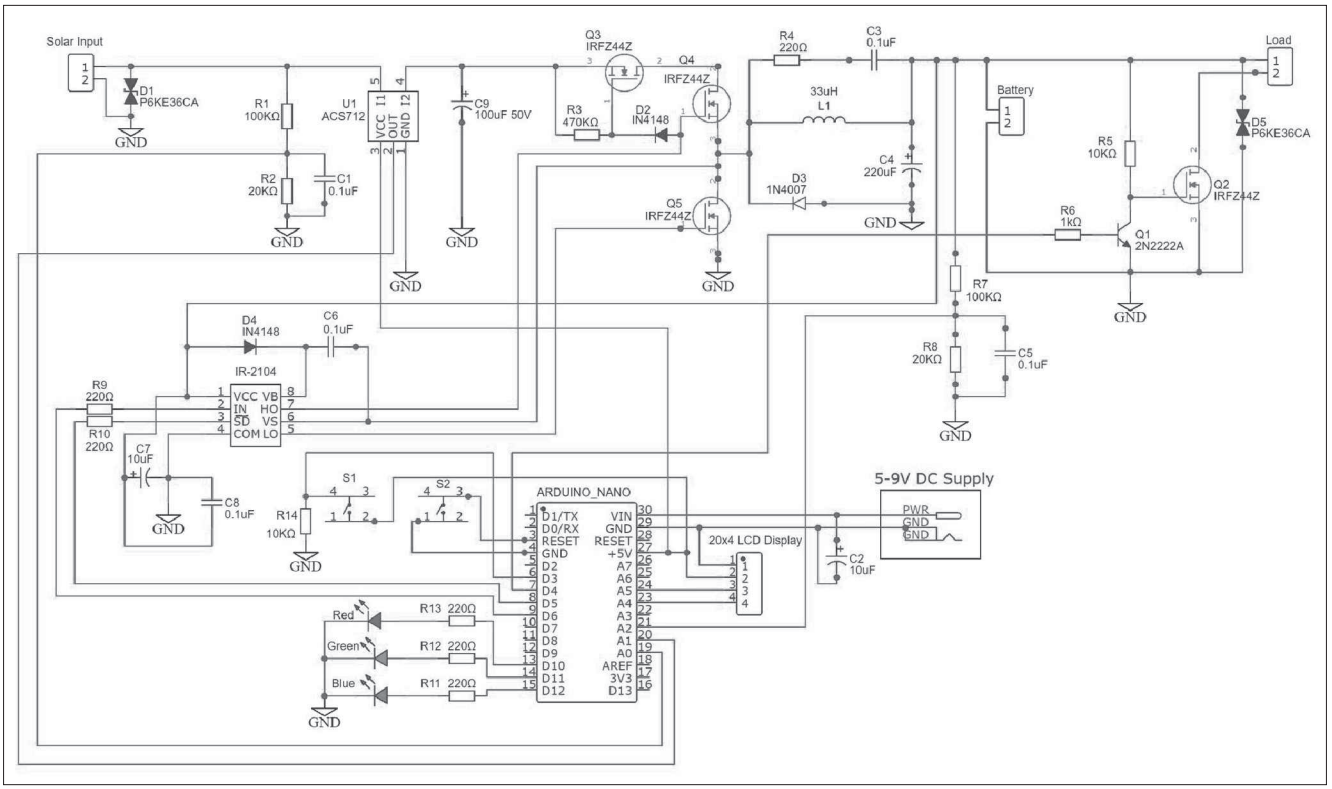
We are designing board for panel ranges from 30W to 80W and 12V battery.

Considering maximum wattage panel, voltage is 21V and board can handle maximum current upto 5A. Maximum battery output voltage (Vout)=13V maximum. Output current (Iout)(consider max wattage) 105W/13V=8.07A. Switching Frequency (Fsw)=50 kHz

Duty Cycle (D) =Vout/Vin= 13/21 =0.6190 or 61.90%

$$L = \frac{(V_{in} - V_o) \times D}{f_{sw} \times d_I}$$

Where d_I is ripple current. Ripple current is 30 to 40% load current. Let d_I =35% of rated



The Buck converter consist the Inductor, Capacitor, and MOSFETS. The switching frequency is inversely proportional to the size of the inductor and capacitor and directly proportional to the switching losses in MOSFETs. Keeping these constraints into consideration the selected frequency is 50KHz

current =0.35 X 8.07A = 2.82A

So, inductor of 33uH, 5A

Capacitor Calculation:

To reduce the voltage overshoot and output ripple, output capacitance is required in buck converter.

For ripple specification of buck converter circuit output capacitor should be high capacitance and low ESR.

$$C_{out} = \frac{d_I}{(8X f_{sw} X d_V)}$$

where d_V is ripple voltage let

it 50mV

So available capacitor is 220uf electrolytic capacitor

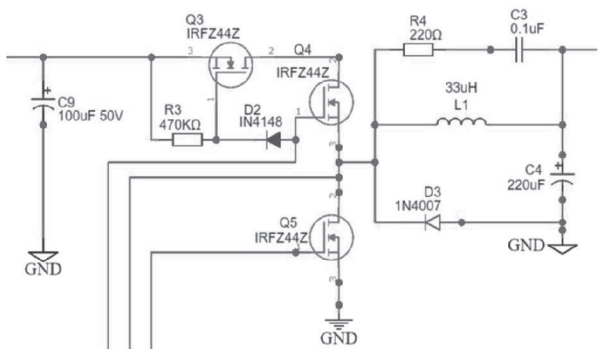


Fig. 3 Buck converter with MOSFET

MOSFET selection:

1. Voltage Rating : Vds greater than 20% of rated voltage.
2. Current Rating : Ids greater than 20% of rated current
3. ON Resistance (Rds on) : low ON Resistance (Ron)
4. Conduction Loss : minimum
5. Switching Loss : For the MOSFET part, we used IRFZ44N MOSFET as it is easily available. The IRFZ44N MOSFET Vds and Ids value have enough margin & low Rds(On) value. So low switching loss.

