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Knowledge, Attitude and Practice (KAP) Study on Food Label Information available on Packed Food Items

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ABSTRACT

Many of the pre-packed food products, imported and locally manufactured, are now provided with nutrition information on their food labels. With the rising interrelationship between nutrition, health and life style among the population, this study was aimed to assess the knowledge, attitude and practices related to food label information available on packed food items. In this study, the association between knowledge, attitude and practice related to food label information among 100 respondents was studied. Self-structured study questionnaire was used. Nutrition education was also imparted through video and pamphlet. Feedback was taken with the help of google form from 100 random respondents. The overall knowledge, attitude and practice did not show significant correlation but association between knowledge and practice showed significant moderate positive correlation, also the association between practice and attitude showed statistically moderate positive correlation. The questionnaire was set to assess the purchase behavior of some common food items like beverages, staples, confectionary foods, spices, bakery, sauces and processed food items. This study also reveals that out of total respondents, majority of them 73 per cent preferred reading food labels because they were concerned about taste and price. The study revealed that male considered taste (97.67% male) the most important factor while female considered expiry date (92.98% female) the most important factor while buying packaged food items.

Keyword: Food labelling, Knowledge, Attitude, Practice, Association

INTRODUCTION

The phrase “You are what you eat” is exactly true. Food carries a variety of nutrients which are important for our bodies to function effectively. Getting consumers to eat healthily is no trivial task. One of the important tools in trying to bring about healthier eating figures has been food labeling. Consumers are highly demanding that they need information to make better choices in the food market. Public health policies aimed to reduce diet-related non-communicable diseases have started focusing more on displaying the nutritional value of food products through the use of “front of pack” nutritional-labeling schemes to increase the visibility of the nutritional quality of the food product so that it helps consumers interpret the healthfulness of food products (*Kleef, and Dagevos,* 2015). Food labels provide information on the nutritional content of packaged foods to consumers, which may influence consumption of nutrients such as energy, fat, saturated fat, trans-fats, sodium and added sugars (*Anastasiou, 2019*).To safeguard the

interest of the consumer, *The Food Safety and Standards (Packaging and Labelling) Regulations, 2011,* provides that every packaged food article has to be labeled and it provide the following information:

- The name of the food
- List of ingredients
- Nutritional information
- Declaration regarding vegetarian or non-vegetarian
- Declaration regarding food additives
- Name and complete address of the manufacturer or packer
- Net quantity
- Code No. / Lot No. / Batch No
- Date of manufacturing or packaging
- Best before and use by date
- Country of origin for imported food and
- Instructions for use

Grunert (2010) conducted a research study on nutrition knowledge, use and understanding of nutrition information on food labels among consumers in the UK and concluded that 27% of shoppers looked for nutrition information on food labels. *Bhilwar (2018)* conducted a study on the use of information on prepacked foods among residents of an urban village in South Delhi in India and concluded that the intention of promoting healthy food choices using food labels is met inadequately. Awareness generation activities were required to improve this behaviour. *Saha (2013)* conducted a study on Knowledge and Practices of using food label information among adolescents attending schools in Kolkata and concluded that there is a need to provide education on different aspects of label information to promote food label use. The effectiveness of alternative methods of nutrition information display should also be investigated. Keeping the above factors in mind, it was decided to a KAP study on food label information available on packed food items to study the knowledge, attitude and practices of the selected male and female consumers about food label information. and find out the association between knowledge, attitude and practice of the consumers about food label information.

MATERIALS AND METHODS

This cross-sectional study was conducted in the District Agra of Uttar Pradesh. The locale of this study was conveniently selected for the present study investigation. The selection of sample was done by random sampling (Uncontrolled Instrument Distribution Technique) with the help of offline survey questionnaire. The sample included 100 adults above 20 years upto 60 years who purchase packed food items.

Data was collected by using self-constructed proforma. The proforma was validated by subject experts. Data collection work was carried out by face-to-face interaction and the respondents, were asked to fill the proforma themselves. It took about 45 days for data collection and managing data entry for statistical analysis.

The proforma basically consists of five sections as section a, b, c, d and e. The sections were divided as demographic profile, food items purchased having food labels, knowledge-based questions, attitude-based questions and practice-based questions. First part included general information of the consumers, i.e., name, address, age, gender, education, occupation, religion, family type, family income per month, family members, vegetarian / non-vegetarian. The second part included the information regarding the food items used/purchased by the consumers having food label information, *i.e.*, beverages, confectionary food items, staple food items, spices items, bakery items, milk and milk products, fats and oil items, meat and egg

food items, processed foods, types of sauces and other food items. Third section included knowledge-based questions to assess the knowledge of consumers. Each question had two options, Yes or No, for each correct answer 1 mark was allotted. The fourth part included attitude-based questions to assess the attitude of consumers. Each question had four options: Most Important, Important, Least Important and not nmportant, fifth part included practice-based questions to assess the practice of the consumers. Each question had four options often, sometimes, rarely and never.

RESULTS AND DISCUSSIONS

A total of 100 respondents participated in the study. Out of which, 43 per cent males and females 57 per cent were in the age group of 20-60 years. Majority of the respondents were in the age group of 20-29 years. Almost all the respondents were literate and the majority (43%) of them were post-graduate. About 16% were students and 44 per cent were employed. Over 20 per cent of the adults and elderly were housewives and about 20 per cent were in business. Majority of the respondents (70%) belonged to nuclear families and 30 per cent belonged to joint families, out of which 90 per cent were Hindus and 10 per cent were Muslims.

A study was conducted on consumer awareness and status of food labelling in selected supermarkets in Puducherry *(Jain S.,et al., 2018)* showed that out of a total of 153 individuals, majority of them were graduate (80.4%) and none of them were illiterate.

Food items used by the consumers with food label information:

Eighty eight percent of respondents preferred to buy packed juices, followed by soft drink and water bottle with 75 per cent and 74 per cent respectively. In the purchase behavior of confectionary items, 73 per cent of the respondents preferred to buy packed cakes and chocolates, followed by 71 per cent who preferred to purchase packed cookies. In the purchase behavior of staple foods, purchase of packed rice and packed wheat flour were almost same about 67 per cent and 66 per cent respectively (Figure 1). In the purchase behavior of packed spices, 91 per cent purchased packed salt, followed by 71 per cent and 55% who preferred to purchase packed turmeric and packed garam masala (Figure 2). 85 per cent purchased white bread and 62 per cent purchased brown bread in purchase behavior of bakery products. In the purchase behavior of milk and milk products, purchase of milkmaid and cheese was highest with 62 per cent and 57 per cent respectively. In the purchase behavior of fats and oil, majority of the respondents preferred to buy butter (81%), followed by ghee and mustard oil with 72 per cent each (Figure 3). Purchase of Kurkure in processed food was highest among all with 97 per cent and purchase of tomato ketchup with 91 per cent was highest in the category of sauces. A cross-sectional study conducted in Delhi, across the generations showed that the purchase of ready-to-eat packaged food was influenced by children in the family (Mediratta, and Mathur, 2023).

Knowledge of the consumers about food label information:

Among all the respondents, 83 per cent of the respondents considered reading food label information while 17 per cent did not read food label information, followed by 23 per cent of the consumers who always bought prepackaged food and 78 per cent did not buy prepackaged food. Eighty nine per cent respondents believed that food label

Figure 1- Distribution of the Respondents according to purchase behaviour of Staple with food label information.

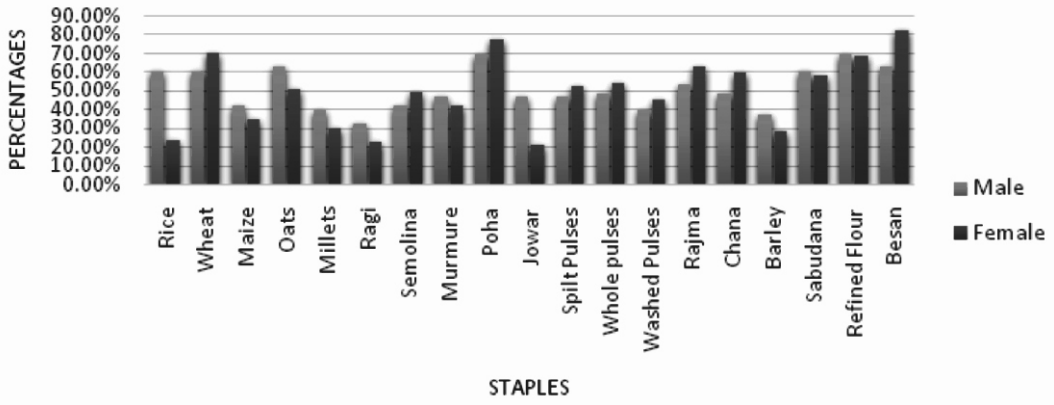


Figure 2- Distribution of the Respondents according to purachse behaviour of Spices with food label information

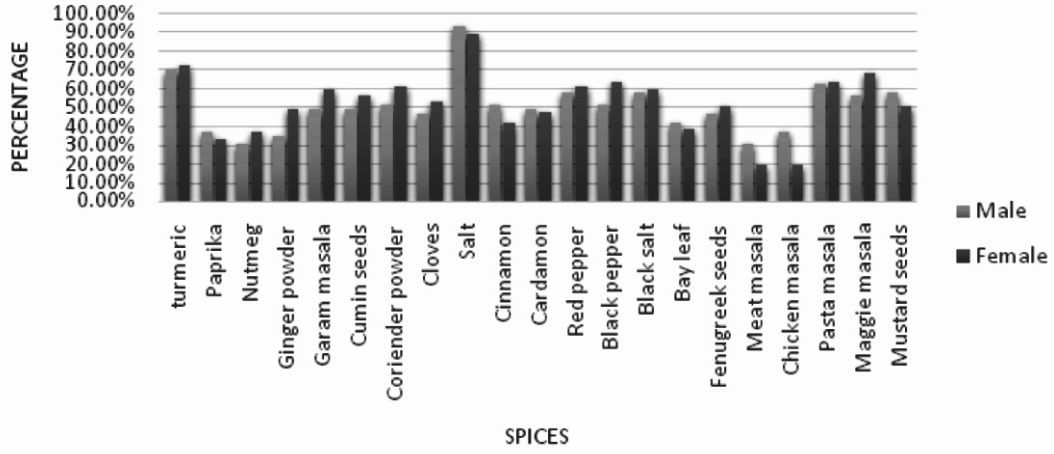
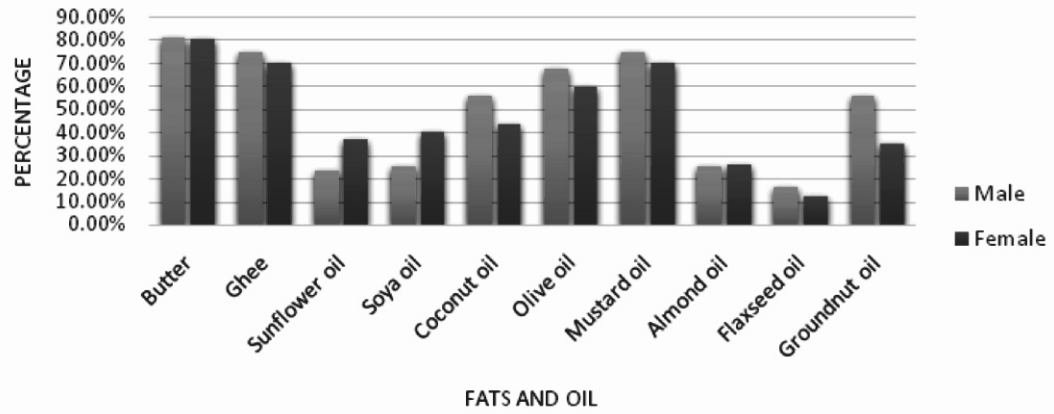


Figure 3- Distribution of the Respondents according to purchase behaviour of Fats and Oil with food label information.



influences nutritional composition, followed by 100 per cent respondents who believed that food labels are useful tool for consumers, only 13 per cent consumers thought that nutrient information is not accurate on food labels, followed by 19 per cent consumers who did not understand food label easily. It was also observed that there is no statistically significant difference between the level of knowledge among males and females on food labelling. Therefore, our hypothesis (H1) is rejected. It means that the level of knowledge among both the group was statistically insignificant i.e.,both group were having equal knowledge on food labelling ($p<0.05$).

A study was conducted on knowledge and practices of using food label information among adolescents attending schools in Kolkata, India by Saha (2013) showed that out of 316 respondents, about 66% believed that nutrition information on labels was too complex to understand, about a half of the adolescents considered labels useful for making food choices, and one third of them thought that the labels were sometimes useful and only 6 per cent thought that labels was not useful for choosing foods.

Attitude of the consumers about food label information

Male considered taste (97.67%) as the most important factor while female considered expiry date (92.98%) as the most important factor. Method of preparation was considered as important factor by only 44.18 per cent male while only 50.87 per cent female considered method of preparation as important. For male, price (9.34%) was least important. Female considered packaging and method of preparation (8.77 female each) as least Important, followed by male who considered method of preparation (11.62% male) as Not Important while female also considered method of preparation (5.26% female) as Not Important (Table 1). It was also observed that there is no statistically significant difference between the level of attitude among male and female on food labelling.

A study was conducted on use of information on prepacked foods among residents of an urban village of South Delhi, India (*Bhilwar, 2018)* showed that a majority (222/368; 60%) of participants brought prepacked food because they liked taste and also because they were easily available (153/368; 41.7%). In a study conducted by *Mediratta and Mathur (2023)* in Delhi was observed that a higher percentage of men (35%) reported that they were influenced by food brands (P = 0.01) as compared with (26%) women and a higher percentage of women reported that they were influenced by recommendations by friends or family (10% compared with 8%, P = 0.02) and advertisements (6% compared with 2%, P = 0.004) as compared with men.

Practice followed by the consumers about food label information:

Male often practised reading production and expiry date (88.37%), female often practised the same (96.49%). Male sometimes practised reading storage and direction of use (58.13%) while females also sometimes practised reading storage conditions (49.12%), while males rarely practised direction of use (9.30%) while female rarely practised reading about allergen information (26.31%). It was also observed that there is no statistically significant difference between the practices among males and females on food labelling. Therefore, our hypothesis (H3) was rejected. It means that the level of practice among both the group was

Table 1: Attitude of the consumers about food label information

Frequency	Most Important		Important		Least Important		Not Important		Total		
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Sum
	%	%	%	%	%	%	%	%	f	f	f
Price	83.72	73.68	6.97	19.29	9.30	7.01	0	0	43	57	100
Taste	97.67	91.22	2.32	8.77	0	0	0	0	43	57	100
Nutrient content	81.39	78.94	16.27	19.29	2.32	1.75	0	0	43	57	100
Ingredient	81.39	64.91	18.60	28.07	0	7.01	0	0%	43	57	100
Packaging	62.79	57.89	32.55	31.57	2.32	8.77	2.32	1.75	43	57	100
Expiry Date	90.69	92.98	9.30	7.01	0	0	0	0	43	57	100
Method of preparation	37.20	35.08	44.18	50.87	6.97	8.77	11.62	5.26	43	57	100

statistically insignificant i.e., both groups were following equal practices related to food labelling ($p<0.05$) (Table 2).

Table 2: Practices followed by the consumers about food label information (n=100)

Frequency	Often		Sometimes		Rarely		Never		Total		
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Sum
	f	f	f	f	f	f	f	f	f	f	f
List of ingredients	37	49	5	6	1	2	0	0	43	57	100
Serving size	23	33	17	19	3	2	0	3	43	57	100
Sugar	25	27	14	24	3	4	1	2	43	57	100
Health claims	25	32	11	19	6	6	1	0	43	57	100
Reading Production and Expiry Date	38	55	4	2	1	0	0	0	43	57	100
Looking at Price	35	48	4	5	1	1	3	3	43	57	100
Looking at Brand Name	34	40	4	16	3	0	2	1	43	57	100
Reading Storage Conditions	13	21	25	28	2	8	3	0	43	57	100
Direction of Use	11	19	25	26	4	10	3	2	43	57	100
Reading Alarms	12	21	21	22	4	12	6	2	43	57	100
Allergen information	14	23	17	15	3	15	9	4	43	57	100
Place of manufacturing/ import	6	17	22	20	3	9	13	10	43	57	100

Association between knowledge, attitude and practice of consumers:

There is no statistically significant correlation between knowledge and attitude. There is a statistically significant moderate positive correlation between knowledge and practice. There is a statistically significant moderate positive correlation between attitude and practice.

Table 3: Association of variables of consumers about food label information

Variable	Mean± S.D	r-Value	p-Value	Remark
Knowledge Attitude	4.62 ±0.956 18.58±2.150	0.150862	0.134232	Not Significant at <0.05
Knowledge Practice	4.62±0.956 28.27±5.373	0.463363	<0.00001	Significant at <0.05
Attitude Practice	18.58±2.150 28.27±5.373	0.277233	0.005271	Significant at <0.05

CONCLUSION

The food and nutrition label provides the nutrition information that helps consumers to make judicious food choices and they can compare different foods easily with food labels with respect to price, nutrition content, ingredients, manufacturing and expiry date.

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Influence of Gender Difference on Information-seeking Behaviour of the Students

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ABSTRACT

This study was an attempt to assess whether gender as a variable is associated with the information-seeking behaviour of post-graduate students. A descriptive survey research design used for study, and data were collected with the help of a structured questionnaire. A total of 378 questionnaires were returned out of a total of 464 that were sent out. Most components of information seeking behaviour were shown to be significantly different between males and females. Findings of the study showed that gender influenced the frequency of library visits, the goal of seeking information, information sources consulted, and the purposes of library visits as the calculated P-values for all these aspects, i.e.,.0037,. 0044, .0029, .0026 respectively are less than the significance level of.01. while not significantly associated with the amount of time students spend in the library, internet usage for specific purposes, browsing of various e-resources, and the extent of e-resource usage, as the calculated P-value for these aspects is higher than the significance level (0.10). The findings could have implications for psychological study and practise, particularly in the areas of educational institutions, library science, cognitive development, and training and performance appraisal.

Key words: Information -seeking behaviour, Post-graduate students, Gender-differences, University

INTRODUCTION

The practise of gathering information from diverse sources, such as formal, informal, and electronic sources, is known as information seeking. It is the process through which people look for and use information in order to meet their information needs. The manner in which people seek information differs from one person to another. The personal, emotional, educational, and demographic aspects of the person seeking information influence his or her information-seeking behaviour. Due to their various characteristics and requirements, students' information-seeking behaviour has also varied. As a result of the digital revolution, information distribution services have changed dramatically. Most people find it challenging to appreciate the dynamic causes, techniques, and implications of these changes in the information environment. Some social psychology depicts physical, mental, and social disparities between men and women in society. Gender has been identified as a possible effect variable in information seeking behaviour in various studies. Hsieh and Wu (2015) discovered that

children's information seeking patterns are influenced by their gender. Boys cared a lot about information accessibility, according to the study, and tended to surf rapidly and accomplish their task by clicking each symbol and haphazardly glancing over the contents. The females, on the other hand, valued information diagnostically more and searched more cautiously, pausing the cursor on the screen for longer periods of time. Ahmad (2019) conducted research with the goal of understanding and analysing the information demands and information seeking behaviour of undergraduate students in the Brarmulla District of Jammu and Kashmir State. The study's findings revealed that there is a significant gap in information demands and information seeking behaviour between men and women. According to the study, the majority of male students go to library twice a week, whereas the majority of female students go once a week. According to research, textbooks are preferred by the majority of students across the genders, with 47.36 per cent of male students and 55.76 per cent of female students preferring them.

Gender as a variable could be useful in better understanding the cognitive and social environment of human information processing, and it could have major implications for information dissemination services and systems. As a result, in order to better support university students in their information-seeking procedures, it is vital to explore gender-specific disparities in their information-seeking behaviours.

MATERIALS AND METHODS

The research was carried out at Rajasthan University in Jaipur. Students enrolling in PG degree programmes for the academic year, 2019-2020 at the University of Rajasthan Jaipur's eight faculties (Science, Arts, Commerce, Education, Fine Arts, Law, Social Science, and Engineering and Technology) were included in the study. A descriptive survey study design was used to collect data via a questionnaire. Questionnaires were distributed to 464 randomly selected postgraduate students from all eight faculties at the University of Rajasthan Jaipur. A total of 378 completed questionnaires were received, reflecting a response rate of 81.46 per cent. Statistical techniques ie:- percentage, Chi-square test, t-value were used for interpreting the data and testing the hypotheses.

RESULTS AND DISCUSSION

Dependent variable of the study is "information seeking behaviour", which means, students' way of information exploration by using the library and the Internet (information resources). The selected aspect of information seeking behaviour taken as dependents variable were: The purpose of seeking information, types of information sources consulted, Frequency visits of using the library, time spent in the library, purposes of use of the library, purpose of using the internet, Information resources browsed on the internet.

The association between independent variable (gender) and information seeking behaviour is presented below:

1. Gender and purpose-wise seeking information: Table- 1 reveals the relation between the gender and the purpose-wise seeking information by the respondents.

Table 1: Gender and purpose-wise seeking information n=378

Purpose of information seeking	Gender				Total
	Male		Female		
	f	%	f	%	
Routine academic information needs	121	48.4	129	51.6	250
Career counselling and employment	128	55.6	102	44.4	230
Students' union election	67	34.7	126	65.3	193
Library related	48	46.6	55	53.4	103
Social security benefits	65	42.5	88	57.5	153
Co-curricular activities	60	54.5	50	45.5	110
Associated curricular activities	94	49.5	96	50.5	190
Competency development	115	56.1	90	43.9	205
Research work	91	48.9	95	51.1	186

X2 value =28.1596, df= 8, The P-Value is .000448. Significant at p < .01.

Table-1 presents that among 250 students who sought information for academic information (routine), 51.6 per cent were female students and 48.4 per cent were male students. In a total of 193 respondents who sought information regarding the students' union election, the majority of students were female (65.3%) as compared to male peers (34.7%). Male respondents made up more than half of those seeking information on career counselling and employment (55.7%), competency development (56.1%), and co-curricular activities (54.6%). Female respondents were more concerned with social security benefits (57.5%), library-related information (53.4%), and research work information (51.1%). The results are in accordance with the findings of Khorsrowjedrdi and Iranshahi (2011).

Thus the results show that there is a considerable difference between respondents' gender and the various purposes for which they seek information.

This can be statistically proved as the P-value (.0004) is less than the significance level (0.01) at 8 degrees of freedom. Hence it is statistically defensible. As a result, the null hypothesis must be rejected. It shows that there is an association between the variables being compared. Thus, we conclude that gender is associated with the purpose of seeking information by the respondents. Tilahun and Natarajan (2016) and Halder (2013) focused that gender as a major factor that influences the information requirements and information-seeking behaviour of students.

As compared to female students, Male students seek information more regarding competency development, career counselling, co-curricular activities, and related to students' union elections, it mbe due to the fact that males are more interested and active for these activities, whereas female students seek information about social security benefits because they appear to be more concerned about security than males.

2. Gender-wise information sources consulted: Figure -1 shows the comparison between type of information sources consulted and gender of the respondents.

Findings indicates that 56.6 per cent of respondents are male and 43.4 per cent are female who consult academic libraries (n=286). Out of the total number of respondents consulting faculty (n=240), female respondents are a bit higher (52.9%) than male respondents (47.1%). The results also show that males have a slightly higher internet score than females. Among respondents consulting informal sources like

family and friends, male and female respondents accounted for 58.0 per cent and 42.0 per cent, respectively. University administration was also consulted by a higher number of male (72.7%) as compared to female respondents (27.9%).

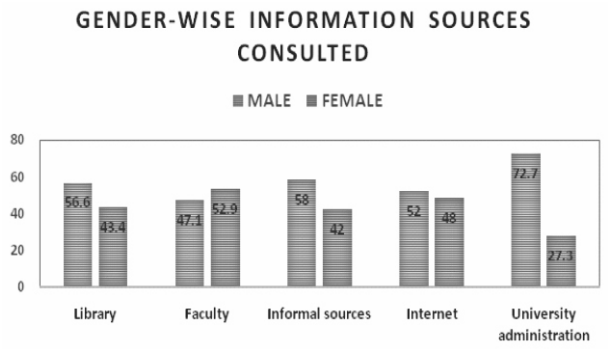


Figure 1 : Gender-wise information sources consulted

X2 value =16.0279, df= 4, The P-Value is. 00298. Significant at p < .01.

The above Figure -1 clearly depicts that the genders of the students and the information sources consulted by the respondents differed significantly. The P-value (.00298) is lower than the significance level (0.01). Thus, it calls for the rejection of the null hypothesis. It reveals that there is an association between the compared variables.

Results show that females respondents consult faculty most for information and males consult university administration more as compared to female respondents. This may be due to that females are more likely to go with hierarchy and males seem to go directly to administration or higher levels of information system.

3. Gender wise usage of library: Figure- 2 explores the relation between gender of respondents and frequency of library usage in a university environment.

It is found from Figure-2 that, out of total number of respondents who are using the library every day (n=133), males account for 64.66 per cent more of the total than female respondents (35.3%). While 55.6 per cent of the students who use library every alternative day are female, 44.4 per cent are male.

Out of 83 respondents using the library once a week, 56.6 per cent are male and 43.4 per cent are female respondents. Among the total number of respondents using the library monthly (n=17) , 64.7 per cent of them are female and 35.3 per cent are male respondents. The ratio of respondents who visited the library fortnightly and occasionally skewed towards female respondents.

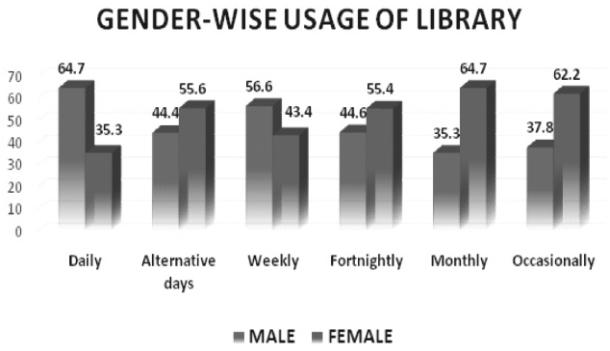


Figure 2: Gender-wise usage of library

X^2 value =17.4189, df= 5, The P-Value is. 00377. Significant at p < .01.

The above Figure-2 shows that there is a significant difference between gender and the frequency with which students use libraries. The P-value (.00377) is less than the significance level (0.01) for 5 degrees of freedom, hence it is statistically defensible. As a result, the null hypothesis must be rejected. It demonstrates that there is a link between the variables being compared. As a result, we conclude that post-graduate students' use of the library is associated to their gender. The study is in line with the study of Tilhahun and Natarajan (2016), which states that gender influences the frequency of visits to the library.

Above description shows that boys are more regular in visiting the library daily and girls visit the library more on alternate days,

occasionally and fortnightly. This may be because of differ lifestyle between males and females. Girls usually study in hostel rooms only as there are fewer disturbances compared to boys' hostels. Day scholar girls may have household responsibility also adverse to boys. Maybe that's why the majority of boys visit the library daily and girls visit on alternate days.

4 Gender-wise purposes of use of library: Table 2 depicts that out of 288 respondents used for borrowing books, a majority were female (52.1%). Among the respondents who visited the library to access reference resources, 56.2 per cent were male and 43.8 per cent were female. Out of 227 respondents using the library to access periodicals, 52.0 per cent were male and 48.0 per cent were female. Similarly, using e-resources , reading newspapers and going to the library solely for study purposes are skewed towards male students.

Furthermore, among 197 respondents who visited libraries for thesis and project reports, the percentage of females (55.3%) was higher than males (44.7%).

Table 2: Gender-wise purposes of use of the library n=378

Purposes of use of the library	Gender				Total
	Male		Female		
	f	%	f	%	
Borrowing books	138	47.9	150	52.1	288
Reprography service	96	66.2	49	33.8	145
Access thesis and project reports	88	44.7	109	55.3	197
Access reference resource	150	56.2	117	43.8	267
Access e-resource	63	60.0	42	40.0	105
Read news paper	132	54.0	113	46	245
Study purpose only	138	53.3	121	46.7	259
Access periodicals	118	52.0	109	48.0	227

X^2 value =21. 9229, df= 7, The P-Value is .00262. Significant at p < .01

Therefore it is clear from the above data , that there was a marginally significant difference between the different purposes of use of the library and the gender of the respondents. It can be statistically justified as the P-value (0.00262) is smaller than the level of significance (0.01) at 7 degrees of freedom. Thus, the null hypothesis must be rejected. It depicts that there is a relation between the compared factors. Thus, we conclude that gender is associated with the purposes of use of libraries by the respondents.

5. Gender-wise time spent in the library: Figure- 3 shows that, out of total of respondents (n=87) who spent time up to one hour per day on library visits, males were 58.6 per cent and females were 41.4 per cent. Results also show that who spent one to two hours per day on library visits (n=117), 54.7 per cent were male and 45.3 per cent were female. Among the respondents 103, who spent two to three hours per day on library visits, males were 57.3 per cent and females, 42.7 per cent.

A higher proportion of male respondents (60.4%) spend three to four hours per day in libraries than females (39.6). A similar trend was also followed in the case of spending more than four hours per day on library visits.

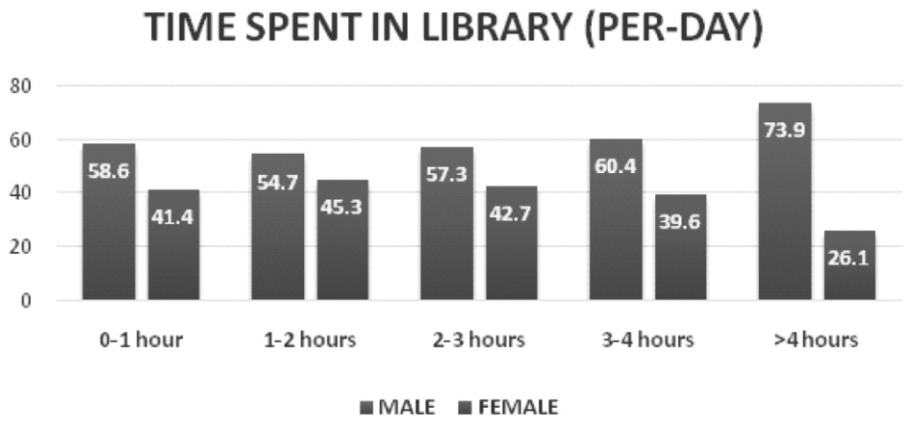


Figure-3 : Gender-wise time spent in the library

X2 value=3.0258, df=4, The P-Value is .5535. Not significant

It is clear from the figure-3 that there was no significant difference between the time spent by students in the library and genders of the respondents.

The P-value (0.5535) is higher than the significance level (0.10) at 4 degrees of freedom, hence it is statistically proved As a result, the null hypothesis must be accepted. Thus, it is concluded that gender has no influence on the time spent by the students at the library.

6. Gender and the purpose-wise usage of internet: Table 3 depicts the respondents' gender and the purpose-wise usage of the internet.

Table 3: Gender and the purpose-wise usage of internet n=378

Purpose of usage of internet	Gender				Total
	Male		Female		
	f	%	f	%	
Reading	111	50.4	109	49.6	220
Literature search	130	46.4	150	53.6	280
Employment/entrepreneurship purpose	76	55.5	61	44.5	137
To update knowledge	137	52.7	123	47.3	260
Means of communication	93	54.7	77	45.3	170
Database search	91	53.5	79	46.5	170
Research purpose	114	48.7	120	51.3	234

X^2 value = 5.612, df = 6, The P-Value is .4679. Not significant at p < .10.

It is found from Table 3 that, among the 220 respondents using the internet for the purpose of reading on the Internet, male students (50.4%) and female students (49.6%) were more or less equal. However, among respondents (280) literature searches, 46.4 per cent were male and 53.6 per cent were female.

Out of 170 respondents using the internet for database search, 53.5 per cent were male and 46.5 per cent were female. However, the per cent of respondents using the internet for

research purposes among females is a bit higher than their male counterparts. Of the 170 respondents using the internet for communication purposes, 54.7% were males and 45.3% were female. Similarly, males were a bit higher than female students in respect to the use of the internet to update knowledge and to access information for entrepreneurship/ employment.

It is clear from the above table that there was no significant difference between the gender of the respondents and the different purposes for which they used the internet. It can be statistically justified as the P-value (.4679) is higher than the significance level (0.10) at 6 degrees of freedom. Thus, it calls for the acceptance of the null hypothesis.

7. Gender-wise browsing of E-sources by the respondents: Table-4 indicates the association between gender and use of e-sources by the Post Graduate students.

Table 4 : Gender-wise browsing of different e-sources n=378

E-information -sources	Gender				Total
	Male		Female		
	f	%	f	%	
E-periodicals (journals & magazines)	79	46.5	91	53.5	170
E-books	108	52.7	97	47.3	205
Digital library	67	53.6	58	46.4	125
E-newspaper	89	52.3	81	47.7	170
E-thesis	77	48.1	83	51.9	160
Website resources	164	51.4	156	48.6	320
Online-database	71	51.4	67	48.6	138
Other information sources	43	53.7	37	46.3	80

X^2 value = 2.880, df= 7, The P-Value is .895. *Not* significant

Table- 4 indicate that, out of 205 students who browse e-books , the proportion of usage among male (52.7%) and female (47.3%) respondents was more or less the same. As far as accessing e-periodicals by the respondents (n=170), more than 50 per cent of the sample population were female (53.5%). Among the respondents using e-thesis, 51.9 per cent were female and 48.1 per cent were male. Among the total number of students (n=125) students assessing the digital library, 53.6 per cent were male and 46.4 per cent were female. A similar trend was found regarding the use of online databases and website resources, which were used by male and female students, similarly. Khan and Nisha (2017) revealed that online journals are more commonly used by female students than their male counterparts, whereas the utilisation of websites among both female and male students is similar.

Results found that there is no considerable variation between gender and the various information resources browsed on the internet by the respondents. It can be statistically proved as the P-value (.895) is greater than the level of significance (0.01) at 7 degree of freedom. Thus, it calls for the acceptance of the null hypothesis.

When the Chi-square test is applied to examine the association between the gender of the respondents and information sources browsed on the internet, it is found that there is no considerable association between the

compared aspects. Thus, it calls for the acceptance of the null hypothesis. Further, when the Pearson's r test is applied to see the level of association, it is found that gender is not associated with various information resources browsed on the internet by the respondents at a 0.10 significance level.

8. Gender-wise extent of E-resources usage: Figure-4 shows the relation between extent of e-resources usage and gender of the respondents.

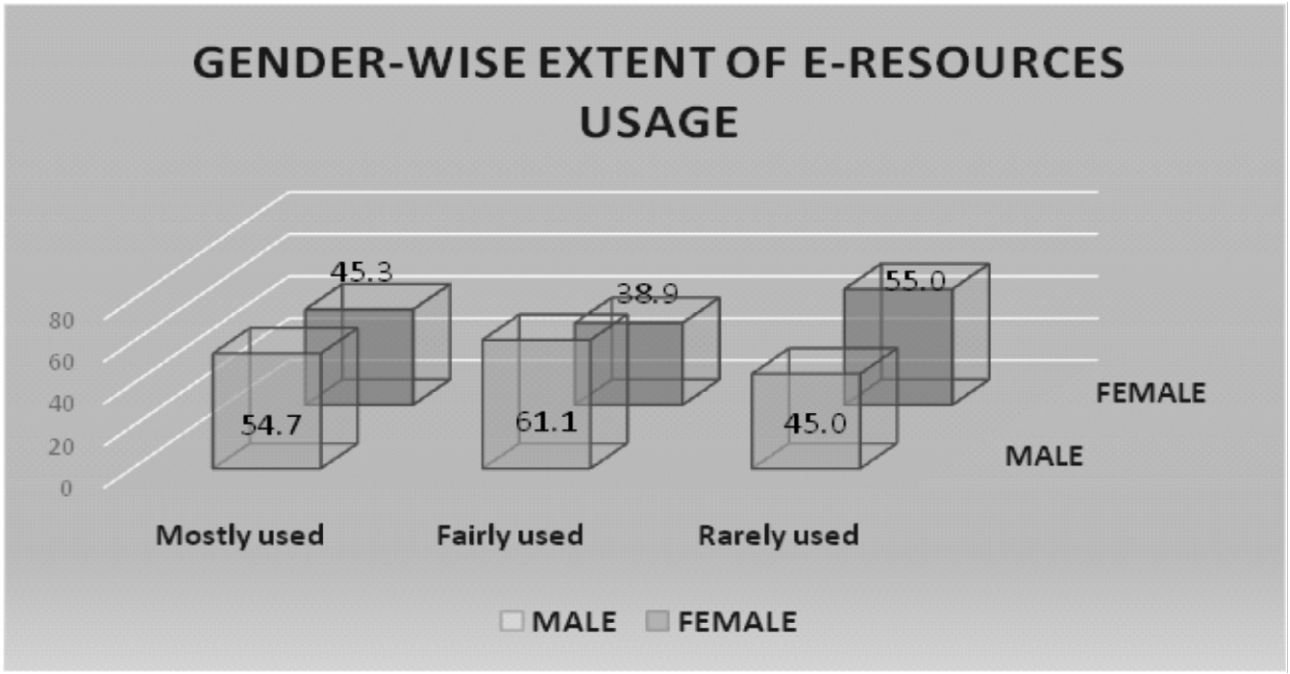


Figure-4: Gender-wise extent of e-resources usage

X2 value = 3.264, df = 2, The P-Value is .1954. Notsignificant at p < .10.