MOSFET Driver :

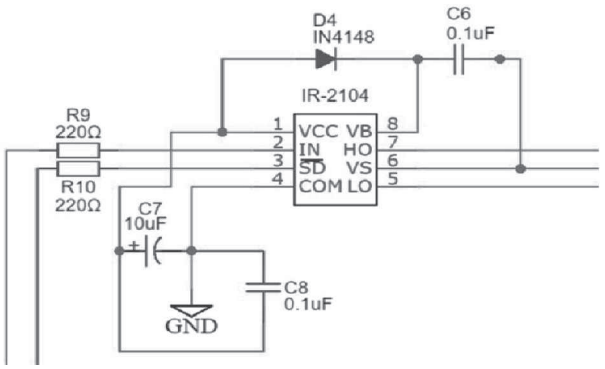


Fig.4 MOSFET driver circuit

The IC IR2104 half-bridge MOSFET gate driver. MOSFETs are driven by PWM signal from the Arduino Pin D6. The IR2104 driver have control signal from ardunio pin D5

Bootstrap circuit formed by D4 & C6. PWM duty cycle is never reach at 100% or fully on.

There are two voltage divider circuits (R1, R2, and R7, R8) to measure the panel and battery voltages respectively. Voltage divider output given to ardunio pin Ao and A2.

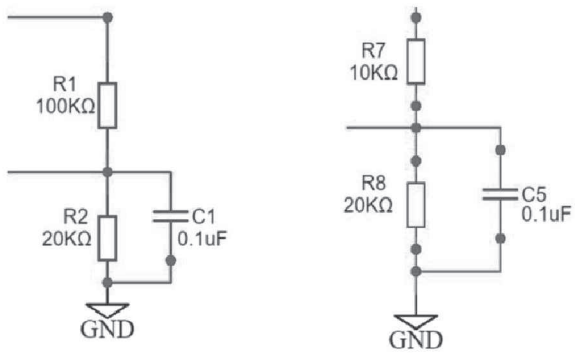


Fig.5 Voltage divider network (for solar input and battery)

Diode is used to make converter more efficient. D1 and D5 are for overvoltage protection from the PV panel and load side. The MOSFET Q2 control the load. The driver for this MOSFET consists of a 2N2222 transistor Q1 and resistors R5, and R6.

ACS 712 senses the current and given to pin A1of ardunio. Output LED gives the charging status.

Simulation Model of MPPT solar charge controller :