Figure- 4 presents that, among the total number of students (n=225) who mostly used E-resources for various information, most of them were male students (54.7%). Similarly, out of 113 respondents (who used e-resources, fairly), 61.1 per cent were male and 38.9 per cent were female. More than fifty per cent of respondents who rarely used e-resources for seeking various types of information were female (55.0%). The findings are similar with the findings of Khan and Nisha (2017).

A Chi-square test is applied to test the association between the extent of E-resources used and the gender of respondents, which reveals that there is no significant variation between the gender of the respondents and the extent of their use of e-resources It can be statistically justified as the P-value (.1954) is greater than the significance level (0.10) at 2 degrees of freedom. Thus, it calls for the acceptance of the null hypothesis.

CONCLUSION

The act of actively seeking information in order to meet an information need is known as information-seeking behaviour. This is influenced by the person seeking

information's personal, emotional, educational, and demographic characteristics. Several research have identified gender as a possible influencing factor in information seeking behaviour. Gender influenced the frequency of library usage, the goal of seeking information, the information sources consulted, and the purposes of library use, according to the study's findings. While not significantly related to the amount of time students spend in the library, internet usage for specific purposes, browsing of various e-resources, and the level of internet usage.

The findings could have implications for psychological research and practise, particularly in the areas of educational institutions, library science, cognitive development, and training and performance evaluation. As a result, in order to effectively help university students in their information-seeking procedures, it is vital to explore gender-specific disparities in their information-seeking behaviours.

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Bio-efficacy of Insecticides against Thrips, *Scirtothrips dorsalis* Hood in Pomegranate

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ABSTRACT

An experiment was conducted on farmers' field at Bhadaran village of Anand district during *Hast bahar* season 2017 and 2019 to determine the efficacy of different insecticides against the thrips, *Scirtothrips dorsalis*in pomegranate (*Bhagwa*) by using Completely Randomized Design (CRD) with four repetitions. The lowest (3.42 thrips/10 cm shoot and 7.91 thrips/fruits) population of thrips were found in trees treated with spinosad 45% SC @ 0.01% and it was at par with cyantraniliprole 10.26% OD @ 0.008% (3.87 thrips/10 cm shoot and 8.38 thrips/fruits) followed by buprofezin 15% + acephate 35% 50 WP @ 0.063% (4.25 thrips/10 cm shoot and 8.80 thrips/fruit). The highest fruit yield was recorded from the plot treated with spinosad 45% SC @ 0.01% (110.50 q/ha) and it was at par with cyantraniliprole 10.26% OD @ 0.008% (106.42 q/ha) and followed by buprofezin 15% + acephate 35% 50 WP @ 0.063% (103.75 q/ha) as compared to the rest of treatments.

Keywords : Thrips, *Scirtothrips dorsalis* insecticides and Pomegranate

INTRODUCTION

The pomegranate crop (*Punica granatum* L.) suffers from the attack of several insect and non-insect pests. Eighty-six species of insect pests infesting pomegranate have been reported from various parts of the world (Zirpe, 1966). It is widely grown in Ahmedabad, Sabarkantha, Banaskantha and Kheda districts of Gujarat state. Among the major pests of pomegranate, Sucking pests infest the crop during flowering and fruiting stage of the crop and thereby reduce the vigour of the plant in addition to excretion of honeydew on the leaves and development of sooty mould on leaves and fruits (Dong *et al.,* 2014). Among sucking pests, thrips cause more damage to the crop by reducing the fruit set and also the quality of the fruit. The main thrips species on pomegranate is *Scirtothrips dorsalis* Hood which is often seen on leaves and also on young fruits causing characteristic scab on fruits and thereby reducing the market and export value as well as developing fruits contributing huge losses both quantitative and qualitative.

There are three main fruiting seasons in a year which are locally known as bahars *viz*. *Ambe* (March to June),*Mrig* (July to October) and *Hasta* (November to February). Among three bahars, *Hasta* bahars is most favourable season to perpetuate the pests during the year but it also remains associated during all the three bahars of the pomegranate. Nymphs and adults lacerate and suck the contents of leaves, buds, flowers, and fruits. As a result, leaf tips turn brown and get curled due to the feeding of thrips on the underside of the leaves by rasping the surface and sucking the oozing cell-sap leading to drying and shedding of flowers. Scrapping on fruits leads to scab

formation. Due to a lack of alternate effective pest control methods, chemical insecticides are widely used by farmers in combating *S. dorsalis* on pomegranate in Gujarat. This crop has great export potential, therefore, newer combi-molecules reported to be effective against thrips on other crops were tested to see their potential in combating *S. dorsalis* on pomegranate. Keeping in view of negligible work on pomegranate, based on the review of literature, the present experiment was proposed to find out effective insecticides for the better control of the pest.

MATERIALS AND METHODS

In order to study the evaluation of different insecticides against the thrips, Scirtothrips *dorsalis*in pomegranate, a field experiment was carried out on farmer's field during *Hast bahar*season 2017 and 2019 at Bhadaran village of Anand district in Completely Randomized Design (CRD) with 6 (Six) treatments and 4 (Four) repetitions (One plant considered as one repetition) each having plot size of 3.0 x 2.0 m. The existing pomegranate orchard variety *Bhagwa* having equal growth, age and canopy at a spacing of 3 m x 2 m. The pomegranate crop was raised by following standard agronomical practices except for pest control measures. The treatments imposed are as follows : T_1Acephate 50% + Imidacloprid 1.8% - 51.8 SP,518 g a.i/ha, T_2Buprofezin 15% + Acephate 35% - 50 WP, 625 g a.i/ha, T_3Profenophos 40% + Cypermethrin 4% - 44 EC, 660 g a.i/ha, T_4Cyantraniliprole 10.26% OD,76.9 g a.i/ha, T_5 Spinosad 45 % SC, 100 g a.i/ha and T_6 control.The first spray was applied when the thrips population reach to 5 thrips/10 cm shoot. The second spray was done after 15 days of the first spray. Spray fluid was applied to the extent of slight runoff using a foot sprayer with triple action nozzle. The population of thrips (nymphs and adults) was recorded on randomly selected four fruits on each tree from each direction (East, West, South and North). The population of thrips on shoots was recorded on four randomly selected shoots from each direction. The counts of thrips on both fruits and shoots were recorded one day before the first spray and subsequently at 5, 10 and 15 days after each spray. Picking wise fruits were collected from each treatment and weighed. Based on these data, the yield of healthy fruits per hectare was calculated for each treatment. The data obtained thus were, subjected to statistical analysis after appropriate transformation to draw valid conclusions.

RESULTS AND DISCUSSION

The periodical data on thrips population per shoot and fruit as well as the yield of fruits in different treatments during 2017 and 2019 are presented in Table 1 to 6, respectively. The efficacy of different insecticides against thrips is adjudged based on pooled over periods.

Thrips population on shoot

First year (2017) First spray

The population of thrips was found non-significant before spray which indicated homogenous distribution of the pest. The results on pooled over the first spray showed the lowest (4.84 thrips/10 cm shoot) number of thrips on trees treated with spinosad 45% SC @ 0.01% and it was at par with cyantraniliprole 10.26% OD @ 0.008% (5.26 thrips/10 cm shoot) and buprofezin 15% + acephate 35% 50 WP @ 0.063% (5.75 thrips/10 cm shoot). Next effective group based on thrips population was acephate 50% + imidacloprid 1.8% - 51.8 SP @ 0.052% andprofenophos 40% + cypermethrin 4% 44 EC @ 0.066% which recorded 8.62 and 9.05 thrips per 10 cm shoots, respectively.

Second spray

Data of pooled over second spray revealed that all the treatments were found significantly superior to the control. The lowest (2.60) number of thrips was found in trees treated with spinosad 45% SC @ 0.01% and it was at par with cyantraniliprole 10.26% OD @ 0.008% (2.96 thrips/10 cm shoot) and buprofezin 15% + acephate 35% 50 WP @ 0.063% (3.34 thrips/10 cm shoot). Acephate 50% + imidacloprid 1.8% - 51.8 SP @ 0.052% andprofenophos 40% + cypermethrin 4% 44 EC @ 0.066% emerged out as next effective group of treatments.

Pooled over sprays

In case of pooled over sprays, the lowest (3.66 thrips/10 cm shoot) thrips population was observed in trees treated with spinosad 45% SC 0.01% and it was at par with cyantraniliprole 10.26% OD @ 0.008% (4.04 thrips/10 cm shoot) and buprofezin 15% + acephate 35% 50 WP @ 0.063% (4.47 thrips/10 cm shoot). Trees treated with acephate 50% + imidacloprid 1.8% 51.8 SP @ 0.052% andprofenophos 40% + cypermethrin 4% - 44 EC @ 0.066% were 7.28 and 7.85 thrips per 10 cm shoot of pomegranate, respectively.

Second year (2019)

First spray

In case of second year, the results on pooled over first spray indicated that the lowest (4.38 thrips/10 cm shoot) number of thrips was noticed in trees treated with spinosad 45% SC @ 0.01% and it was at par with cyantraniliprole 10.26% OD @ 0.008% (4.93 thrips/10 cm shoot) followed by buprofezin 15% + acephate 35% 50 WP @ 0.063% (5.60 thrips/10 cm shoot). Next effective group based on thrips population was acephate 50% + imidacloprid 1.8% 51.8 SP @ 0.052% andprofenophos 40% + cypermethrin 4% 44 EC @ 0.066% which recorded 7.85 and 8.68 thrips per 10 cm shoots, respectively.

Second spray

Data of pooled over second spray showed that all the treatments were found significantly superior to the control. The lowest (2.19 thrips/10 cm shoot) number of thrips was recorded in trees treated with spinosad 45% SC 0.01% and it was at par with cyantraniliprole 10.26% OD @ 0.008% (2.60 thrips/10 cm shoot) followed by buprofezin 15% + acephate 35% 50 WP @ 0.063% (2.85 thrips/10 cm shoot). Acephate 50% + imidacloprid 1.8% 51.8 SP @ 0.052% andprofenophos 40% + cypermethrin 4% 44 EC @ 0.066% emerged out as next effective group of treatments by recording 5.65 and 6.31 thrips per 10 cm shoots of pomegranate.

Pooled over sprays

Results of pooled over sprays exhibited that the lowest (3.22 thrips/10 cm shoot) number of thrips were found in trees treated with spinosad 45% SC 0.01% and it was at par with cyantraniliprole 10.26% OD @ 0.008% (3.70 thrips/10 cm shoot) followed by buprofezin 15% + acephate 35% 50 WP @ 0.063% (4.12 thrips/10 cm shoot). Remaining treatments showed more or less similar trend of effectiveness as observed in first year.

Pooled over year

Overall, the data on pooled over year showed the lowest (3.42 thrips/10 cm shoot) number of thrips were recorded in trees treated with spinosad 45% SC 0.01% and it was at par with cyantraniliprole 10.26% OD @ 0.008% (3.87 thrips/10 cm shoot) followed by buprofezin 15% + acephate 35% 50 WP @ 0.063% (4.25 thrips/10 cm shoot). The profenophos 40% + cypermethrin 4% 44 EC @ 0.066 % recorded the highest (7.62 thrips/10 cm shoot) thrips population and it was at par with acephate 50% + imidacloprid 1.8% 51.8 SP @ 0.052% (6.95 thrips/10 cm shoot) in pomegranate.

Thrips population on fruits

First year (2017) First spray

The population of thrips was found non-significant before spray which indicated homogenous distribution of the pest. The results on pooled over first spray showed that the lowest (11.33 thrips/fruit) number of thrips were observed in trees treated with spinosad 45% SC @ 0.01% and it was at par with cyantraniliprole 10.26% OD @ 0.008% (11.82 thrips/fruit) and buprofezin 15% + acephate 35% 50 WP @ 0.063% (12.32 thrips/fruit). Next effective group based on thrips population was acephate 50% + imidacloprid 1.8% 51.8 SP @ 0.052% andprofenophos 40% + cypermethrin 4% - 44 EC @ 0.066% which recorded thrips population between 16.23 and 16.64 thrips per fruit.

Second spray

Data of pooled over second spray revealed that all the treatments were found significantly superior to the control. The lowest (5.36 thrips/fruit)number of thrips was found in trees treated with spinosad 45% SC @ 0.01% and it was at par with cyantraniliprole 10.26% OD @ 0.008% (5.70 thrips/fruit) and buprofezin 15% + acephate 35% 50 WP @ 0.063% (6.10 thrips/fruit). Acephate 50% + imidacloprid 1.8% - 51.8 SP @ 0.052% andprofenophos 40% + cypermethrin 4% 44 EC @ 0.066% emerged out as next effective group of treatments.

Pooled over sprays

In case of pooled over sprays, the lowest (8.08 thrips/fruits) thrips population was recorded in trees treated with spinosad 45% SC 0.01% and it was at par with cyantraniliprole 10.26% OD @ 0.008% (8.50 thrips/fruit)and buprofezin 15% + acephate 35% 50 WP @ 0.063% (8.89 thrips/fruit). Trees treated with acephate 50% + imidacloprid 1.8% 51.8 SP @ 0.052% andprofenophos 40% + cypermethrin 4% 44 EC @ 0.066% recorded thrips population between 12.32 and 12.82 per fruit.

Second year (2019)

First spray

In case of second year, the results on pooled over first spray showed that the lowest (10.66 thrips/fruit) number of thrips were noticed in trees treated with spinosad 45% SC @ 0.01% and it was at par with cyantraniliprole 10.26% OD @ 0.008% (11.13 thrips/fruit) and buprofezin 15% + acephate 35% 50 WP @ 0.063% (11.61 thrips/fruit). Next effective group based on thrips population was acephate 50% + imidacloprid 1.8% 51.8 SP @ 0.052% andprofenophos 40% + cypermethrin 4% 44 EC @ 0.066% which recorded 15.42 and 15.98 thrips per 10 cm shoots of pomegranate.

Second spray

Data on pooled over second spray showed that all the treatments were found significantly superior to the control. Of the tested insecticides the lowest (5.40 thrips/fruit) number of thrips was observed in trees treated with spinosad 45% SC @ 0.01% and it was at par with cyantraniliprole 10.26% OD @ 0.008% (5.80 thrips/ fruit) and buprofezin 15% + acephate 35% 50 WP @ 0.063% (6.31 thrips/ fruit). The highest population of thrips was noticed in trees treated with acephate 50% + imidacloprid 1.8% 51.8 SP @ 0.052% and it was at par withprofenophos 40% + cypermethrin 4% 44 EC @ 0.066%.

Pooled over sprays

Results of pooled over sprays exhibited the lowest (7.79 thrips/ fruit)number of thrips in trees treated with spinosad 45% SC @ 0.01% and it was at par with cyantraniliprole 10.26% OD @ 0.008% (8.26 thrips/ fruit) and buprofezin 15% + acephate 35% 50 WP @ 0.063% (8.74 thrips/ fruit). Among the tested insecticides the highest (12.68) population of

Table 1: Bio-efficacy of insecticides against thrips, *S. dorsalis* infesting shoots of pomegranate during 2017

Sr. No.	Treatments	No. of thrips/ 10 cm shoot days after spray									Pooled over sprays
			First				Second				
		Before spray	5	10	15	Pooled	5	10	15	Pooled	
1	Acephate 50%+ Imidacloprid 1.8% - 51.8 SP, 0.052%	3.15 (9.42)	3.07 (8.92)	2.82 (7.45)	3.19 (9.68)	3.02 (8.62)	2.63 (6.42)	2.60 (6.26)	2.44 (5.45)	2.56 (6.05)	2.79 (7.28)
2	Buprofezin 15% + Acephate 35% - 50 WP, 0.063%	3.26 (10.13)	2.50 (5.75)	2.32 (4.88)	2.68 (6.68)	2.50 (5.75)	2.07 (3.78)	2.01 (3.54)	1.81 (2.78)	1.96 (3.34)	2.23 (4.47)
3	Profenophos 40% + Cypermethrin 4% - 44 EC, 0.066%	3.08 (8.99)	3.11 (9.17)	2.90 (7.91)	3.27 (10.19)	3.09 (9.05)	2.75 (7.06)	2.67 (6.63)	2.61 (6.31)	2.68 (6.68)	2.89 (7.85)
4	Cyantraniliprole 10.26% OD, 0.0077%	3.43 (11.26)	2.37 (5.12)	2.25 (4.56)	2.58 (6.16)	2.40 (5.26)	1.97 (3.38)	1.93 (3.22)	1.70 (2.39)	1.86 (2.96)	2.13 (4.04)
5	Spinosad 45 % SC, 0.010%	3.10 (9.11)	2.26 (4.61)	2.15 (4.12)	2.51 (5.80)	2.31 (4.84)	1.90 (3.11)	1.82 (2.81)	1.58 (2.00)	1.76 (2.60)	2.04 (3.66)
6	Control (water spray)	3.14 (9.36)	3.61 (12.53)	3.67 (12.97)	3.79 (13.86)	3.69 (13.12)	3.35 (10.72)	3.55 (12.10)	3.52 (11.89)	3.47 (11.54)	3.58 (12.32)
	S. Em. ± T	0.13	0.16	0.14	0.15	0.09	0.17	0.17	0.12	0.09	6.19
	P					0.06				0.06	4.38
	S	-	-	-	-	-	-	-	-	-	0.04
	S x P	-	-	-	-	-	-	-	-	-	6.19
	S x T	-	-	-	-	-	-	-	-	-	8.75
	T x P	-	-	-	-	0.15	-	-	-	0.15	0.11
	S x P x T	-	-	-	-	-	-	-	-	-	0.15
	C. D. at T	-	0.48	0.42	0.43	0.25	0.50	0.50	0.36	0.25	0.17
	0.05 % P					0.17				NS	NS
	S	-	-	-	-	-	-	-	-	-	0.10
	S x P	-	-	-	-	-	-	-	-	-	0.17
	S x T	-	-	-	-	-	-	-	-	-	NS
	T x P	-	-	-	-	NS	-	-	-	NS	NS
	S x P x T	-	-	-	-	-	-	-	-	-	NS
	C. V. % 8.38	11.43	10.63	9.71	10.60	13.64	13.70	10.71	12.85	11.62	

Note: Figures in parenthesis are retransformed values, those outside are $\sqrt{x + 0.5}$ transformed values.

Table 2: Bio-efficacy of insecticides against thrips, *S. dorsalis* infesting shoots of pomegranate during 2019

Sr. No.	Treatments	No. of thrips/ 10 cm shoot days after spray									Pooled over sprays
			First				Second				
		Before spray	5	10	15	Pooled	5	10	15	Pooled	
1	Acephate 50% + Imidacloprid 1.8% - 51.8 SP, 0.052%	2.97 (8.32)	2.86 (7.68)	2.71 (6.84)	3.09 (9.05)	2.89 (7.85)	2.56 (6.05)	2.52 (5.85)	2.37 (5.12)	2.48 (5.65)	2.69 (6.74)
2	Buprofezin 15% + Acephate 35% - 50 WP, 0.063%	3.07 (8.92)	2.41 (5.31)	2.27 (4.65)	2.71 (6.84)	2.47 (5.60)	1.97 (3.38)	1.85 (2.92)	1.68 (2.32)	1.83 (2.85)	2.15 (4.12)
3	Profenophos 40% + Cypermethrin 4% - 44 EC, 0.066%	3.11 (9.17)	3.05 (8.80)	2.83 (7.51)	3.23 (9.93)	3.03 (8.68)	2.71 (6.84)	2.58 (6.16)	2.53 (5.90)	2.61 (6.31)	2.82 (7.45)
4	Cyantraniliprole 10.26% OD, 0.0077%	3.26 (10.13)	2.32 (4.88)	2.17 (4.21)	2.51 (5.80)	2.33 (4.93)	1.90 (3.11)	1.82 (2.81)	1.56 (1.93)	1.76 (2.60)	2.05 (3.70)
5	Spinosad 45 % SC, 0.010%	2.99 (8.44)	2.24 (4.52)	2.08 (3.83)	2.32 (4.88)	2.21 (4.38)	1.83 (2.85)	1.69 (2.36)	1.41 (1.49)	1.64 (2.19)	1.93 (3.22)
6	Control (water spray)	2.93 (8.08)	3.41 (11.13)	3.51 (9.42)	3.66 (12.90)	3.53 (11.96)	3.46 (11.47)	3.51 (11.82)	3.52 (11.89)	3.50 (11.75)	3.51 (11.82)
	S. Em. ± T	0.10	0.14	0.13	0.13	0.08	0.10	0.09	0.08	0.05	4.72
	P	-	-	-	-	0.06	-	-	-	0.04	3.33
	S	-	-	-	-	-	-	-	-	-	2.72
	S x P	-	-	-	-	-	-	-	-	-	4.72
	S x T	-	-	-	-	-	-	-	-	-	6.67
	T x P	-	-	-	-	0.14	-	-	-	0.09	8.17
	S x P x T	-	-	-	-	-	-	-	-		0.11
	C. D. at 0.05 % T	NS	0.42	0.40	0.39	0.22	0.31	0.26	0.23	0.15	0.13
	P		-	-	-	0.16	-	-	-	0.10	NS
	S	-	-	-	-	-	-	-	-	-	7.64
	S x P	-	-	-	-	-	-	-	-	-	0.13
	S x T	-	-	-	-	-	-	-	-	-	0.18
	T x P	-	-	-	-	NS	-	-	-	NS	NS
	S x P x T	-	-	-	-	-	-	-	-	-	NS
	C. V. % 6.27	10.53	10.26	9.00	9.91	8.70	7.49	7.21	7.88	9.16	

Note: Figures in parenthesis are retransformed values, those outside are $\sqrt{x + 0.5}$ transformed values.

Table 3 : Bio-efficacy of insecticides against thrips, *S. dorsalis* infesting shoots of pomegranate (Pooled over years)

Sr. No.	Treatments		No. of thrips/ 10 cm shoot		
			2017	2019	Pooled over years
1	Acephate 50% + Imidacloprid 1.8% - 51.8 SP, 0.052%		2.79 (7.28)*	2.69 (6.74)	2.73 (6.95)
2	Buprofezin 15% + Acephate 35% - 50 WP, 0.063%		2.23 (4.47)	2.15 (4.12)	2.18 (4.25)
3	Profenophos 40% + Cypermethrin 4% - 44 EC, 0.066%		2.89 (7.85)	2.82 (7.45)	2.85 (7.62)
4	Cyantraniliprole 10.26% OD, 0.0077%		2.13 (4.04)	2.05 (3.70)	2.09 (3.87)
5	Spinosad 45 % SC, 0.010%		2.04 (3.66)	1.93 (3.22)	1.98 (3.42)
6	Control (water spray)		3.58 (12.32)	3.51 (11.82)	3.55 (12.10)
	S. Em. ±	T	6.19	4.72	0.03
		P	4.38	3.33	0.02
		S	0.04	2.72	0.02
		S x P	6.19	4.72	0.03
		S x T	8.75	6.67	0.05
		T x P	0.11	8.17	0.06
		S x P x T	0.15	0.11	0.57
	C. D. at 0.05 %	T	0.17	0.13	0.10
		P	NS	NS	0.07
		S	0.10	7.64	6.21
		S x P	0.17	0.13	0.10
		S x T	NS	0.18	0.15
		T x P	NS	NS	NS
		S x P x T	NS	NS	NS
	C.V. %		11.62	9.16	10.50

Note: *Figures in parenthesis are retransformed values; those outside are $\sqrt{(x+0.5)}$ transformed values.

Table 4: Bio-efficacy of insecticides against thrips, *S. dorsalis* infesting fruits of pomegranate during 2017

Sr. No.	Treatments	No. of thrips/fruit days after spray										Yield (q/ha)
			First				Second				Pooled	
		Before spray	5	10	15	Pooled	5	10	15	Pooled	over sprays	
1	Acephate 50% + Imidacloprid 1.8% - 51.8 SP, 0.052%	4.92 (23.71)	4.23 (17.39)	3.84 (14.25)	4.21 (17.22)	4.09 (16.23)	3.37 (10.86)	3.04 (8.74)	2.78 (7.23)	3.06 (8.86)	3.58 (12.32)	86.81

2	Buprofezin 15% + Acephate 35% - 50 WP, 0.063%	4.99 (24.40)	3.74 (13.49)	3.32 (10.52)	3.67 (12.97)	3.58 (12.32)	2.88 (7.79)	2.57 (6.10)	2.24 (4.52)	2.57 (6.10)	3.07 (8.92)	103.74
3	Profenophos 40% + Cypermethrin 4% - 44 EC, 0.066%	4.82 (22.73)	4.26 (17.65)	3.92 (14.87)	4.26 (17.65)	4.14 (16.64)	3.43 (11.26)	3.10 (9.11)	2.93 (8.08)	3.16 (9.49)	3.65 (12.82)	83.86
4	Cyantraniliprole 10.26% OD, 0.0077%	5.11 (25.61)	3.68 (13.04)	3.25 (10.06)	3.60 (12.46)	3.51 (11.82)	2.82 (7.45)	2.50 (5.75)	2.16 (4.17)	2.49 (5.70)	3.00 (8.50)	106.42
5	Spinosad 45 % SC, 0.010%	5.06 (25.10)	3.59 (12.39)	3.19 (9.68)	3.54 (12.03)	3.44 (11.33)	2.77 (7.17)	2.42 (5.36)	2.05 (3.70)	2.42 (5.36)	2.93 (8.08)	110.50
6	Control (water spray)	4.97 (24.20)	4.78 (22.35)	4.53 (20.02)	4.77 (22.25)	4.70 (21.59)	4.02 (15.66)	3.88 (14.55)	3.90 (14.71)	3.94 (15.02)	4.32 (18.16)	65.78
	S. Em. ± T	0.19	0.15	0.17	0.17	0.09	0.16	0.13	0.13	0.08	6.23	5.89
	P	-	-	-	-	0.07	-	-	-	0.06	4.40	-
	S	-	-	-	-	-	-	-	-	-	0.04	-
	S x P	-	-	-	-	-	-	-	-	-	6.23	-
	S x T	-	-	-	-	-	-	-	-	-	8.81	-
	T x P	-	-	-	-	0.16	-	-	-	0.14	0.11	-
	S x P x T	-	-	-	-	-	-	-	-	-	0.15	-
	C. D. at 0.05 % T	-	0.45	0.49	0.51	0.27	0.48	0.40	0.38	0.23	0.17	14.51
	P	-	-	-	-	0.19	-	-	-	0.16	0.12	-
	S	-	-	-	-	-	-	-	-	-	0.10	-
	S x P	-	-	-	-	-	-	-	-	-	0.17	-
	S x T	-	-	-	-	-	-	-	-	-	NS	-
	T x P	-	-	-	-	NS	-	-	-	NS	NS	-
	S x P x T	-	-	-	-	-	-	-	-	-	NS	-
	C.V. % 7.63	7.39	9.03	8.53	8.30	10.12	9.15	9.54	9.68	8.91	12.70	

Note: *Figures in parenthesis are retransformed values; those outside are $\sqrt{(x+0.5)}$ transformed values.

Table 5: Bio-efficacy of insecticides against thrips, *S. dorsalis* infesting fruits of pomegranate during 2018

Sr. No.	Treatments	No. of thrips/fruit days after spray									
		Before spray	First				Second				Pooled over sprays
			5	10	15	Pooled	5	10	15	Pooled	
1	Acephate 50% + Imidacloprid 1.8% - 51.8 SP, 0.052%	4.84 (22.93)	4.16 (16.81)	3.73 (13.41)	4.07 (16.06)	3.99 (15.42)	3.54 (12.03)	3.09 (9.05)	2.81 (7.40)	3.15 (9.42)	3.57 (12.24)
2	Buprofezin 15% + Acephate 35% - 50 WP, 0.063%	3.85 (14.32)	3.63 (12.68)	3.27 (10.19)	3.55 (12.10)	3.48 (11.61)	3.08 (8.99)	2.54 (5.95)	2.20 (4.34)	2.61 (6.31)	3.04 (8.74)

3	Profenophos 40% + Cypermethrin 4% - 44 EC, 0.066%	3.86 (14.40)	4.19 (17.06)	3.82 (14.09)	4.18 (16.97)	4.06 (15.98)	3.58 (12.32)	3.12 (9.23)	2.91 (7.97)	3.20 (9.74)	3.63 (12.68)
4	Cyantraniliprole 10.26% OD, 0.0077%	3.62 (12.60)	3.58 (12.32)	3.17 (9.55)	3.49 (11.68)	3.41 (11.13)	2.98 (8.38)	2.42 (5.36)	2.12 (3.99)	2.51 (5.80)	2.96 (8.26)
5	Spinosad 45 % SC, 0.010%	4.04 (15.82)	3.51 (11.82)	3.11 (9.17)	3.39 (10.99)	3.34 (10.66)	2.93 (8.08)	2.34 (4.98)	2.03 (3.62)	2.43 (5.40)	2.88 (7.79)
6	Control (water spray)	4.98 (24.30)	4.78 (22.35)	4.80 (22.54)	4.84 (22.93)	4.81 (22.64)	4.25 (17.56)	4.16 (16.81)	3.95 (15.10)	4.12 (16.47)	4.46 (19.39)
	S. Em. ± T	0.87	0.14	0.17	0.14	0.09	0.14	0.11	0.13	0.07	0.05
	P	-	-		-	0.06	-	-	-	0.05	0.04
	S	-	-	-	-	-	-	-	-	-	3.32
	S x P	-	-	-	-	-	-	-	-	-	0.05
	S x T	-	-	-	-	-	-	-	-	-	0.08
	T x P	-	-	-	-	0.15	-	-	-	0.13	9.98
	S x P x T	-	-	-	-	-	-	-	-	-	0.14
	C. D. at 0.05 % T	NS	0.43	0.52	0.41	0.25	0.43	0.32	0.37	0.21	0.16
	P	-	-	-	-	0.18	-	-	-	0.15	0.11
	S	-	-	-	-	-	-	-	-	-	9.33
	S x P	-	-	-	-	-	-	-	-	-	0.16
	S x T	-	-	-	-	-	-	-	-	-	NS
	T x P	-	-	-	-	NS	-	-	-	NS	NS
	S x P x T	-	-	-	-	-	-	-	-	-	NS
	C.V. % 41.28	7.26	9.56	7.13	7.99	8.56	7.41	9.41	8.49	8.24	

Note: *Figures in parenthesis are retransformed values; those outside are $\sqrt{(x+0.5)}$ transformed values.

Table 6: Bio-efficacy of insecticides against thrips, *S. dorsalis* infesting fruit of pomegranate (Pooled over years)

Sr. No.	Treatments	No. of thrips/fruit		
		2017	2019	Pooled over Years
1	Acephate 50% + Imidacloprid 1.8% - 51.8 SP, 0.052%	3.58 (12.32)*	3.57(12.24)	3.57 (12.24)
2	Buprofezin 15% + Acephate 35% - 50 WP, 0.063%	3.07 (8.92)	3.04 (8.74)	3.05 (8.80)
3	Profenophos 40% + Cypermethrin 4% - 44 EC, 0.066%	3.65 (12.82)	3.63(12.68)	3.64 (12.75)
4	Cyantraniliprole 10.26% OD,0.0077%	3.00 (8.50)	2.96 (8.26)	2.98 (8.38)
5	Spinosad 45 % SC, 0.010%	2.93 (8.08)	2.88 (7.79)	2.90 (7.91)
6	Control (water spray)	4.32 (18.16)	4.46(19.39)	4.38 (18.68)
	S. Em. ±	T 6.23		0.050.04
		P 4.40		0.040.03
		S 0.04		3.320.02
		S x P 6.23		0.050.04
		S x T 8.81		0.080.05

	T x P	0.11	9.980.07
	S x P x T	0.15	0.140.10
C. D. at 0.05 %	T	0.17	0.160.11
	P	0.12	0.118.30
	S	0.10	9.336.82
	S x P	0.17	0.160.11
	S x T	NS	NSNS
	T x P	NS	NSNS
	S x P x T	NS	NSNS
C.V. %		8.91	8.24	8.58

Note: *Figures in parenthesis are retransformed values; those outside are $\sqrt{(x+0.5)}$ transformed values.

thrips recorded in trees treated with Profenophos 40% + Cypermethrin 4% 44 EC @ 0.066% and it was at par with Acephate 50% + Imidacloprid 1.8% 51.8 SP @ 0.052%.

Pooled over year

Overall, the data on pooled over year showed the lowest (7.91 thrips/fruit) number of thrips were recorded in trees treated with Spinosad 45% SC @ 0.01% and it was at par with Cyantraniliprole 10.26% OD @ 0.008% (8.38 thrips/fruit) followed by Buprofezin 15% + acephate 35% 50 WP @ 0.063% (8.80 thrips/fruit). The Profenophos 40% + Cypermethrin 4% 44 EC @ 0.066 % recorded the highest (12.75 thrips/10 fruit) thrips population and it was at par with acephate 50% + imidacloprid 1.8% - 51.8 SP, 0.052% (12.24 thrips/fruit) in pomegranate.

Yield

The highest yield of pomegranate was recorded from the treatment of Spinosad 45% SC (110.50 q/ha) which was at par with cyantraniliprole 10.26% OD (106.42 q/ha) and Buprofezin 15% + Acephate 35% 50 WP (103.74 q/ha). The experiment was conducted at a farmer's field and the farmer had removed the orchard due to some personal social issues. Hence, during second-year pomegranate yield was not possible to record.

The present results conform to the findings of Kadam (2006) who reported the effectiveness of spinosad (0.025% and 0.018%) and Kadam (2012) reported Spinosad @ 56.25 g a.i. ha-1 was the most effective treatment (4.26 thrips/fruit) at 14 DAS on par with fipronil @ 25 g a.i. ha-1 (4.42 thrips/fruit), while Jadhav (2003) found that fipronil 5 SC 100 g a.i. ha-1 was more effective than imidacloprid 17.8 SL @ 20 g a.i. ha-1 against chilli thrips. According to Jagginavar *et al.,* (2018) significantly lowest number thrips per 10 cm tender new shoots of pomegranate was recorded in higher dose of Alanto 240 SC (1.25ml/l) and Cyantraniliprole 10.26 % OD @ 0.75ml/l. Whereas, Patil *et al.,* 2017 minimum thrips population were recorded in spinosad 45% SC and cyantraniliprole 10.26% OD and found at par with each other in sweet orange. Cyantraniliprole 10.26 % w/w OD @ 90 g a.i/ha registered lowest number of thrips (5.16/leaf) at 10 days after application and highest yield of 49.70 t/ha in brinjal and watermelon (Savitha *et al.,* 2018).

CONCLUSION

Based on thrips incidence on shoots and fruits of pomegranate as well as the yield of fruits, the treatments of Spinosad 45 % SC 0.01%, Cyantraniliprole 10.26% OD @ 0.008% and buprofezin 15% + Acephate 35% 50 WP @ 0.063% was found more effective in checking the pest of pomegranate.

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Vermicomposting of leaf litter in E. eugeniae and its effects on epidermal and pharyngeal extract

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ABSTRACT

Vermicompost, when used as fertilizer, not only bears positive impact on soil quality, plant growth and yield but also enhances nutritional value of crops produced. *E. eugenia* earthworms are renowned for producing vermicompost quickly and effectively for cellular growth needs. This study analyzed the vermicompost of leaf litter, phytochemical analysis, and phytotoxicity of *E. eugeniae,* FT-IR, Brine shrimp analysis, and enzymatic activity. The Phytochemical analyses were recorded and the plant growth parameters were significantly higher than the control.FT-IR analysis, functional groups like alcohol, phenol, carboxylic acid can be found in *E. eugenia's* epidermis extract and pharynx extract. The brine shrimp assay reveals lower toxicity for both extracts. Pharynx extracts results are less toxic when compared to epidermal extract.

Key words: *E. Eugenia*, Epidermal fluid, pharyngeal fluid, phtyotoxicity

INTRODUCTION

Vermicomposting biotechnologies involve the bio-oxidation and stabilization of organic matter through the joint action of earthworms and microorganisms under aerobic and mesophilic conditions (Lorimor *et al*., 2001). The goals of vermicomposting are to continually increase the number and weight of worms and to convert the substrate material into vermicompost in the shortest time and highest recovery as possible (Rupani, *et al.,* 2013). The leaf litter biomass, which is one of the lignocellulose wastes generated in enormous quantity in hill towns in India, has always been a problem for municipal workers and city resource managers (Gairola and Suthar, 2014). Thermal composting or cool composting using earthworm (vermi-composting) are proposed as low-cost technologies to recover the valuable nutrients from leaf biomass under the anaerobic condition with the help of detritus micro- and macro communities under humid environmental conditions (Gairola and Suthar, 2014; Harindint wali *et al*., 2020). The reactors were started by introducing desired number of *Eudriluseugeniae* adults, which, in turn were randomly picked from a cowdung-fed culture of over 5000 animals. *E. eugeniae* Kinberg were used in this study as they had earlier proved more effective against water hyacinth, and nearly as effective against paper waste, as *Lampitomauritii* Kinberg (Gajalakshmi *et al*., 2001a, Gajalakshmi *et al.*,2001b).