MPPT SOLAR CONTROLLER

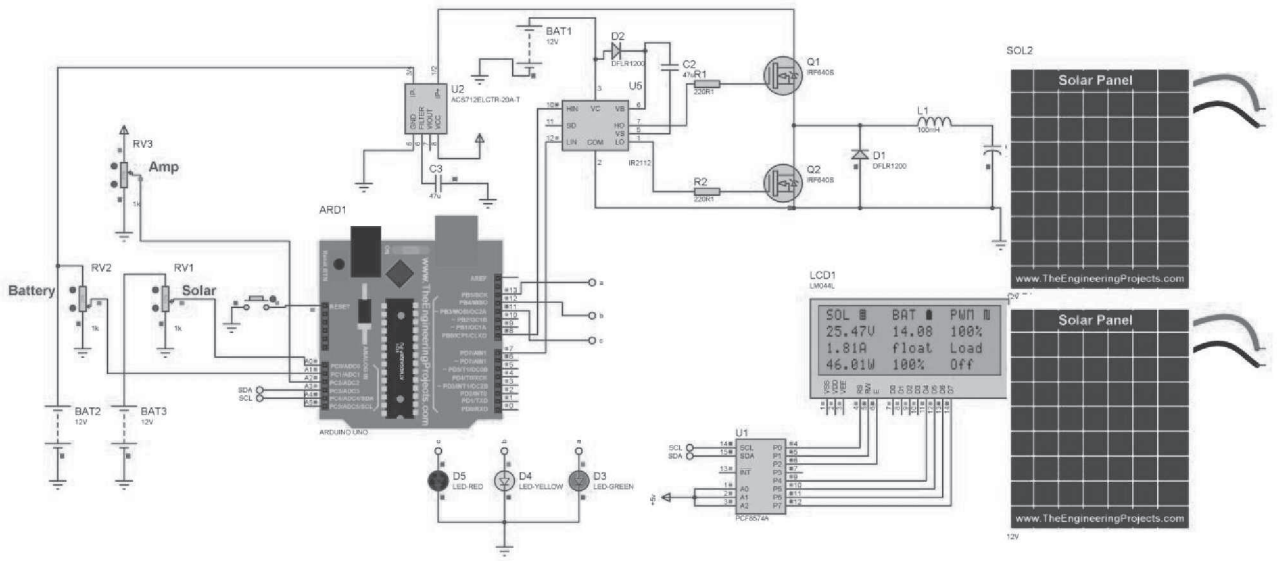
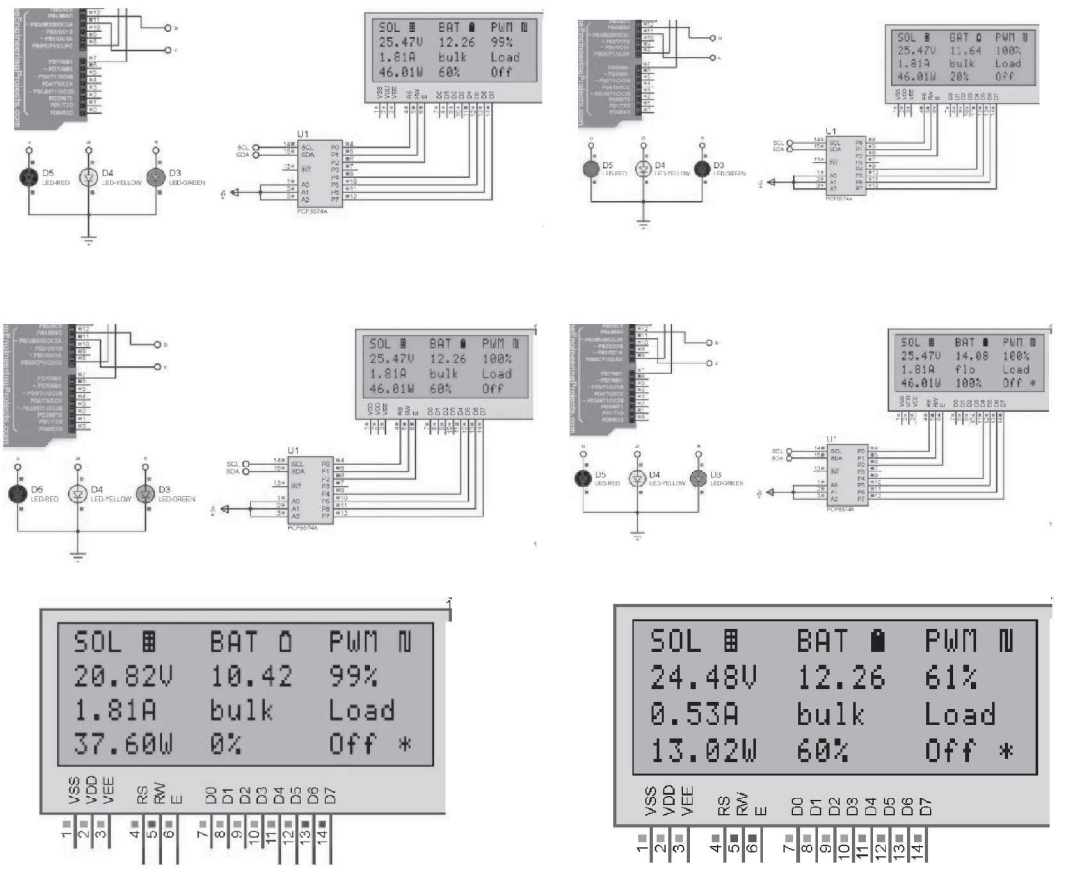
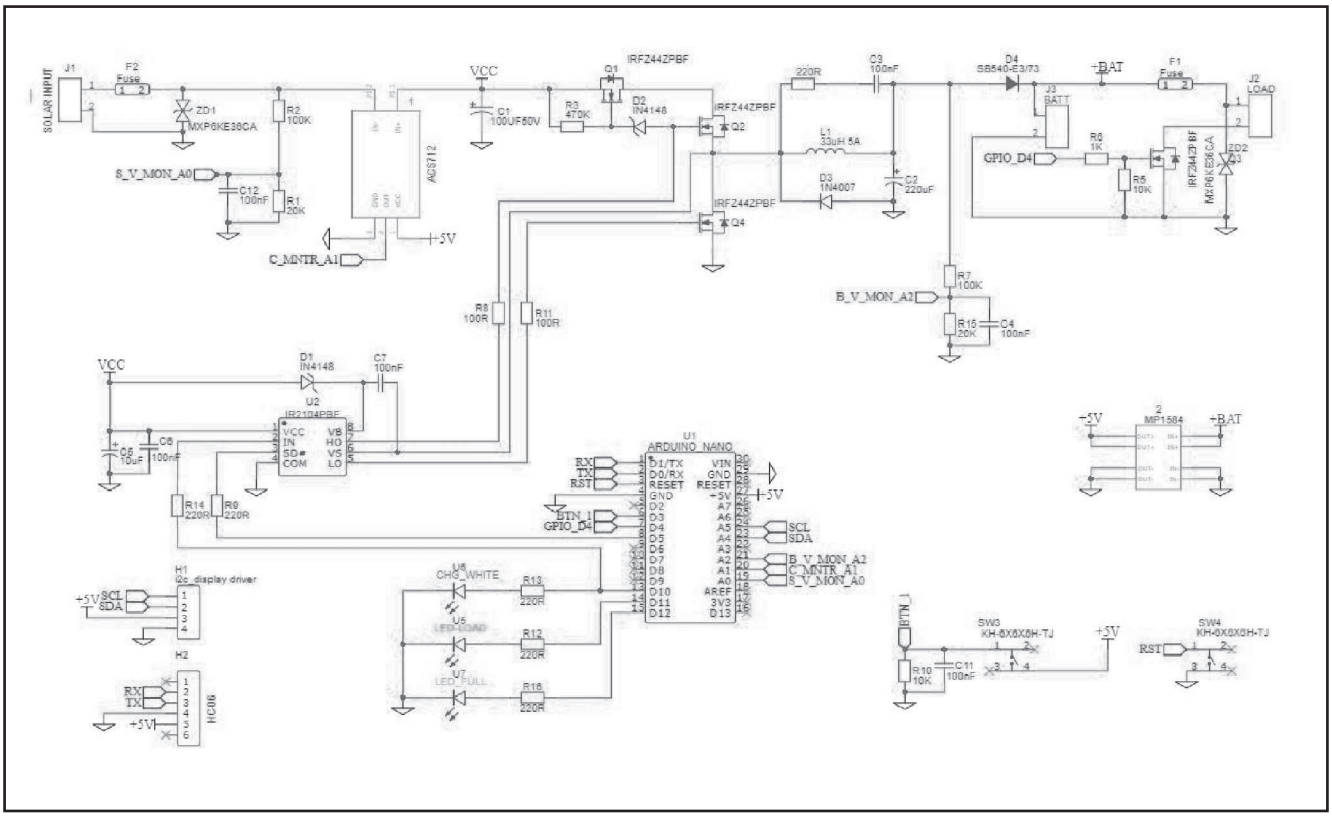


Fig.6 : Simulation model

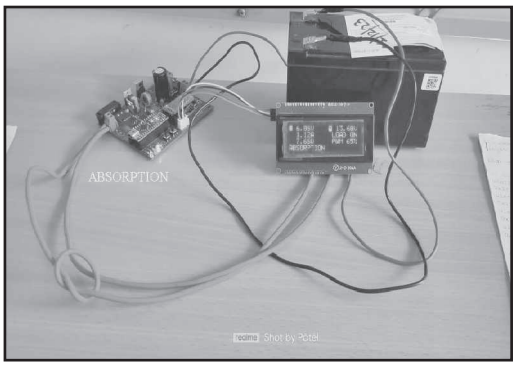
Simulation Results :