Many metabolic processes occur in the coelom which contains coelomic fluid, which also serves as a site for temporary food storage, nitrogen-containing was tesex cretion, and gametes' maturation, and reproduction is either by both external and

internal types. Because annelids are hermaphroditic, development soccur without alarval stage (*Phillipsetal.*,2019). The bodily fluid that exists between the earthworm's body wall and intestine is called coelomic fluid. The coelomic fluid of the earthworm includes a variety of molecules and demonstrates significant biological characteristics like antimicrobials, hemagglutination, and anticoagulant agents. The main function of the earthworm's coelomic fluid is to demolish the cell membranes of foreign organisms (*Umamaheswari and Murugan,* 2021). Eisenia fetida is a typical and significant element of the earth ecosystem. Their physiological functions are aided by the coelomic fluid they produce. They release this liquid under stress, and it has a big impact on soil heath and ecotoxicity. The results of this research seek to determine the quantity of protein and carbohydrates found in E. eugenia (Demirkan *et al.*, 2022).

In this study, to evaluate the suitable leaf litter for vermicomposting, the physiochemical analysis; induce the germination on fenugreek; and the collected of epidermalandpharynx extract from *E. eugeniae* in FT-IR, Brine shrimp analysis and Enzymatic activity.

MATERIALS AND METHODS

Experimental design

Vermibed substrates were done in a circular plastic pot with a 12 cm height and 13 cm diameter. Precomposted various leaf litter was separately crushed and mixed with Cow dung, in the ratio of 1:1 and all the experimental setups were maintained in triplicates. Each vermibin was loaded with 2 kg of vermibed substrates and introduced to twenty mature earthworms. During this period, moisture content was monitored periodically and maintained at 70–80% kept at a temperature of 27 °C. Then the 0th day (initial mixture) of vermibed substrates were kept as control and also after 60th day (vermicompost) substrates were collected, dried, sieved and then stored in air-tight zip lock covers at cool condition for further analysis (Ramalingam and Thilagar, 2000).

Phytochemical analysis of leaves

Litter leaves of plants consists of enormous organic chemicals. Lower organisms like invertebrtaes are feeding on decomposed plant products eg., earthworms . They procure food by intaking by decayed matter of plant byproducts and assimilate in their bodies, so plants play major role in the energy flow in the ecosystem. All the organisms directly or indirectly depending on (producers) plants Raman (2006).

Test used for qualitative analysis

Testforalkaloids

Mayer's test

The Mayers reagent was applied to a test tube containing 1 ml of leaf extract. The appearance of green precipitate indicates the presence of alkaloids.

Test Wagner

Wagner's reagent and 1 ml of the material were combined in a test tube. When a reddish-brown sediment forms, alkaloids are present.

Test of Dragendorff

A test tube containing 1 ml of material, 1 ml of Dragendorff reagent, and 2 ml of diluted HCl was used. Alkaloids are present when a variety of colored precipitates emerge.

Test for carbohydrates

Fehling's test

A minimum of 1 milliliter of the sample was combined with distilled water before being filtered through filter paper. Fehling's fluid was

diluted slightly and heated for a while. Brick red color formation suggests the presence of sugar.

Test for Benedict

To 2 milliliter of Benedict's solution, 1 ml of the sample extract was added. There are carbs present when a reddish-brown precipitate forms.

Test for flavonoids

Ferricchloride test

1ml of the sample was taken inatesttube and a few drops of neutralferricchloride was added. The appearance of a blackish-redprecipitatein dicates the presence of flavonoids.

Alkalinereagent test

1ml sample was treated with the aqueous NaOH and HCl. The appearance of yellow-orangecolourindicatesthepresenceofflavonoids.

Test for Glycosides

Keller killani test:

1ml of the sample was taken and 1ml of glacialacetic acid was added and then cooled. Then 2-3 drops of ferric chloride and 2ml of concentric sulphuric acid were added along the sides of the test tube. The appearance of a bluish-brown ring at the junction of two layer sindicates the presence of' Cardiac' glycosides.

Test for proteins Biuret test

1ml sample was mixed with 0.4ml of NaOH and 2 drops of Cu sulphate wereadded.The appearance of pink or purple colour indicates the presence of proteins.

Warming test

1ml sample was taken in a test tube and heated in a water bath for some time. The appearance of coagulation indicates the presence of proteins.

Test for Sterols

Salkowski's test

1ml sample was treated with a few drops of concentrated sulphuric acid and shaken well and the solution was allowed to stand for sometime. The appearance of red colour in the lower layer indicates the presence of sterols or the appearance of yellow colour in the lower layer indicates the presence of triterpenoids.

Liebermann-Burchard test

1ml sample was treated with chloroform, aceticanhydride, and drops of sulphuric acid were added. The formation of dark pink or red colour indicates the presence of sterols.

Test for Triterpenoids

Salkowski's test

1ml sample was treated with a few drops of concentrated sulphuric acid andshaken well and the solution was allowed to stand for some time. The appearance of red colour in the lower layer indicates the presence of sterols whereas the formation of yellow colour in the lower layer indicates the presence of triterpenoids.

Liebermann-Burchard test

1ml sample was boiled with the 2 drops of aceticanhydride and cooled then the concentrated sulphuric acid was added along the side of the test tube. The Appearance of the brown ring at the junction of two layers is taken as a reference, and the appearance of the brown ring at the junction of two layers is taken as a reference. If the upper layer turns green, sterols are present whereas the formation of deep red colour indicates the presence of triterpenoids.

Test for Tannins

Ferric chloride test

1ml of the sample was added with a few drops of 5% ferricchloride solution was added. The appearance of intense green or blue colour indicates the presence of tannins.

Lead acetate test

To the 5ml sample solution, a few drops of 10% lead acetate were added. The appearance of a yellow colour precipitate indicates the presence of tannins.

Phytotoxicity Assay

Experiments were carried out at the Laboratory condition, Different treatment of vermicompost, homogenized to a fine texture and removing inert matter before being used for seed sowing. Red soil and caste of tea dust powder 1:1 (75g: 75g=150g). 10 Fenugreek seeds of each treatment were sown in each pot for germination. Three replications of each treatment were arranged in a completely randomized design (CRD). Watering was done every evening until completion of the experiment (Annapoorani and Sindhu, 2019).

Preparation of epidermal and pharynx extract

Earthworms were collected from the vermicomposting pits and washed with distilled water. The coelomic fluid will ooze out to the epidermal region from epidermal surface the secretion was collected. They were centrifuged at 6000 rpm for 10 minutes. The pharynx is a white region that extends to the 4^{th} segment of the body and were used to collect the pharynx and mixed with the 5ml of Distilled Water. Then it was ground well using a mortar and pestle. The obtained filtrate was centrifuged for 15 min at 6000 rpm and the samples were analyzed.

Fourier Transform-Infrared (FT-IR)

Spectrum Analysis

Fourier-transform infrared (FTIR) spectroscopy was applied for chemical structure analysis of Pharynx Extract and Epidermal Extract in a wave number range of 400–4000 cm−1 by spectrometer of Shimadzu (IRAffinity) with a resolution of 4 cm−1, and 64 scans.

Brine shrimp analysis

Brine shrimp analysis of Pharynx Extract and Epidermal Extract in different volumes (10, 50, 100, 250, and 500 l) is added to each beaker containing saline solution. This was repeated for both samples. 30 shrimp were introduced into the sample solution at various concentrations. The movement of shrimp is monitored at intervals of 2, 4, 6, 8, and 24 hours. In the blank solution, 30 shrimp were introduced into the brine solution. For positive control, potassium dichromate (100 g/ml) was taken. The mortality of shrimp was observed and calculated after 24 hours. For each of the samples, 30 shrimp were added to 25 ml of the brine solution. Shrimp mortality was tracked as a blank and positive control.

Method for enzyme activity test

To know the presence of different enzymes in the body of Earth wormanen zymatic test was done. The Earthworm was dissected into Foregut, Midgut, Hindgut and coelom or epidermal region for the enzymatic test. They were separately grounded using a motor and pistol with 2ml of Distilled Water. The nit was filtered using filter paper. The obtained sample was then centrifuge at 3000rpm for 10 minutes. Then the sediment was taken as a sample. This procedure is repeated for the Midgut, Hindgut and coelomor epidermal region crushed samples.

Test for Invertase

1ml of 5% sucrose was taken in the test tube along with the sample of 5 drops. This was kept at room temperature for half an hr. After half an hour 4 drops of DW were added. Into that 1 drop of Fehling's solution, A and B were added. Then the mixture was kept in the water bath for 5 minutes. The brown - reddish colour precipitate indicates the presence of the Invertase enzyme. This was repeated for three samples, Foregut, Midgut, Hindgut and coelom or epidermalregion.

Test for Amylase

1ml of starch along with 5-10 drops of the sample was taken in a porcelain tile. Then it was kept at room temperature for 20 minutes. At every 2 minutes, a drop of Iodine solution was added to the mixture. No colour change indicates the presence of maltose which is an automatic point. If there is a colour change, it indicates the presence of amylase. If the blue-black colour appears it indicates the presence of starch. After the blue-black colour, reddish violet appears indicating the starch gets hydrolyzed into dextrin. This was repeated separately for three samples, Foregut, Midgut, Hindgut and coelomore pidermal region.

5ml of Benedict solution was taken in the test tube and along with that 8 drops of the sample were taken in the test tube. To that few drops of 1%, starch was added and Boiled in the water bath for 2 minutes. The appearance of a red colour precipitate indicates that starch hydrolysis into monosaccharides. This was repeated separately for three samples, Foregut, Midgut, Hindgut and coelomor epidermal region.

Test for Protease

Photographic film was taken and in that film 1 drop of the sample was placed. This setup was kept in the moist chamber for 2 hours. After 2 hours the photographic film was washed off. The drop digests the gelatin emulsion was noted. The disappearance of the Black colour of the film negative indicates the presence of enzyme-protease.This was repeated separately for three samples, Foregut, Midgut, Hindgut and coelomor epidermal region.

Test for Lipase

1ml of Methyl salicylate was taken in the test tube and kept at room temperature for half an hr. After half an hour the colour change to white precipitate indicates the presence of the Lipase enzyme. This was repeated separately for three samples, Foregut, Midgut, Hindgut and coelomor epidermal region.

RESULTS AND DISCUSSION

Vermicompost is a good soil fertilizer made up of worms digested excretory product known as worm caste which contains essential nutrients for plants to germinate, growth and development. It increase the soil richness, water holding, softening, aeration and porosity of the soil due to the movement of earthworms turns upside movements of top soil and sub-soil.

Phytochemical analysis

A phytochemical analysis was made in the leaflitter vermicompost, and the results were obtained by observing the colour changes in the alkaloid, carbohydrate, flavonoid, glycosides, proteins, sterols, triterpenoids, and tannins samples represented in (Table 1).Karmegam and Daniel, (2011) have reported that leaflitter and cowdungisa good source and has the ability to improve the quality of vermicomposting. Daniel and Karmegam, (1999) reported that vermicomposting prepared by *E. eugeniae* using specific three types of leaf litter *Azadirachta indica, Ceiba pentandra,* and *Morindatinctoria* with the cow dung in the ratio of 1:1 shows higher nutrients and micro-organisms and can be used for crop production and maintenance of soil fertility. The above results are similar to our present study as the different number of microbial colonies was developed.

Joseph and Kathireswari, (2020) stated that in addition to the animal dung, in some leaflitter substrates the reproductive potential and biomass got enhanced but in some leaf

litter, reproductive potential got reduced and leading to the death of Earthworms. This is because the variation in chemical contents of different leaf litters may affect health and reproductive potential. This result shows controversially with our present study whereas, in our vermicomposting pits all were active.

Table 1: Phytochemical analysis of dired Leaf extract

Phyto chemicals	Name of the Test	Result
Alkaloids	Mayer's	Negative
	Wagner's	Positive
	Dragen droff's	Negative
Carbohydrates	Benedict's	Positive
	Fehling's	Negative
Flavonoids	FeCl3	Positive
	Alkalinere agent	Negative
Glycosides	Keller Killani	Negative
Proteins	Biuret	Positive
	Warming	Positive
Sterols	Salkowski's	Positive
	Libermann-Buchard's	Negative
Triterpenoids	Salkowski's	Negative
	Libermann-Buchard's	Positive
Tannins	FeCl3	Positive
	Lead Acetate	Positive

Phtyotoxicity as say of Vermicompost

The present findings showed that fenugreek seeds were completely germinated. The effectively protected the ability of seeds to germinate capacity during the storage period, revealing non-phytotoxic nature. Thus, vermicompost of seeds may be further utilized for sowing or consumption. At concentration, leaf litter vermicompost were significantly increased up to 55.9% and 33.10% growth, plumule and radicle length of fenugreek seeds between the experiment and control groups of leaf litter vermicompost, there were no significant alterations in fenugreek seeds for germination or radicles.

The beneficial effect of vermicompost on these parameters might be due to its contribution in supplying additional plant nutrients and increasing availability of native soil nutrients with increased microbial activity. The results of the present investigation proved with that of (Khiriya *et al.,* 2001 and Jat *et al.*, 2006). The positive response of vemicompost may probably due to enhanced supply of macro as well as micro nutrients which led to high assimilation of food and its subsequent partitioning in sink. It improved yield components due to vegetative and reproductive growth led to higher seed and yields (Chaturvedi and Chandel, 2005 and Suman *et al*., 2007).

FT-IR Results for Epidermal Extract and Pharynx Extract of *E. Eugeniae*

The functional groups present in the epidermis extract and pharynx extract of E. eugeniae were analysed. The FTIR of epidermal extract displays peeks at (Fig. 1) 3317.56 cm^{1}, 1635.64 cm^{1}, 1211.30 cm^{1},

686.66 cm^1, 601.79 cm^1, 532.35 cm^1, and 478.35 cm^1, with it containing strong functional groups of alcohols, phenols, carboxylic acid, ether, and alkenes and medium functional groups of primary amine and alkyl halides. Similarly, 3317.56 cm^1, 1635.64 cm^1, 686 cm^1, 601.79 cm^1, 532.35 cm^1, 501.49 cm^1, 478.35 cm^1, and 455.20 cm^1 show peaks. It contains strong functional groups of alcohols and phenols, and medium functional groups of primary amines and alkyl halides were observed.

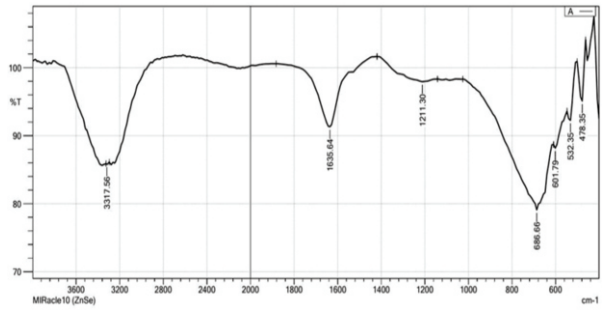


Figure1: FT-IR peaks recorded for Epidermal extract

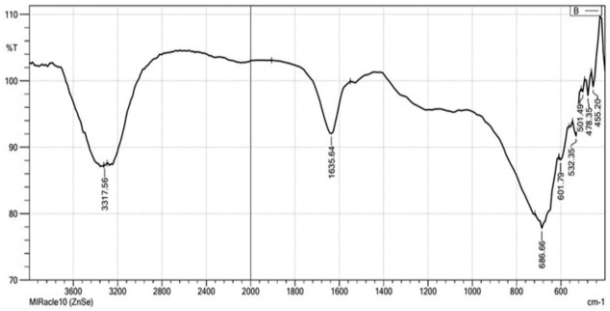


Figure 2: FT-IR peaks recorded for Pharynx extract

The peak responses of *E. eugeniae* epidermal extract and pharynx extract were similar, as observed at 3317.56 cm1, 1635.64 cm1, 686.66 cm1, 601.79 cm1, 532.35 cm1, and 478.35 cm^1 (Fig.2). Scullion *et al*., (2003) reported that the FT-IR spectroscopic technique is used todetermine the biomolecular profile of micro-samples of coelomic fluid. A few reports on the FT-IR studies relating to oligochaetes mainly focus on earthworm compost. Our study findings depicted as containing functional groups of alcohols, phenols, carboxylic acid, ether, and alkenes. Aja *et al.,* (2014) reported that the coelomic fluid of *Perionyx sansibaricus* showed characteristic peaks at 480 cm^{-1}, 566 cm^{-1}, 731 cm^{-1}, and 1663 (amide I) cm^{-1}, 2081 cm^{-1},2143cm^{-1},3122cm^{-1},and3504cm^{-1}.Inthe present study, the peaks observed in the ceolomic fluidof *E. eugeniae* was dissimilar to the above findings.

Brining shrimp assay result for epidermal extract:

The epidermal extract was taken in different concentrations for the analysis of the cytotoxicity of earthworms. They were taken in concentrations of 10, 50, 100, 250, and 500 g/ml. Less toxicity is seen in the brine shrimp assay at both extracts. Pharynx extracts results are less toxic when compared to epidermal extract.

A comparison assay of *E. euginae* pharynx and epidermal extract using brine shrimp is shown in (Table 2). Due to the organism's epidermal secretion, creates a slightly acidic level for the brine shrimps because of the acidic climatic conditions noted in samples 10, 50, 100 concentrati on results were recorded as the number of mortality recorded thirteen and 250 and mortality rate was increased as sixteen in 500 concentration. When compared to the control, this was more toxic.

In the digestive system, the pharynx plays a vital role in the process of taking in food and transforming it into energy. It is a tube-like structure located between the mouth and oesophagus that serves as a passageway for both food and air. The pharynx also has an alkaline climate, as seen in 10, 50, and three mortality were recorded in concentration 100. There is no mortality rate in 250; in 500, the mortality rate increased to ten, which was toxic compared to the control.

Table 2: comparative brine shrimp assay of epidermal extract and pharynx extract of *E.euginae.*

SAMPLE	CONCENTRATION (µg/ml)	Mortality of Brineshrimps (no of shrimps dead)(h)					
		2	4	6	8	24	% Mortality (at 24 h)
A	10	0	0	0	0	4	13
	50	0	0	0	0	4	13
	100	0	0	0	0	4	13
	250	0	0	0	0	5	16
	500	0	0	0	0	5	16
B	10	0	0	0	0	1	3
	50	0	0	0	0	1	3
	100	0	0	0	0	1	3
	250	0	0	0	0	0	0
	500	0	0	0	0	3	10
Control	100	0	0	12	21	30	100
Blank	SW	0	0	0	0	0	0

A - Epidermal extract ; B - Pharynx extract; SW – Saline Water

The brine shrimp lethality test, which is a brine shrimp assay, was conducted to determine the cytotoxic nature obtained from *E. eugeniae*. Because of its high survival rate, shrimp were used to test the cytotoxic nature. Shrimp in vinegar a micro crustacean called Artemia is well suited to surviving in and reproducing in habitats that are extremely hypersaline (*Gajardo and Beardmore, 2012*). Chanda, (2011) states that brine shrimp assay gives the cytotoxicity effects of a certain sample and gives the result of EW has fewer cytotoxicity effects comparing some other Oligochaeta. In our present study brine shrimp assay was used to test the cytotoxicity of earth worm parts. Using epidermal and pharynx extract gives the result of the pharynx extract is less toxicthan epidermal because the mortality rate of shrimps in the pharynx is less after 24 hrs of observation compared to the mortality rate in the epidermal extract.

Enzymatic test

The enzymatic test was done separately for three samples: foregut, midgut, hindgut, and coelom, or epidermal region. The lipase enzyme was absent in the foregut, hindgut, and coelom, or epidermal region, but present in the midgut. Amylase, invertase, and protease enzymes were present in all three gut and coelomic or epidermal regions (Table 3) shows enzymes present in different parts of intestine present in *E. euginae.*

The gelatin observation was washed away from the photographic film. The appearance of a brown or reddish precipitate suggests that invertase. Invertase is present in all three guts and the coelom, or epidermal, regions. Colour changes or slight precipitate formation indicate the presence of amylase. The blue coloration indicates the presence of starch. The presence of the lipase enzyme is indicated by the formation of a thick blue colour. The lipase enzyme is present in the colon, epidermis, and midgut. Earthworms secreted body fluids through epidermal dorsal pores onto the exposed body surfaces. The epidermal mucus layers protected body surfaces from contacting soil directly while earthworms move in soil. The living environment of earthworms is rich in pathogens and other potential threats (Wang *et al*., 2007).

Table 3: Enzymes present in different part of *E.euginae*

Extract	Enzymes			
	Invertase	Amylase	Protease	Lipase
Foregut	+	+	+	_
Midgut	+	+	+	+
Hindgut	+	+	+	_
Coelomic or epidermal region	+	+	+	_

CONCLUSION

The vermicompost produced can be of significant value to the end users like farmers for replacement of chemical fertilizers and procuring better prices for the organic produce using such composting material locally available at much lower cost. The earthworm *E. eugeniae* is known as a soil engineer. Because they travel from the topsoil and subsoil and penetrate deep soil layers, they rotate the soil again and again for feeding and also to make the soil smooth. These organisms feed on dead and decaying matter, absorb the nutrients, and disperse the remaining material as casts and its impact on seed germination and plant growth parameters. The pharynx of an earthworm has a pharyngeal gland that secretes pharyngeal fluid, which contains more microbial colonies that help the digestion of decayed parts and break them down into simple substances known as secondary metabolites.

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Optimization and Production of Amylase Enzyme from Crab Gut Microflora

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ABSTRACT

Crab receives bacteria in the gut from their aquatic environment through water and food that are populated with bacteria. Being rich in nutrients, the environment of the crab gut confers favourable conditions for the microorganisms. Enzymes are biological catalysts exceedingly esoteric with extraordinary catalytic power and embrace remarkable specificity. The objective of the present study was to improve the synthesis of amylase that was isolated from *Bacillus* sp., a type of crab intestine bacteria. In addition, many variables, including pH, temperature, and substrate concentration, were tuned. To generate higher and better enzyme activity, additional ions in the reaction media were categorised as activators or inhibitors. Based on this fact, crab waste can be effectively utilized for the production of amylases. The results illustrated in the table indicate that the optimum temperature for amylase activity was 33 ˚ C. The optimum temperature for amylase activity from MBK2 was similar to or quite higher than that of the optimum temperature of amylase from MBK1. The enzyme was highly active at pH 4.0 and it lost that optimum temperature for amylase activity was 33 ˚ C. The optimum temperature for amylase activity from MBK2 was similar to or quite higher than that of the optimum temperature of amylase from MBK1. The enzyme was highly active at pH 4.0 and it lost its activity considerably at pH 7. This pH optimum was higher than the pH optimum (11) of MBK1 and MBK2.

Keywords : Amylase, Starch, Rice-bran, *Bacillus* sp.

INTRODUCTION

All living things have the gut microflora as a necessary component. Numerous endogenous and external variables impact the physiological and biochemical characteristics of the microorganisms themselves and define the quantity and species composition of the microbial population. Various microorganisms from the environment infiltrate living things. The bacteria present in the aquatic environment have an impact on the composition of the gut biota and vice versa since the host and microorganisms share the ecosystem, but only those bacteria survive and find appropriate living circumstances in that organism. This is because of natural selection. Crab receives bacteria in the gut from their aquatic environment through water and food that are populated with bacteria. Being rich in nutrients, the environment of the crab gut confers favourable conditions for the microorganisms. The importance of gut bacteria in the nutrition and well-being of their hosts has been established for homeothermic species, such as birds and mammals. Bacterial species of the gut can influence the health and development of the host. By creating an ecological niche for these species, the milieu of crustaceans' digestive tracts promotes favourable development for

bacteria. It has been demonstrated that the micro biota of crustaceans is heavily reliant on bacterial colonisation during early growth, environmental factors, nutritional composition, and dietary alterations. These bacteria have a higher chance of surviving because they feed on the food that the host animal absorbs, digestive fluids, and pieces of mucosal epithelium that have shed their scales. Microbes are identified in classical microbiology using both gross and microscopic morphologies. Colony size, form, and surface characteristics were all part of the gross morphology. With proper sample preparation and light microscopy, the shapes attributed to bacterial cocci (round), bacilli (rods), or spirochetes (corkscrew) may be easily seen. One of the most effective and often used methods for discriminating bacteria above the genus level is gram staining. Enzymes are biological catalysts exceedingly esoteric with extraordinary catalytic power and embrace remarkable specificity. A wide range of living things, including bacteria, plants, animals, and people, create amylases. Amylases are secreted by bacteria and fungi outside of the cells to perform extracellular digestion. In both academic and industrial settings, microbial amylases derived from bacteria, fungi, and yeast has been employed. The amount of amylase generated also varies according to the microbe's place of origin, with strains from environments rich in starch or amylase naturally producing more enzymes . Generally, enzymes are elected over chemical catalysts. Therefore, thermo-phillic microorganisms are conceived to be potentially good alternative sources of thermo-stable thermophiles . Thermo-stable enzymes have been delineated to have inflated stability to organic solvents, acidic and alkaline pH, and detergents . Enzymes are recognised as a crucial resource used by the food, chemical, and related sectors to build a wide range of biotechnology goods. They have so far been acknowledged as a priceless catalyst for numerous organic transformations as well as the creation of quality chemicals and medications . Due to their low cost, high productivity, chemical stability, preservation of the environment, adaptability, and wide availability, microbial enzymes are frequently utilised in industrial processes. The pace of amylase production, particularly in fermentation processes, is greatly influenced by variables including pH, temperature, and supplies of carbon and nitrogen. Microorganisms are amenable to genetic engineering; therefore strains can be modified to produce larger amylase yields . Agro-industrial residues are generally considered the best substrates for the solid-state fermentation process because the metabolites are produced concentrated and purification processes are less costly . Solid state fermentation by agro-industrial waste makes an attractive method as support carbon sources for the production of industrial enzymes and various value-added products such as Organic acids, flavour compounds, biofuel, and secondary metabolites. When agro residues are used as primary substrates in solid-state fermentation, there is a limitation in the availability and accessibility of nutrients. Therefore, supplements of sugars and nitrogenous compounds in the form of diluents should be provided to enhance microbial growth and enzyme productivity. Moreover, for maximum production formation, microorganisms also need to facilitate optimum cultural conditions such as incubation time, growth temperature, and, initial pH of diluents, moisture content and inoculum level. The major factors that affect the microbial synthesis of enzymes in a solid-state fermentation system include the selection of a suitable substrate and

microorganism, the weight of the substrate, inoculum concentration and the moisture level of the substrate. Thus it involves the screening of several agro-industrial materials for microbial growth and product formation . In the industrial setting, they are employed in the manufacturing of digestive aids, cheese, baking, brewing, de-hairing, and tenderising of meat . It is also preferable to avoid adding calcium during starch hydrolysis for many industrial operations, such as brewing or syrup manufacture, as its presence lowers product quality and necessitates further calcium removal. Reduced danger of contamination, lower internal cooling costs, and higher diffusion rate are some benefits of employing thermo-stable - amylases in industrial processes. Nevertheless, practically all known - amylases have Ca^{2+} in their structure and need calcium to be added during hydrolysis to maintain their stability and activity . The creation of extracellular enzymes by Bacillus sp., one of the many non-pathogenic microbes capable of creating helpful enzymes, makes them particularly intriguing. With a vast number of them becoming commercially accessible, microbial amylases satisfy industrial demands and have almost entirely replaced the chemical hydrolysis of starch in the starch processing sector. The capacity to create amylases in large quantities and the simplicity with which they may be modified to produce desired products are the main benefits of employing microbes for amylase synthesis. Using a plasma enzyme test to identify diseases in various body sections. The pancreas produces the digesting enzyme - amylase, and in some pancreatic disorders, notably acute pancreatitis, this enzyme is present in plasma in elevated concentrations. The easiest enzyme to test is -amylase since it uses starch as a substrate and turns it into reducing sugars as a by-product. Being one of the few proteins small enough to pass past the glomerulus of the kidney and be eliminated in the urine is a peculiar property of pancreatic -amylase in macro-amylasaemia, an uncommon disease. The plasma-amylase activity increases as a result of the plasma-amylase forming complexes with other proteins and becoming unable to be eliminated in this manner. However, due to the highly distinct clinical manifestations of macro-amylasaemia, acute pancretitis could not be mistaken for it. The major factors that affect the microbial synthesis of enzymes in an SSF system include the selection of a suitable substrate and microorganism, the particle size of the substrate, inoculum concentration and moisture level of the substrate. Thus, it involves the screening of a number of agro-industrial materials for microbial growth and product formation. Temperature and pH are known to be important parameters in the production of enzymes from bacteria; hence, the thermal and the pH stability of the enzyme, which is a function of the exposure time, must also be taken into account. The goal of the current study was to improve the synthesis of amylase that was isolated from *Bacillus* sp., a type of crab intestine bacteria. In addition, a number of variables, including pH, temperature, and substrate concentration, were tuned. To generate higher and better enzyme activity, additional ions in the reaction media were categorised as activators or inhibitors. Additionally, we wanted to assess the enzymes' ability to remove stains by employing low-cost raw materials to extract enzymes from the amylase that the detergent industry uses extensively. Investigating the cleaning effectiveness of animal amylase mixed with laundry detergent and its unique compatibility as a very effective novel additive was the major contain of the enzyme application raised in this study.

MATERIALS AND METHODS

SAMPLE COLLECTION

Samples of marine crab species, blue swimmer crab (Portunuspelagicus) (Herbst, 1783) and three-spotted crab (Portunussanguinolentus) (Herbst, 1783)were collected from the Mannapad Estuary, Tuticorin district, which had been transported to the laboratory under iced condition. Crabs 12+1 cm in length were selected for this study. They were cleaned in chlorinated water and the length and breadth of their carapaces were measured. The surfaces of the crabs were sterilized using 70% ethanol. The intestines were aseptically dissected from the musculature and placed into a 2-mL sterile centrifuge tube.

ISOLATION OF GUT BACTERIA

The *Bacillus* species strains (MK1 and MK6) used in the present study were isolated as potent amylolytic bacteria from the gut of Portunussanguinolentus and Portunuspelagicus (figure.1). Approximately 9 mL of distilled water was then sterilized. To this 1mL stock, the solution was added and the following dilutions were prepared i.e. 10-1, 10-2, 10-3, 10-4, 10-5, 10-6, 10-7 from each dilution 0.1 mL of sample was spread into the Petri plates with nutrient agar medium (peptone-5.0; NaCl-5.0; HM peptone B-1.5; yeast extract-1.5; agar-15.0; pH-7.4±0.2). The plates were incubated at 37°C in an inverted position for approximately 24 hours. After incubation, the number of colonies that formed was counted. A single colony was isolated and streaked on the prepared agar slants. Agar slants were maintained at 4°C in the refrigerator for further experimental studies.



Figure.1 plate showing the gut microflora of the crab species

BIOCHEMICAL ANALYSIS

KB002-HiAssortedTM Biochemical test (By kit)

The biochemical test was carried out for Citrate utilization test, Lysine utilization test, Ornithine utilization test, Urease detection test, Phenylalanine deamination (TDA), Nitrate reduction test, H_2S production test, Glucose utilization test, Adonitol, Lactose, Arabinose, Sorbitol according to company instruction (Hi Media Mumbai) 50 µl of the isolated organism was inoculated into each well in the test kit by surface inoculations method. The kit was incubated at 37 ° C for 24 hrs based on the principle of pH change after substrate utilization. On incubation, organisms undergo metabolic changes which were indicated by colour changes in the media that can be either interrupted visually or after the addition of a reagent.

KB009- HiCarboTM Utilization test (by Kit)

The carbohydrate test is based on the principle of pH change and substrate utilization. On incubation, the organism undergoes metabolic changes which were indicated by a spontaneous colour change in the media. The organisms were analysed for

the utilization of (i) Lactose, (ii) Xylose, (iii) Maltose (iv) Fructoe (v) Dextrose (vi) Galactose (vii) Raffinose (viii) Trehalose (ix) Melibiose (x) Sucrose (xi) L-Arabinose, (12) Mannose, (13) Inulin, (14) Sodium gluconate, (1) Glycerol, (16) Salicin, (17) Dulcitol, (18) Inositol, (19) Sorbitol, (20) Mannitol, (21) Adonitol, (22) Arabitol, (23) Erythritol, (24) α-Methyl-D-glucoside, (25) Rhamnose, (26) Cellubiose, (27) Melezitose, (28) α-Methyl-D-mannoside, (29) Xylitol, (30) ONPG, (31) Esculin hydrolysis, (32) D-Arabinose, (33) Citrate utilization, (34) Malonate utilization, (35) Sorbose 50µl of broth containing the sample was inoculated into the well provided in the kit and incubation was carried out at 37 ° C for 24 h

IDENTIFICATION AND MOLECULAR CHARACTERIZATION OF BACTERIA

16S ribosomal RNA gene sequencing

Sequencing the purified PCR products with the help of the Rajiv Gandhi Centre for Biotechnology. Nucleotide sequences were compared with sequences in the National Centre for Biotechnology Information (NCBI). GenBank database using the BLASTn program database using the sequence matching program.

Phylogenetic analysis

Once the sequencing was done, the resultant nucleotide sequence was subjected to n-BLAST analysis in National Centre for Biotechnology Information (NCBI) and deposited in the GenBank database for universal accession.

QUALITATIVE TEST OF AMYLASE-PRODUCING STRAIN

By performing a starch hydrolysis test on a starch agar plate (peptic digest of animal tissue-5.0; meat extract-3.0; starch, soluble-2.0; agar-15.0; pH-7.20.1), bacteria were isolated and their amylolytic potential was evaluated. Starch agar plates with the microbial isolates were streaked, and the plates were incubated at 37°C for 24 h. Following incubation, the starch agar plate was treated with freshly prepared 1% iodine solution. The blue colour zone around the growth denotes a poor outcome, whereas a clear zone of hydrolysis around the growth denotes a good outcome. These isolates were further examined because they exhibited distinct zones of hydrolysis and were thought to be amylase producers.

Substrate

Two different types of substrates were used one standard starch and another one agro-industrial waste to be used as substrate viz., rice bran was procured from the local market and powdered to obtain a particle size of 1.0 to 2.0 mm. SSF was performed with all two substrates and their enzyme production was checked by assay.

SUBMERGED FERMENTATION

Bacterial inoculums were inoculated into 50mL of the culture medium. The fermentation medium comprised starch (10 g/L), Peptone (10 g/L), yeast extract (20 g/L), KH_2PO_4 – (0.05 g/L); $MnCl_2$. H_2O (0.015 g/L); $MgSO_4.7H_2O$ (0.25 g/L); $CaCl_2.2H_2O$ (0.05 g/L); $FeSO_4.7H_2O$ (0.01 g/L). Submerged fermentation is used to make amylase. 10 mL of bacterial inoculum was added to 500 mL of the production medium for inoculation. The flask was put into a rotating shaker incubator and run for 24 hours at a particular speed, temperature, and pH. After incubation, the fermented broth was centrifuged in a cooling centrifuge for 15 minutes at 10,000 rpm. The amylase concentration in the supernatant was measured.

pH, temperature, and incubation time effects on growth and amylase production

The effect of pH value and temperature on amylase production was carried out using the following pH range (4-11) and incubation temperature (28, 33, 38, 43 and 48°C) for bacterial isolates Bacillus sp. For the test isolation, 100 mL of sterile medium (starch-10g/L; peptone-10g/L; yeast extract-20g/L; KH2PO4-0.05g/L; MnCl2.4H2O-0.015g/L; MgSO4.7H2O-0.25g/L; CaCl2.2H2O-0.05g/L; FeSO4.7H2O-0.01g/L) was produced in several conical flasks and infected for 24-72 hours with 2% inoculum (starch/rice bran)

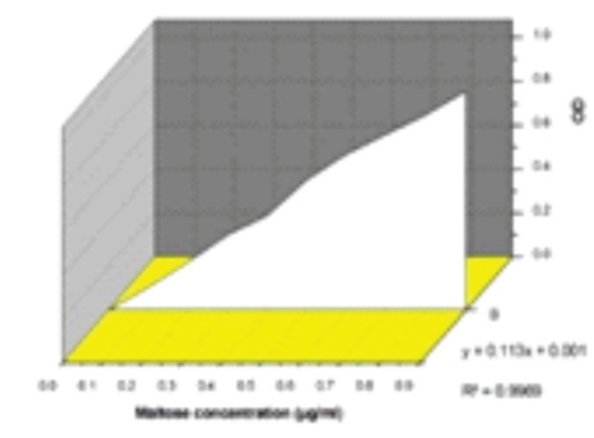
QUANTITATIVE TEST FOR AMYLASE

Based on the methods, amylase activity was estimated. According to Gomes *et al.*, amylase was mainly measured using soluble starch, 1% (w/v), as substrate in phosphate buffer (pH 6.5). The reaction mixture, which included 0.2mL of adequately diluted enzyme solution and 1.8mL of the substrate solution, was incubated at 50°C for 10 min. By adding 3mL of dinitrosalicylic acid (DNS), the process was halted. The Miller technique was used to determine the amount of reducing sugar released (1959). Absorbency of the solution was made at 540 nm using UV-Vis Spectrophotometer against a blank prepared by a control fermentation medium. The absorbency of the enzyme concentration of reducing sugar was determined by comparing the OD value with a standard graph prepared using maltose. In all circumstances, the quantity of enzyme releasing 1 g of reducing sugar as maltose per minute under test conditions is referred to as one unit (U) of enzyme activity.