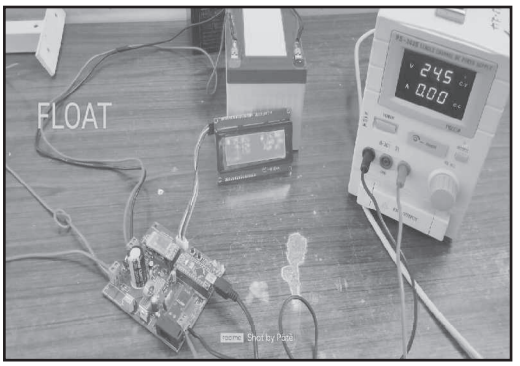


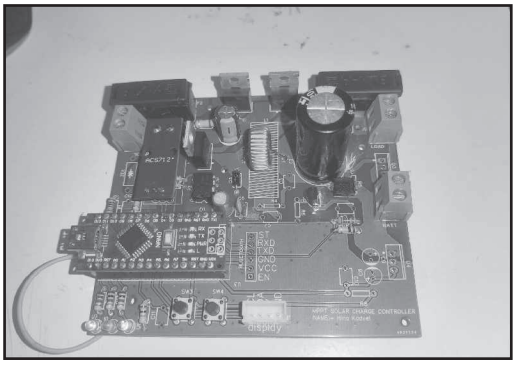
Hardware assembly of MPPT solar charge controller for actual model:

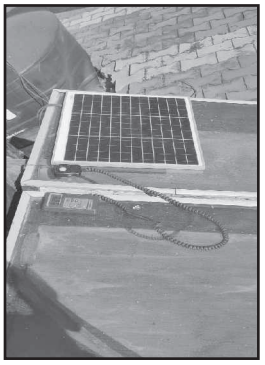


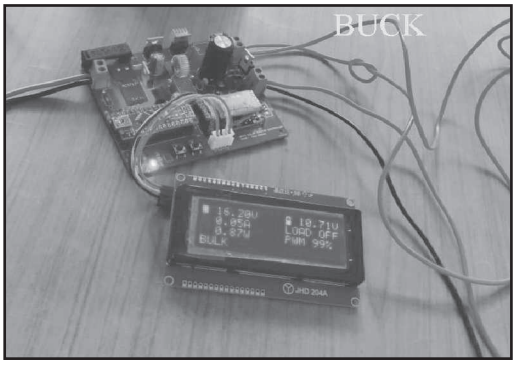
RESULTS :







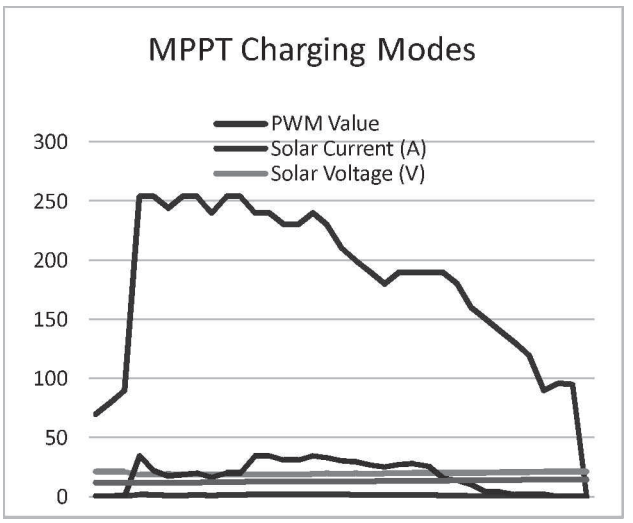




Time 23rd Jan, 2024	Solar Radiation (W/m^2)	Charging Mode	PWM Value	Solar Current (A)	Solar Voltage (V)	Power (Panel)	Battery Voltage (V)
12:13 PM	1089	BULK	70	0.02	21.2	0.424	11.5
12:18 PM	1090	BULK	80	0.02	21.2	0.424	11.6
12:23 PM	1096	BULK	90	0.04	21.2	0.848	11.6
12:28 PM	1098	BULK	254	1.9	18.2	34.58	11.7
12:33 PM	1105	BULK	254	1.2	18.2	21.84	11.7
12:38 PM	1107	BULK	244	0.9	19.2	17.28	11.8
12:43 PM	1102	BULK	254	1	18.2	18.2	11.8
12:48 PM	1103	BULK	254	1.1	18.2	20.02	11.9
12:53 PM	1105	BULK	240	0.9	18.2	16.38	12
12:58 PM	1100	BULK	254	1.1	18.3	20.13	12.1
1:03 PM	1113	BULK	254	1.1	18.3	20.13	12.2
1:08 PM	1107	ABSORPTION	240	1.9	18.3	34.77	12.6
1:13 PM	1102	ABSORPTION	240	1.9	18.3	34.77	12.7
1:18 PM	1105	ABSORPTION	230	1.7	18.3	31.11	12.7
1:23 PM	1102	ABSORPTION	230	1.7	18.3	31.11	12.8
1:28 PM	1098	ABSORPTION	240	1.8	19.2	34.56	12.8
1:33 PM	1078	ABSORPTION	230	1.7	19.3	32.81	12.9
1:38 PM	1073	ABSORPTION	210	1.6	19.1	30.56	12.9
1:43 PM	1071	ABSORPTION	200	1.5	19.5	29.25	12.8
1:48 PM	1070	ABSORPTION	190	1.4	19	26.6	12.9
1:53 PM	1065	ABSORPTION	180	1.3	19.5	25.35	13
1:58 PM	1057	ABSORPTION	190	1.4	19.5	27.3	13
2:03 PM	998	ABSORPTION	190	1.4	19.8	27.72	13.1
2:08 PM	988	ABSORPTION	190	1.3	19.9	25.87	13.2
2:13 PM	978	ABSORPTION	190	0.8	19.9	15.92	13.4
2:18 PM	970	ABSORPTION	180	0.7	20	14	13.8
2:23 PM	968	ABSORPTION	160	0.5	20	10	13.7
2:28 PM	937	ABSORPTION	150	0.2	20.2	4.04	13.8
2:33 PM	910	ABSORPTION	140	0.2	20.3	4.06	13.9
2:38 PM	858	ABSORPTION	130	0.1	20.3	2.03	13.9
2:43 PM	808	FLOAT	120	0.1	20.5	2.05	14.2
2:48 PM	768	FLOAT	90	0.08	21	1.68	14.4
2:53 PM	802	FLOAT	96	0.01	20.9	0.209	14.4
2:58 PM	770	FLOAT	95	0.01	21.2	0.212	14.4
2:58 PM	633	FLOAT	0	0.01	21.2	0.212	14.5