STANDARD PREPARATION

Maltose working solution:

180mg of maltose is weighed and made up to 100mL with d. H_2O.



Maltose standard graph for amylase enzyme

Maltose standards are shown in the graph above. Given that the line is straight, the photometry was successful in demonstrating that the variation in optical density relies on the concentration of maltose as the concentration of maltose rises. Using Microsoft Excel, the R (correlation coefficient), which measures the strength of the association between the two variables and aids in determining the linear regression between maltose concentration and absorbance, was computed. Its square value was R_2 = 0.9969. The concentration of maltose emitted during this experiment was calculated using the maltose standard curve equation, y=0.113x - 0.0015, where y stands for maltose absorbance and x for concentration.

RESULTS AND DISCUSSION

The study was performed to isolate, characterize and optimise of growth and enzymatic activity of amylolytic bacteria

isolated from the gut of Portunussanguinolentus and Portunuspelagicus. Enrichment of samples, selective plating, microscopy, biochemical tests and the different enzymatic assay was applied for this purpose.

Morphological characterization

The selected antagonistic isolate MBK1 was gram-positive rod-shaped bacilli with square ends. They are single rod-shaped or appear in short chains. MBK2 was an aerobic gram-positive, round shape, white, smooth edges and raised elevation, spore-forming motile bacteria. Gram-positive, rod-shaped bacteria that differentiate into heat-resistant endospores under aerobic conditions were placed in the genus *Bacillus*. Bacteria belonging to the genus *Bacillus* have a long and distinguished history in the realms of biotechnology. Manufacture of amylases and proteases for industrial applications began early this century, but significant production and usage were delayed until after the 1950s when the advantages of including the alkaline protease of *Bacillus licheniformis* in washing detergents were realized. This was followed by developments in the starch processing industry based on the α-amylase from *Bacillus licheniformis*, particularly the conversion of starch to high-fructose corn syrups as sucrose replacements in foods and beverages. described the genomic information that can be employed in the development of other researchers, including comparative analyses employing genes and genomes, origin and dispersion of strains. It was reported as a *Bacillus cereus* isolated from the digestive tract in a saline environment. Reported that all the microbes were grouped into six families and seven genera, which showed a good relationship between the bacterial populations associated with crabs; *Bacillus* was the dominant genus, followed by *Staphylococcus, Pseudomonas*, *Enterobacter, Aeromonas, Alcaligenes* and *Acinetobacter*. reported from the gut of fifteen mangrove crab species 523 bacterial strains were isolated and classified into ten taxonomic groups including the highest number of strains *Bacillus* (113).

Biochemical characterization

The isolate MBK1 exhibited positive lysine utilization, ornithine utilization, urease detection, H_2S production test, adonitol, lactose, arabinose, sorbitol and starch. Further, this isolate showed negative for the following reactions, such as citrate utilization test, phenylalanine deamination (TDA), nitrate reduction and glucose. The isolate MBK2 exhibited positive lysine utilization, ornithine utilization, urease detection, adonitol and starch. Further, this isolate showed negative for the following reactions, such as citrate utilization test, phenylalanine deamination (TDA), nitrate reduction, H2S production test, glucose, lactose, arabinose and sorbitol. These results suggested that all the isolates belong to the genus Bacillus sp(figure.3).

Analysis for carbon utility test

Among the 35 carbon sources, this MBK1 strain used and ferment the Lactose, Dextrose, Galactose, Mannitol, Adonitol, Arabitol, Erythritol, α-Methyl-D-glucoside, ONPG, Esculin hydrolysis, D-Arabinose, Citrate utilization and Malonate utilization. However, they could not hydrolyze Xylose, Maltose, Fructose, Raffinose, Trehalose, Melibiose, Sucrose, L-Arabinose, Mannose, Inulin, Sodium gluconate, Glycerol, Salicin, Dulcitol, Inositol, Sorbitol, Rhamnose, Cellobiose, Melezitose, α-Methyl-D-mannoside, Xylitol and Sorbose. The MBK2 strain was used and ferments the Lactose, Xylose, Maltose, Fructose, Dextrose, Galactose, Raffinose, Trehalose, Melibiose, Sucrose, Mannose, Inulin, Sodium gluconate, Glycerol, Salicin, Dulcitol, Inositol, ONPG,

Esculin hydrolysis, D-Arabinose, Citrate utilization and Malonate utilization. However, they could not hydrolyze L-Arabinose, Sorbitol, Mannitol, Adonitol, Arabitol, Erythritol, α-Methyl-D-glucoside, Rhamnose, Cellobiose, Melezitose, α-Methyl-D-mannoside, Xylitol and Sorbose. (figure.4)

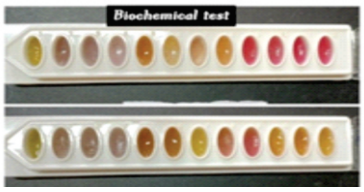


Figure.3 Biochemical characterization of MBK1 and MBK2

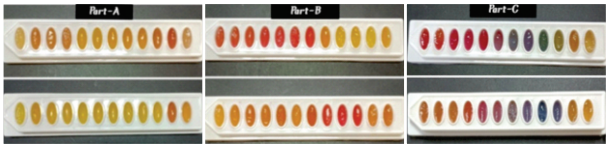


Figure.4 Characterization of carbohydrate utilization for MBK1 and MBK2

Molecular identification

The 16S rRNA gene of the MBK1 & MBK2 strains were amplified by PCR techniques using the 16S rDNA universal primers and both the strands were sequenced. The resultant nucleotide sequences were subjected to n-BLAST analysis in the National Center of Biotechnology Information (NCBI) and deposited in the GenBank database for universal accession. The 16S rRNA sequence of isolated strains MBK1 & MBK2 was subjected to alignment using BLAST to find out the sequence similarities. With the help of the BLAST Nucleotide tool, the sequence alignment drawn by the slanted cladogram phylogenetic tree of MBK1 and MBK2 showed in Figures 5 & 6. The strain MBK1 closest sequence to the Bacillus thuringiensis strain with 99.29% similarity, assession number MT435558 and the MBK2 strain is closely similar with 99.86% KX610819 *Bacillus cereus*.


05/04/2023, 19:06 Bacillus thuringiensis strain MBK1 16S ribosomal RNA gene, partial seq - Nucleotide - NCBI

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Here's how you know

Log in

Nucleotide

GenBank

Bacillus thuringiensis strain MBK1 16S ribosomal RNA gene, partial sequence

GenBank: OP518457.1

FASTA Graphics

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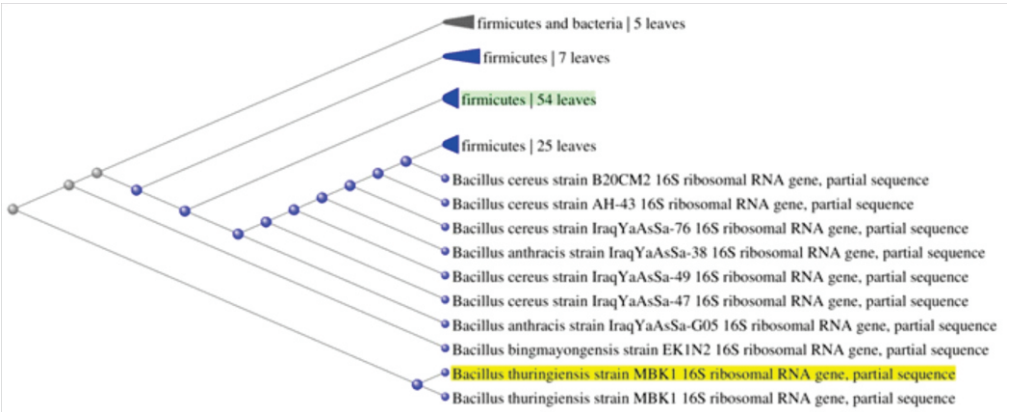


Figure:5 Phylogenetic tree of MBK1

06/03/2023, 12:59 Bacillus cereus strain MBK2 16S ribosomal RNA gene, partial sequence - Nucleotide - NCBI

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Here's how you know

Nucleotide

GenBank

Bacillus cereus strain MBK2 16S ribosomal RNA gene, partial sequence

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FASTA Graphics

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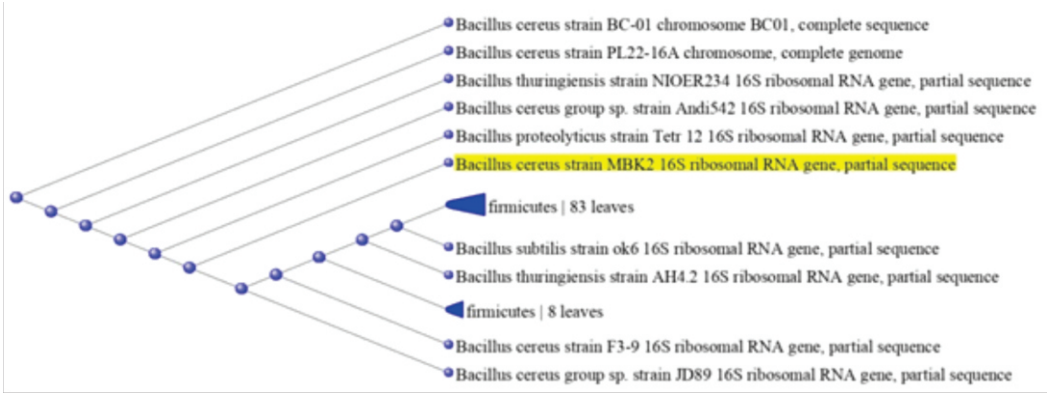


Figure: 6. Phylogenetic tree of MBK2

Selection of probiotic microorganisms that produce amylase

After the screening, it was discovered that only 2 of the 6 bacterial strains produced the most amylase. The strain from these two that produced the zone most effectively was chosen for further study. Fogarty and Kelly (1979), as well as Iverson and Millis (1974), described the use of starch agar and idoine for the detection of amylase (hydrolytic enzyme) generating bacteria. Starch hydrolysis may be observed on plates as a clear zone encircling a colony. The amylase generated during the organisms' development has hydrolyzed starch around the colony, hence testing negative when inundated with iodine is the mechanism behind the clean zone that was seen (Figure.7).The unhydrolyzed portion of the plate tested positive for starch (amylose), which is why it was blue-black. According to Akpan *et al*. (1999), a quick screening approach like Remazol Brilliant Blue (R.B.B.) will be more effective because the starch hydrolysis method is time-consuming and difficult for direct separation of intact cells. After all, the cells die after being flooded with iodine. R.B.B. starch agar offers a quick and easy method for searching for amylolytic bacteria. It doesn't call for floods or previous slant-based colony reproduction. The zones stand out vividly against the blue-black background.

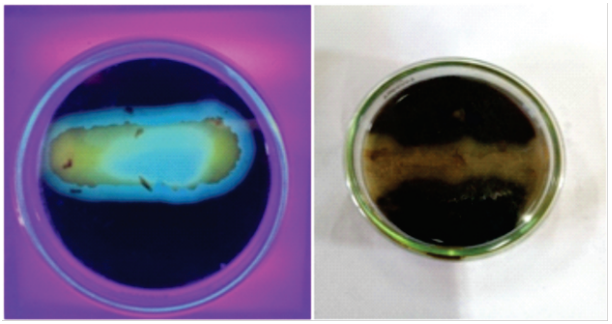


Figure. 7 Streaking of Bacterial strains on starch agar plate showed clear surrounding zone after flooding the plate with iodine solution

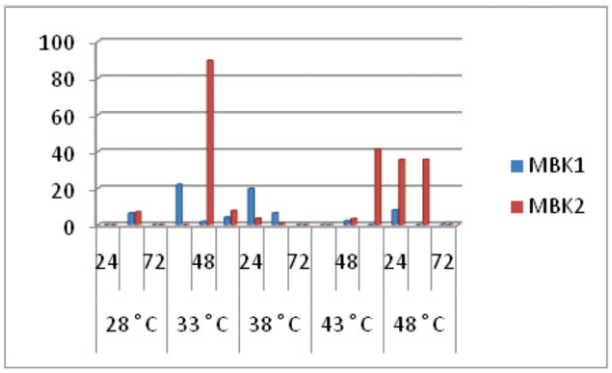


Figure 8; Effect on enzyme production with pH – 4 at different temperatures of MBK1 & MBK2 strains with different time intervals

Figure 8, shows the effect of temperature of pH 4, and starch as a substrate on the activity of α-amylase whereby, maximum enzyme activity was observed on MBK2 strain at 33˚ C at 48 h. MBK1 showed the highest amylase activity at 21.91 ± 0.07 at 33˚ C, 24 h. MBK2 showed the highest amylase activity 89.55 ± 0.08 at 33˚ C at 48 h. The result of the temperature effect on the growth of selected bacterial strains MBK1 and MBK2 showed maximum growth at 28 ˚ C 6.42 ± 0.04 of 48 h incubation and 7.00 ± 0.24 of 48 h incubation; 33˚ C 21.91 ± 0.07 of 24 h incubation and 89.55 ± 0.08 of 48 h incubation; 38˚ C 19.79 ± 0.09 of 24 h incubation and 3.5 ± 0.12 of 24 h incubation; 43˚ C 2.16 ± 0.51 of 48 h incubation and 41.07 ± 0.20 of 72 h incubation; 48˚ C 8.07 ± 0.23 of 24 h incubation and 35.52 ± 0.55 of 24 h and 48h incubation.

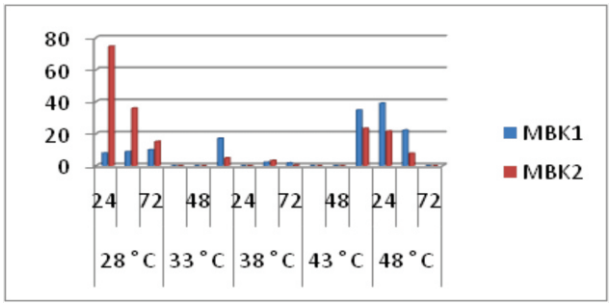


Figure 9; Effect on enzyme production with pH – 4 at different temperatures of MBK1 & MBK2 strains with different time intervals

Figure 9, shows the effect of temperature of pH 7 on the activity of α-amylase whereby, maximum enzyme activity was observed on MBK2 strain at 28˚C at 24 h. MBK1 showed the highest amylase activity at 39.01 ± 0.47 at 48˚C, 24 h. MBK2 showed the highest amylase activity 74.64 ± 0.31 at 28˚C at 24 h. The result of the temperature effect on the growth of selected bacterial strains MBK1 and MBK2 showed maximum growth at 28˚C 9.91 ± 0.21 of 72 h incubation and 74.64 ± 0.31 of 24 h incubation; 33˚C 17.04 ± 0.13 of 72 h incubation and 4.82 ± 0.04 of 72 h incubation; 38˚C 2.08 ± 0.08 of 48 h incubation and 3.21 ± 0.04 of 48 h incubation; 43˚C 34.77 ± 0.16 of 72 h incubation and 23.38 ± 0.08 of 72 h incubation; 48˚C 39.01 ± 0.47 of 24 h incubation and 21.6 ± 0.32 of 24 h incubation.

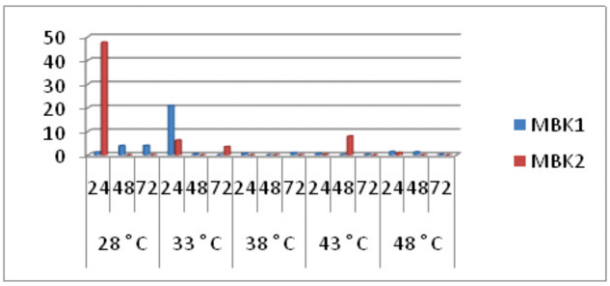


Figure 10; Effect on enzyme production with pH – 4 at different temperatures of MBK1 & MBK2 strains with different time intervals

Figure 10, shows the effect of temperature of pH 11 on the activity of α-amylase whereby, maximum enzyme activity was observed on MBK2 strain at 28˚C at 24 h. MBK1 showed the highest amylase activity 20.92 ± 0.39 at 33˚C, 24 h. MBK2 showed the highest amylase activity 47.58 ± 1.01 at 28˚C at 24 h. The result of the temperature effect on the growth of selected bacterial strains MBK1 and MBK2 showed maximum growth at 28˚C 4.01 ± 0.04 of 72 h incubation and 47.58 ± 1.01 of 24 h incubation; 33˚C 20.92 ± 0.39 of 24 h incubation and 6.25 ± 0.31 of 24 h incubation; 38˚C 0.98 ± 0.05 of 72 h incubation and nil activity at the temperature; 43˚C 0.66 ± 0.07 of 24 h incubation and 7.94 ± 0.39 of 48 h incubation; 48˚C 1.44 ± 0.07 of 24 h incubation and 1 ± 0.08 of 24 h incubation.

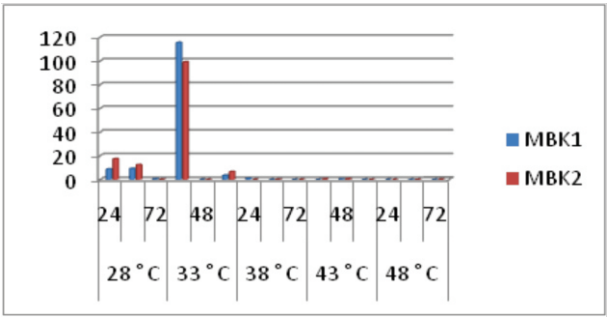


Figure 11; Effect on enzyme production with pH – 4 at different temperatures of MBK1 & MBK2 strains with different time intervals

Figure 11, shows the effect of temperature of pH 4 on the activity of α-amylase whereby, maximum enzyme activity was observed on MBK1 strain at 33˚C at 24 h. MBK1 showed the highest amylase activity at 115.69 ± 0.15 at 33˚C, 24 h. MBK2 showed the highest amylase activity 99.15 ± 0.07 at 33˚C at 24 h. The result of the temperature effect on the growth of selected bacterial strains MBK1 and MBK2 showed maximum growth at 28˚C 9.29 ± 0.82 of 24 h incubation and 17.484 ± 0.23 of 24 h incubation; 33˚C 115.69 ± 0.15 of 24 h incubation and 99.15 ± 0.07 of 24 h incubation; 38˚C 1.03 ± 0.03 of 24 h incubation and 0.11 ± 0.02 of 72 h incubation; 43˚C 0.44 ± 0.03 of 48 h incubation and 0.54 ± 0.05 of 48 h incubation; 48˚C no activity at the incubation temperature and 0.20 ± 0.05 of 72 h incubation.