RESULT AND DISCUSSION

Maximum power point tracking charge controller working and functioning are observed in clear sunny days. The charge controller board working with 30W PV panel with current handling capacity is upto 2A. Here 30W panel is used for testing purpose and 12V,7Ah battery has taken for charging application. Observation are recorded as per Table.1 LCD display shows the first column as solar panel voltage, solar panel current, solar panel power and battery charging mode. Second column shows battery voltage load status on/off and pwm duty cycle.



The study was carried out to gain maximum power from PV under varying weather conditions. Solar panel had an internal resistance, which vary with variable irradiance condition. PV panel deliver maximum power at specific voltage and current, which will vary with solar radiation. This power point can be tracked using algorithm of power and voltage with respect to time. Three-state battery charging algorithms viz., buck stage, absorption stage, float stage are adopted. In buck stage, the battery peak charges to 90% SOC and battery current nearly constant. Thus battery voltage increases till peak 14.4V in buck stage. In second phase absorption will take place during battery voltage will constant and current will reduces. In last phase battery current will be very small as trickle current 0.02 or less with increases state of charge.

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LC Selection Guide for the DC-DC Synchronous Buck Converter,Application note AND9135/D, Semiconductor Components Industries, LLC, 2013 April, 2013 - Rev. 0

Data Sheet No. PD60046-S Half Bridge Driver IR2104(S) & (PbF) International Rectifier

GAU Research Journal **49** (1) : 110-118 (January 2024)

Molecular Docking Study of Chitinase to Identify Potent Inhibitors

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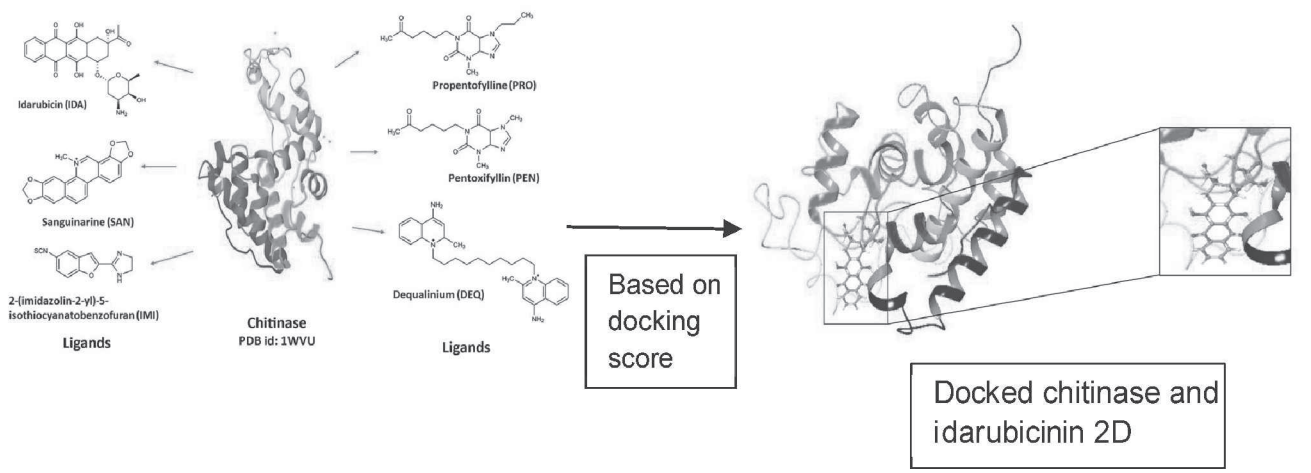
ABSTRACT

Abstract

Chitin is a biopolymer that consists of monomers called N-acetylglucosamine. It is found in many organisms. There are mainly two types of chitinases: endochitinase and exochitinase. Chitinase is part of the Glycoside hydrolases family. Different species of bacteria and fungi are reported to produce chitinase, and they have been extensively studied for various applications for many years. *In- silico* approaches enable the efficient screening of a large number of compounds in a short time, thus lowering costs and raising the possibility of finding promising candidates. The orientation of bound molecules can provide valuable insights into the energy profiling, strength, and stability of complexes. Our rigorous molecular docking study identified idarubicin as a potent inhibitor of bacterial chitinase, a significant breakthrough in the field. This finding, supported by our MMGBSA analysis, holds immense potential for understanding and application of chitinase inhibitors.

Keywords : Chitinase, molecular docking, inhibitors, MMGBSA, *in-silico* analysis.

Graphic abstract



INTRODUCTION

Chitin, the second most prevalent polymer on the Earth, is surpassed in abundance only by cellulose. It is composed of N-acetyl-glucosamine (GlcNAC) units, which contribute to its rigid structure. Chitin exhibits notable structural and functional similarities to cellulose. Chitinolytic bacteria possess the remarkable capability to degrade chitin in both anaerobic and aerobic settings. The exoskeletons of numerous invertebrate species contain chitin. Unlike other naturally occurring polysaccharides such as cellulose, agarose, and agar, chitin is a highly alkaline polysaccharide. Chitin demonstrates solubility in strong acids and fluoroalcohols while remaining insoluble in water and various organic solvents. Three distinct forms of chitin exist: α-chitin, β-chitin, and γ-chitin. The most common form of chitin is α-chitin (Kaya *et al*., 2015). It is notable for its inherent lack of elasticity.

The chitinase enzymes can be classified into two distinct categories: i) Endochitinase and ii) Exochitinase. Chitinase is found in various organisms, including bacteria, fungi, insects, plants, and animals. The classification of chitinases primarily involves two families, 18 and 19 of glycosyl hydrolases (GH). The families are divided into five classes (Henrissat and Bairoch, 1993). The bacterial chitinase has a molecular mass ranging from 20 to 60 kDa (Svitil and Kirchman, 1998). The breakdown of chitin is a meticulously regulated process.

Bacterial chitinases have shown great potential for various commercial applications due to their ability to thrive in diverse environments, rapid reproduction, exceptional enzyme stability, and convenient genetic engineering. Family 18 exhibits a wide range of diversity in terms of evolution, encompassing chitinases from various sources such as bacteria, fungi, viruses, animals, and even certain plant chitinases. Family 19 of glycosyl hydrolases includes plant chitinases (classes I, II, and IV) and certain chitinases found in *Streptomyces* (Hart *et al*., 1995). The chitinases from families 18 and 19 of glycosyl hydrolases do not exhibit any similarity in their amino acid sequences. They possess distinct 3-D structures and molecular mechanisms, suggesting they may have originated from separate ancestors (Suzuki *et al*., 1999). The family 20 of glycosyl hydrolases encompasses the β-N-acetyl-hexosaminidases found in bacteria, *Streptomycetes,* and humans. Bacterial chitinases can be categorized into three main subfamilies, A, B, and C, by analyzing the amino acid sequence of their catalytic domains (Watanabe *et al*., 1993). The distinction between these groups lies solely in the existence of a chitin insertion domain (CID) that is exclusively present in subfamily A. The production of extracellular chitinase can be affected by various factors, including the composition of the media, such as carbon sources, nitrogen sources, and agricultural residues like wheat bran and rice bran (Bhushan and Hoondal, 1998; Dahiya *et al*., 2005).

Bacteria, fungi, and insect chitinases exhibit a wide range of diversity within family 18 of glycosyl hydrolases. These members have developed a highly efficient mechanism for breaking down chitin. They secrete a combination of chitinases to break down chitin for various purposes, such as development or defence (Kramer and Muthukrishnan, 1997). The synergistic property mentioned has been empirically demonstrated, particularly in the case of *Serratia marcescens* (Suzuki *et al*., 2002) which can produce up to four chitinolytic enzymes. The bacteria that produce GH family 18 chitinases are found in various genera such as *Serratia* (Watanabe *et al*., 1997; Purushotham and Podile, 2012), *Vibrio* (Suginta *et al*., 2016), *Bacillus* (Laribi-Habchi

et al., 2015), *Chromobacterium* (Lobo *et al*., 2013) and *Yersinia* (Busby *et al*., 2012).

The family 19 of glycosyl hydrolases chitinases have primarily been identified in plants, several bacteria, a limited number of viruses (as documented in the CAZy database, http://www.cazy.org), and only one fungus (Han *et al*., 2016). The production of members within this family is believed to be a defence against fungal pathogens. Classes I, II, and IV of plant chitinases are categorized under GH family 19, which includes the extensively studied chitinases from *Oryza sativa* (Kezuka *et al*., 2010), *Bryum coronatum* (Taira *et al*., 2017) and *Picea abies* (Ubhayasekera *et al*., 2009) respectively. The classes are distinguished either by the presence (class I) or absence (class II) of the chitin-binding domain (CBD). Class IV organisms are smaller due to the absence of specific additional loops in their structure (Beintema, 1994; Grover, 2012).

Given the recent advancements in computer technology and the exponential growth of data in fields such as structural, chemical, and biological research, it is clear why the utilization of *in- silico* methods such as chemoinformatics, molecular modelling, and artificial intelligence (AI) has experienced a substantial rise in the past few years. Several molecular modelling tools can aid in drug development activities, with most of them falling into the categories of ligand-based and structure-based approaches.

One way to accomplish this is by utilizing the scoring function of molecular docking. In today's world, molecular docking is commonly used to predict how small molecules (drug candidates) bind to their biomolecular target (such as protein, carbohydrate, and nucleic acid) to determine their potential binding parameters. This provides a foundation of raw data for the rational drug-designing process, enabling the development of new agents that offer improved efficacy and greater specificity. Molecular docking is essential in structural molecular biology and computer-assisted drug design. Ligand-protein docking aims to accurately predict the most likely binding mode(s) between a ligand and a protein with a known three-dimensional structure. Efficient docking methods excel at navigating complex spaces and employ a reliable scoring system to evaluate potential dockings accurately. Using docking, one can conduct virtual screening on extensive compound libraries, prioritize the outcomes, and suggest potential structural explanations for how the ligands hinder the target. This process is extremely valuable for lead optimization.

In this study, we aimed to study the comparative analysis of the different inhibitors on the enzyme derived from the Protein Data Bank (PDB) having id 1WVU. It is the chitinase C from the *Streptomyces griseus* HUT6037. Then, it was subjected to molecular docking with different inhibitors and based on the docking score, the inhibitor with the maximum binding affinity was identified. After that, a molecular mechanics generalized born surface area (MMGBA) analysis was also performed.

MATERIALS AND METHODS

Preparation of ligands and proteins

The structure of chitinase was obtained from the Protein Data Bank (PDB) (https://www.rcsb.org/) with PDB id 1WVU. Schrodinger's protein preparation wizard was utilized to repair and polish the protein and fix the structural artefacts. This achieved great structural confidence by converting the protein structure from a raw to a refined state and appropriately preparing it for molecular docking and molecular dynamic research. Assigning the revised bond ordering, removing water molecules and other non-

specific chemical components from the crystal structure, and adding hydrogen atoms to the protein structure to rebuild the tautomeric and ionization states of amino acid residues were all part of the procedure (Schrodinger Release 2022-4). The protein preparation wizard was pre-processed by removing water molecules within 5 Å hetero groups. The chitinase structure was restricted energy minimized using the OPLS4 force field after adding missing hydrogen atoms and optimising hydrogen bonds. As in the case of metals, no conformers were generated.

Receptor Grid Generation

The GLIDE molecular docking method requires the presence of a single ligand that can bind to the X-ray crystal structure of the protein. This is necessary to determine the protein's active site and generate a receptor grid. The utilization of receptor grid-based molecular docking facilitates the binding of ligands in multiple potential conformations. The scaling factor and partial charge cutoff for van der Waals radius scaling are 0.25 and 1 Å, respectively. The default settings of Maestro 12.8 include other parameters such as sites, constraints, rotatable groups, and excluded volume (Friesner *et al*., 2006; Repasky *et al*., 2007).

Molecular Docking

The ligand and protein were prepared, and the grid on the protein's active site was defined. Subsequently, molecular docking procedures were conducted. The GLIDE employ a systematic computational simulation method to evaluate specific poses and ligand flexibility. The GLIDE systematic method is a novel, rapid, and accurate molecular docking approach. This method produces an output that is highly reliable and efficient. GScore, an empirical scoring function, is constructed by combining several parameters. The GScore is computed in units of kcal/mol and encompasses various factors such as internal energy, ligand-protein interaction energies, hydrogen bonds, hydrophobic interactions, p-p stacking interactions, and desolvation. The GLIDE module of the XP visualizer analyses the specific interactions between ligands and proteins (Friesner *et al*., 2004; Halgren *et al*., 2004).

Calculation of binding free energy by using MM-GBSA

Molecular mechanics generalized Born surface area (MM/GBSA) calculates the binding free energy using the single trajectory method. The MM-GBSA technique is often used to analyze the binding free energy of ligands-protein or protein-metal molecules. The Prime MMGBSA technique calculated $\Delta G_{(bind)}$ (relative binding-free energy) for each ligand molecule.

The formula is as follows:

$$\Delta G_{(bind)} = \Delta G_{(solv)} + \Delta E_{(MM)} + \Delta G_{(SA)}$$

Where:

$\Delta G_{(solv)}$ is the difference in GBSA solvation energy of the Chitinase-inhibitor complex and the sum of the solvation energies for unliganded chitinase and inhibitor.

$\Delta E_{(MM)}$ is a difference in the minimized energies between the Chitinase-inhibitor complex and the sum of the energies of the unliganded Chitinase and metal.

$\Delta G_{(SA)}$ is a difference in the surface area energies of the complex and the sum of the surface area energies for the unliganded Chitinase and inhibitor.

The Prime MM-GBSA software calculates the energy of optimized free ligands and receptors and the complex formed by the ligand and receptor. The ligand strain energy is calculated by placing the ligand in a solution automatically generated by the VSGB 2.0 suite. The prime energy visualizer provides a comprehensive visualization of energy.

RESULT AND DISCUSSION

Using the lower energy score, we identified the highest-ranking compounds in the set of inhibitors. When the binding energy decreases, the binding efficiency correspondingly increases, resulting in a more potent inhibition. Details of the docking score are mentioned in table 1.

Table 1 : Docking score of the different inhibitors

	Docking score (kcal/mol)	XP GScore (kcal/mol)	Glide emodel (kcal/mol)	Glide energy (kcal/mol)
Idarubicin	-7.906	-7.906	-71.653	-49.247
Propentofylline	-4.22	-4.220	-40.126	-32.002
Pentoxifylline	-4.071	-4.071	-40.203	-31.946
Dequalinium	-3.868	-3.868	-54.478	-43.967
IMI	-3.332	-3.332	-38.264	-29.47
Sangunarine	-2.672	-2.672	-34.332	-27.367

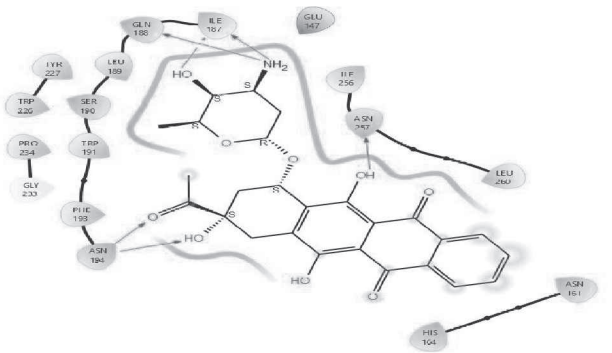


Figure 1 : 2D image of the Idarubicin docked with the chitinase.

The hydrophobic areas around the active site of chitinase are made up of amino acid residues such as ILE187, LEU 189, TRP 191, PHE 193, TRP 226, TYR 227, PRO 234, ILE 256, and LEU 260, whereas ASN 161, HIS 164, GLN 188, SER 190, and ASN 194 are polar amino acids. There is one negatively charged amino acid, GLU 147.

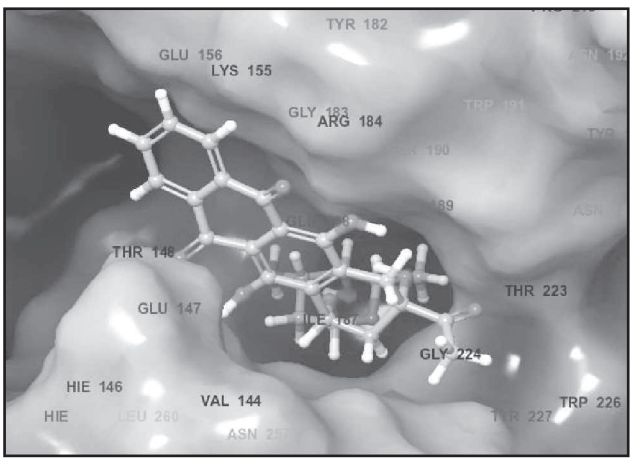


Figure 2: 3D image of the Idarubicin docked with the chitinase.

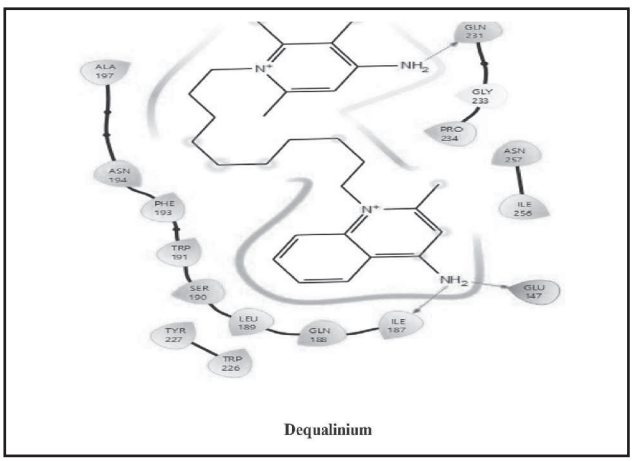


Figure 3: 2D image of different compounds docked with the chitinase.

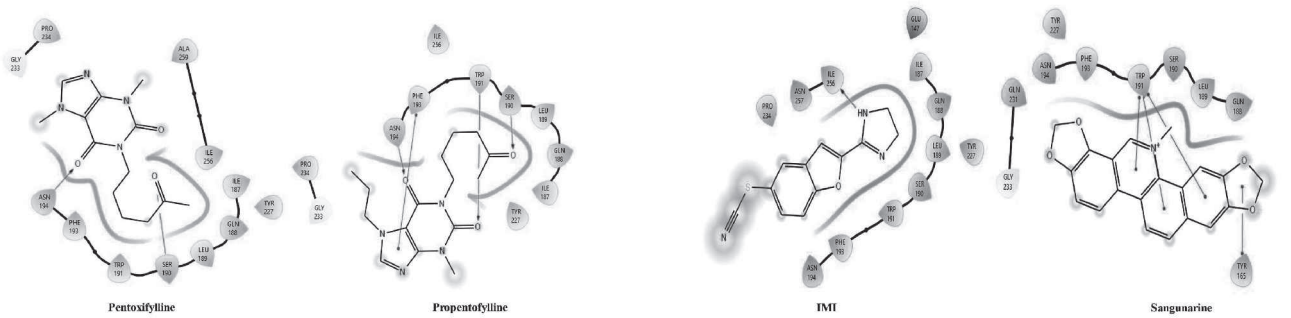


Figure 3: 2D image of different compounds docked with the chitinase.

MMGBSA calculations tend to support the estimation of molecular docking-derived binding energy. The MMGBSA dG Bind values in kcal/mol of different inhibitors are Idarubicin -42.16, Propentofylline -42.92, Pentoxifylline -36.39, Dequalinium -62.66, IMI -25.03, Sangunarine -45.41. Details are mentioned in table 2.

Table 2 : Calculation of binding free energy using MM-GBSA of different inhibitors.

	Docking score (kcal/mol)	Prime Energy (kcal/mol)	MMGBSA dG Bind (kcal/mol)
Idarubicin	-7.906	-8703.0	-42.16
Propentofylline	-4.220	-8794.3	-42.92
Pentoxifylline	-4.071	-8799.1	-36.39
Dequalinium	-3.868	-8719.3	-62.66
IMI	-3.332	-8667.9	-25.03
Sangunarine	-2.672	-8646.4	-45.41

A quantifiable binding pose or conformation of the ligand confined to the protein is predicted by the molecular interaction between the protein and the ligand-based on electrostatic interaction and its shape. The estimated sum of all observed interactions is the docking score of the ligand within the protein's binding pocket. The docking score is quantified in negative energy units (Kcal/mol), with a higher negative total energy indicating a more robust interaction between ligand and protein. The docking technique utilizes computational methods to determine the optimal binding conformation of substances within the protein's binding pocket. It also analyses the interaction between ligand and residues located at the enzyme's active site. Using extra precision (XP) ensures a highly accurate docking result. The inhibition of the enzyme relies on forming various types of bonds between amino acid residues and the ligand at the active site.

The initial aim of docking analysis is to determine the optimal conformation of a drug/compound of interest during interactions with the receptor, in order to minimize the binding free energy (Ferreira *et al*., 2015). Biomolecular docking algorithms often compute score functions to assess the binding energies of interactions between drugs and receptors (Tripathi and Bankaitis,

2017). The resulting binding energy (ΔG bind) is determined by combining many energies including torsional free vibrations, Hydrogen bonds, total internal, van der Waals dispersion, core repulsion, unbound system's desolation, electrostatic interactions, etc. The dissociation constant (Kd) is a measure of the binding energy represented by Gibbs' free energy (ΔG) (Hall *et al*., 2020). Drug-receptor binding prediction is influenced by several mechanisms including intermolecular interactions, solitude, and entropic effects. By increasing the estimate of the physio-chemical parameters, the precision of the scoring function is correspondingly enhanced (Agrawal *et al*., 2019).

MMGBSA

The binding strength of biological macromolecule and compound of interest is governed by the free energy of binding, denoted as ΔGbind. Conventionally, design campaigns are conducted through experimentation, which is a laborious and expensive approach. Hence, computational techniques have been devised to decrease the time and expenses involved in designing novel pharmaceuticals. The selection of the approach relies on the specific phase of the campaign in which it will be used, therefore including a standard balance between precision and effectiveness. Consequently, they fall between the accuracy and computing complexity of empirical scoring and rigorous alchemical perturbation approaches. These techniques have been implemented in several systems with different degrees of success. The integration of molecular mechanics energies with the Poisson-Boltzmann or generalized Born and surface area continuum solution (MM/PBSA and MM/GBSA) techniques is widely used for determining the free energy associated with the interaction between biological macromolecules and small ligands. Their effective use relies in the replication and rationalization of experimental results, as well as the enhancement of virtual screening and docking outcomes.

CONCLUSION

Here, we carried out the molecular docking of the bacterial chitinase (chitinase C from *Streptomyces griseus* HUT6037) with different inhibitors, such as Idarubicin, Propentofylline, Pentoxifylline, Dequalinium, IMI, and Sangunarine. Among all of them, Idarubicin has the highest binding affinity. The MMGBSA analysis also supports the same.

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Conflict of Interest

The authors declare no competing interests.

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