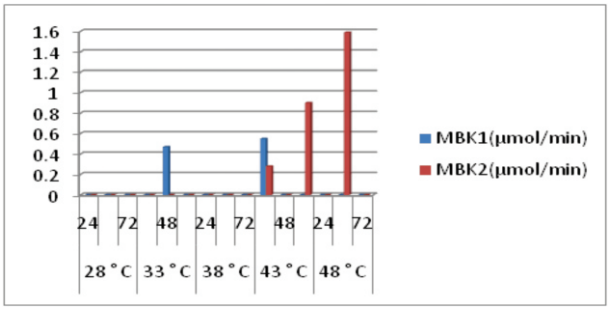


Figure 12; Effect on enzyme production with pH – 7 at different temperatures of MBK1 & MBK2 strains with different time intervals

Figure 12, shows the effect of temperature of pH 7 on the activity of α-amylase whereby, maximum enzyme activity was observed on MBK2 strain at 48˚C at 48 h. MBK1 showed the highest amylase activity 0.55 ± 0.07 at 43˚C, 24 h. MBK2 showed the highest amylase activity 1.59 ± 0.8 at 48˚C at 48 h. The result of the temperature effect on the growth of selected bacterial strain MBK1 showed maximum growth at 33˚C 0.47 ± 0.15 of 48 h incubation; 43˚C 0.55 ± 0.07 of 24 h incubation, and no production at 28˚C, 38˚C and 48˚C. The result of the temperature effect on the growth of selected bacterial strain MBK2 showed maximum growth at 43˚C 0.90 ± 0.05 of 72 h incubation; 48˚C 1.59 ± 0.8 of 48 h incubation and no activity at 28˚ C, 33˚C and 38˚C.

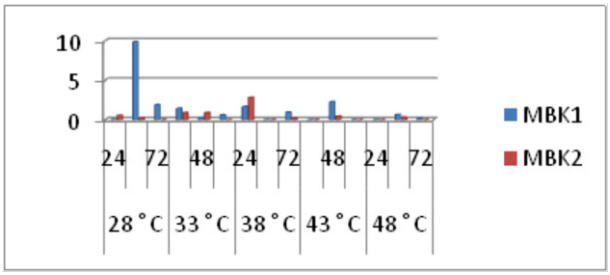


Figure 13; Effect on enzyme production with pH – 11 at different temperatures of MBK1 & MBK2 strains with different time intervals

Figure 13, shows the effect of temperature of pH 11 on the activity of α-amylase whereby, maximum enzyme activity was observed on MBK1 strain at 28˚C at 48 h. MBK1 showed the highest amylase activity 9.88 ± 0.84 at 28˚C, 48 h. MBK2 showed the highest amylase activity 2.82 ± 0.31 at 38˚C at 24 h. The result of the temperature effect on the growth of selected bacterial strains MBK1 and MBK2 showed maximum growth at 28˚C 9.88 ± 0.84 of 48 h incubation and 0.55 ± 0.07 of 24 h incubation; 33˚C 1.44 ± 0.7 of 24 h incubation and 0.89 ± 0.23 of 24 h incubation; 38˚C 1.66 ± 0.2 of 24 h incubation and 2.82 ± 0.31 of 24 h incubation; 43˚C 2.29 ± 0.07 of 48 h incubation and 0.44 ± 0.04 of 48 h incubation; 48˚C 0.64 ± 0.07 of 48 h incubation and 0.33 ± 0.12 of 48 h incubation.

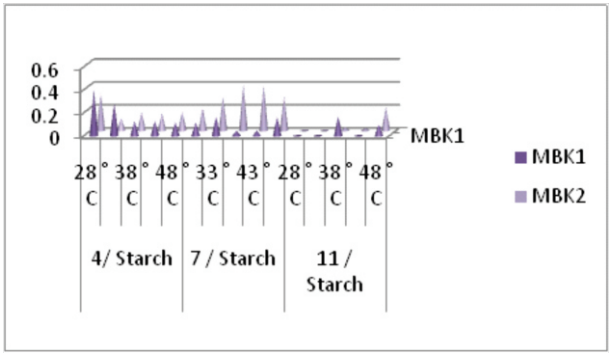


Figure 14; Effect on protein estimation with pH – 4,7&8 at different temperature of MBK1 & MBK2 strains

Figure 14, showed the effect of various temperatures on incubation at pH 4 and substrate as a starch. The result showed the highest protein estimated on pH 4 at 28˚C MBK1 - 0.403 and MBK2-0.329; pH 7 at 33˚C MBK1 - 0.170 and 43˚ C MBK2 - 0.404; pH 11 at 38˚C MBK1 - 0.171 and 48˚ C MBK2 - 0.205.

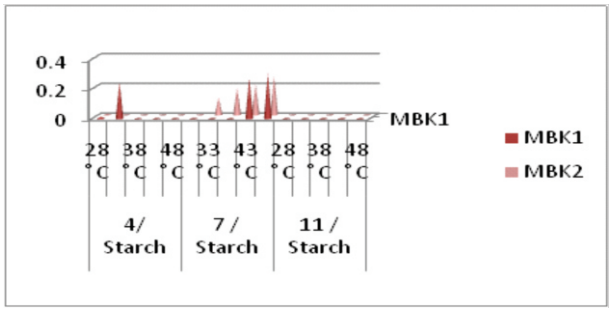


Figure 15; Effect on protein estimation with pH – 4,7&8 at different temperature of MBK1 and MBK2 strains

Figure 15, showed the effect of various temperatures on incubation at pH 4 and substrate as rice bran. The result showed the highest protein estimated on pH 4 at 33˚C MBK1 - 0.244 and MBK2 - 0.008; pH 7 at 48˚C MBK1 - 0.308 MBK2 - 0.263; and no protein on pH 11.

(Filho *et al.*, 2018) described the genomic information that can be employed in the

development of other researchers, including comparative analyses employing genes and genomes, origin and dispersion of strains. It was reported as a Bacillus cereus isolated from the digestive tract in a saline environment. –(Zote *et al.*, 2018) reported that all the microbes were grouped into six families and seven genera, which showed a good relationship between the bacterial populations associated with crabs; Bacillus was the dominant genus, followed by *Staphylococcus, Pseudomonas, Enterobacter, Aeromonas, Alcaligenes* and *Acinetobacter*. In present study, the crab digestive flora contains Bacillus thuringiensis (OP518457) and Bacillus cereus (OP526970) strains. (K *et al.*, 2017) reported from the gut of fifteen mangrove crab species 523 bacterial strains were isolated and classified into ten taxonomic groups including the highest number of strains *Bacillus* (113). Several reports have described amylase production from this particular genus isolated from various sources (Božić *et al.*, 2014) (R, 2017)–(Tamamura *et al.*, 2014). Bacteria belonging to the genus *Bacillus* have a long and distinguished history in the realms of biotechnology. Manufacture of amylases and proteases for industrial applications began early this century, but significant production and usage were delayed until after the 1950s when the advantages of including the alkaline protease of *Bacillus licheniformis* in washing detergents were realized. This was followed by developments in the starch processing industry based on the α-amylase from *Bacillus licheniformis*, particularly the conversion of starch to high-fructose corn syrups as sucrose replacements in foods and beverages.

The production of extracellular amylase was found to be significantly influenced by the composition of the medium and the culture conditions. Carbon is not only an essential component of the medium but also one of the elements required for bacterial growth and metabolism, which in turn stimulates the production of enzymes. *Bacillus* sp. produced amylase at the highest level when starch was the only carbon source used in the current situation. Aygan *et al.*, 2008 reported that an enzyme obtained from *Bacillus* sp. AB68 was active in a broad temperature range between 20 and 90˚C, with an optimum of 50˚C. temperature is an important limiting factor for the storage of enzymes. (Deb *et al.*, 2013) reported the effect of temperature on enzyme activity ranging from 35˚C - 70˚C at optimum pH.Yordan Stefanov described 31% of the studied Bacillus strains showed amylolytic activity after cultivation on a starch agar (61% of *Bacillus cereus*, 36% of *Bacillus thuringiensis* and 3% of *Bacillus sphaericus*). (Vijayaraghavan *et al.*, 2015) also revealed maximum amylase production by Bacillus cereus IND4 was attained by response surface statistical optimization methods employing different process parameters under SSF. The selection of an ideal agro-biotech waste for enzyme production depends upon several factors, mainly related to the cost and availability of the substrate material (Singh Nee Nigam *et al.*, 2009) In the present study, maximum enzyme production (115.69 μmol.min^{-1}) was observed with rice-bran than starch 89.55 μmol. min^{-1}. The results described here are highly significant because no reports so far evidenced the use of crab waste and rice bran as the substrate for the production of amylases. Based on this fact, crab waste can be effectively utilized for the production of amylases. The results illustrated in the table indicate that the optimum temperature for amylase activity was 33˚C. The optimum temperature for amylase activity from MBK2 was similar to or quite higher than that of the optimum temperature of amylase from MBK1. The

enzyme was highly active at pH 4.0 and it lost its activity considerably at pH 7. This pH optimum was higher than the pH optimum (11) of MBK1 and MBK2.

CONCLUSION

The results obtained in the present study indicated that Bacillus sp. could be a potential strain for amylase production in submerged fermentation using starch and rice bran-like easily available substrates. The optimization processes parameters such as pH (4), temperature (33˚C) and inoculum size 1% for attaining a higher yield of amylase.

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Effects of Institutional Agricultural Credit on Agriculture of in India

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ABSTRACT

This study examined the nature of relationship between institutional agricultural credit and agricultural gross value added in India, using the time series data covering the period from 2011-12 to 2021-22. The study used the Ordinary Least Square (OLS) estimation technique to assess the effect of institutional credit on agricultural output in India. The findings from the investigation suggest that over this period, all selected independent variables significantly and positively impacted the agricultural output. But all other things remain constant; individually, a 10 percent increase in institutional production (short term) credit and medium-term/long-term credit led to the rise of 4.5 percent and 1.1 percent in the agricultural output, respectively. Thus, institutional credit seems to be an effective tool for agricultural growth, but its effectiveness is weakened by improper utilization and low productivity. Further results showed that the government expenditure on agriculture positively and significantly affected the agricultural output by 1.9 percent.

Keywords: Institutional Credit, Agricultural Gross Value Added, Agricultural growth in India.

INTRODUCTION

Agriculture is considered an essential building block of the Indian economy. The sector has been playing a very crucial role in employment, poverty reduction, and food security in India. Notwithstanding its contribution, the sector faces multiple constraints in various stages (Chatterjee & Oza, 2017). One of the main constraints is access to formal sector institutional credit. Farmers, mainly marginal and small farmers, often have access to non-institutional credit sources, even though they are usurious because they are easy to access comparatively (Kumar et al., 2007; Sidhu & Gill, 2006). To address the above challenges Government of India (GoI) introduced RRBs, Kisan Credit Card and other financial inclusion policies such as Pradhan Mantri Jan Dhan Yojana and the Pradhan Mantri Mudra Yojana. It resulted in a continuous increase in institutional credit to the agriculture sector. Despite this increase, the Agricultural Gross Value Added (AGVA) as a percentage of total Gross Domestic Product (GDP) stagnantly remained low, 18.3 per cent, 17.6 percent, 18.3 percent, 20 percent, 18.6 percent only from the past five years (Agricultural Statistics at a Glance 2021). This observed phenomenon in institutional credit and agricultural gross value added raises the question of whether the credit had really impacted the agriculture sector. A profound analysis of the credit type, production (Short Term) credit, and Medium-term/Long-term credit discloses a significant degree of disparity with the greater share of production

(ST) credit. It raises another question of whether imbalance causes the ir-responsiveness of AGVA in India at a macro level. Thus, the primary objective of the study is to investigate the impact of intuitional agricultural credit on agricultural growth in India. Particularly this study seeks to 1) Examine the impact of institutional short-term agricultural credit on India's agricultural output. 2) Assess the impact of institutional medium/long-term agricultural credit on India's agricultural output. 3) Determine the impact of the government's expenditure in the sector on agricultural production in India.

MATERIALS AND METHODS

The study mainly relied upon secondary data. The time-series data were collected on an annual basis from Agricultural Statistics at a Glance 2021, Department of Agriculture, Cooperation & Farmers Welfare Directorate of Economics and Statistics, GoI. This study adopted descriptive and analytical methods in analyzing and estimating the causal relationship between independent variables and dependent variable. The descriptive methodology employed descriptive statistics, simple tables, and graphs to analyze the trend performance of the variables. Further, to estimate the relationship among the chosen variables, Ordinary least square (OLS) estimation techniques were used with the help of a statistical package for social sciences (SPSS) version 25. An increase in credit to the agriculture sector by the financial institutions paves the way to invest in the industry, which leads to a rise in the Indian agriculture sector. Based on this, the study quantified agriculture sector production as a linear function of Production (ST) credit, MT/LT credit provided by the formal institutions and government expenditure on agriculture. It is considered the agriculture output as a dependent variable and is being proxied by the Agriculture Gross valued Added (AGVA). Based on these factors, the functional model for this study is

AGOUT = f (ISTC, IMT/LTC, GEXPA)

The estimated form of the model is

$AGOUT = a_0 + a_1 ISTC + a_2 IMT/LTC + a_3 GEXPA + U$

$LOG (AGOUT) = a_0 + a_1 LOG (ISTC) + a_2 LOG (IMT/LTC) + a_3 LOG (GEXPA) + U$

Where;

AGOUT= Agricultural output measured by AGVA in India.

ISTC = Institutional Production (short-term) credit to the agriculture sector in India.

IMT/LTC = Institutional Medium term/ Long term credit to agriculture sector in India.

GEXPA = Government expenditure on Agriculture in India.

a_0 = The autonomous intercept.

a_1 to a_3 = Parameter to be estimated.

U = Random error term.

RESULTS AND DISCUSSIONS

Table 1 Data presentation on selected variables

(Rupees in crore)

Year	AGVA	ISTC	IMT/LTC	GEXPA
2011-12	1501947	396158	114871	16753.1
2012-13	1675107	473500	133875	18028.38
2013-14	1926372	548435	181687	19027.58
2014-15	2093612	635412	209916	19513.58
2015-16	2227533	665312	250197	15333.92
2016-17	2518662	689457	376298	37029.81
2017-18	2829826	753214	409403	37538.77
2018-19	3029925	752209	504620	46583.5
2019-20	3358364	825151	567578	94251.62
2020-21	3609494	893757	681641	108272.8
2021-22	3964312	720736	476369	118294.2

Source; Agricultural Statistics at a Glance 2021.

Table 2 Descriptive statistics for model variables (Rupees in crore)

Variables	Mean	SD	Mini.	Max.
Agriculture GVA	2612286.73	812910.49	1501947	3964312
Institutional production (ST) Credit to Agriculture	668485.55	148441.05	396158	893757
Institutional MT/LT Credit to Agriculture	355132.27	190011.07	114871	681641
Government total expenditure on Agriculture	48238.84	39417.10	15333.92	118294.20

Source: Authors' Calculation.

Table 2 Shows that the agriculture GVA for the period under review averaged 2612286.73crore rupees, institutional production (ST) term and MT/LT credit to the sector averaged 668485.55 and 355132.27 crore rupees respectively. The GOI's total expenditure on agriculture averaged 48238.84 crore rupees. The distribution of intuitional credit to agriculture between production (ST) and MT/LT has been uneven, where a major share is given to production (ST) purposes. The short-term credit increases input use (Fertilizers, pesticides and seeds) but it doesn't increase the agricultural output (Narayanan, S. 2016). Therefore, along with the short-term credit, the MT/LT credit lending rates should also pace up to increase the adoption of modern methods and technology to improve agricultural productivity. The sector performed well in 2021-22, with the highest gross value added of 3964312 crore rupees. GOI had spent significantly less, i.e., 15333.92 crore rupees in 2015-16, whereas in 2021-22, 118294.20 crore rupees have been spent in the sector, which is the highest compared to all other years.

Table 3 Student t-test results

Institutional Credit	N	Mean	St. Deviation	t value	Sig.
ISTC	11	668485.55	148441.05	4.310	0.0003
IMT/LTC	11	355132.27	190011.07		

Source: Authors' calculation

The results obtained from the t-test are summarized in table 3. The independent sample t-test results revealed that the t value was 4.310 and the calculated p-value of 0.0003, which is lesser than the 0.05 significance level; it indicates a significant difference between the means of IST and IMT/LTC. Therefore, it can be inferred that the formal institutions have lent more short-term credits than the medium-term/long-term credit to the agriculture sector.

Table 4 Pattern of the flow of Institutional credit to the agriculture sector

(Rupees in crore)

Year	Production (ST) Credit			MT/LT Credit		
	Co-operative Banks	RRBs	CBs	Co-operative Banks	RRBs	CBs
2011-12	81829	47401	266928	6134	7049	101688
2012-13	102592	55957	314951	8611	7724	117540
2013-14	113574	70697	364164	6389	11956	163342
2014-15	130350	89326	415736	8119	13157	188640
2015-16	143803	101579	419930	9492	17681	223024
2016-17	131880	105001	452576	10878	18215	347205
2017-18	136102	119790	497322	14219	21426	373758
2018-19	142750	125654	483805	9591	24013	471017
2019-20	148287	138069	538795	9080	27257	531241
2020-21	558121	179267	156369	636583	11415	33643
2021-22	462501	134579	123656	436368	12484	27517
Total	2151789	1167320	4034232	1155464	172377	2578615

Source: Agricultural Statistics at a Glance 2021

From Table 4, it is evident that commercial banks have lent the highest production (ST) credit, *i.e.*, 4034232crore rupees to the sector, followed by co-operative banks and RRBs with 2151789 and 1167320 crore rupees, respectively. In MT/LT credit, again commercial banks are the leading banks followed by co-operative banks and RRBs in terms of lending to the sector. Here RRBs have to penetrate their role in lending to the sector since they have been established in rural areas and one of the main reasons is to grant the loan for agriculture purposes in India. From figure 1, it is clear that there is a massive difference in production (ST) credit and MT/LT credit over the period of time. It also shows a steep down in the distribution of the institutional output (ST) credit and MT/LT credit to the sector during 2021-22.

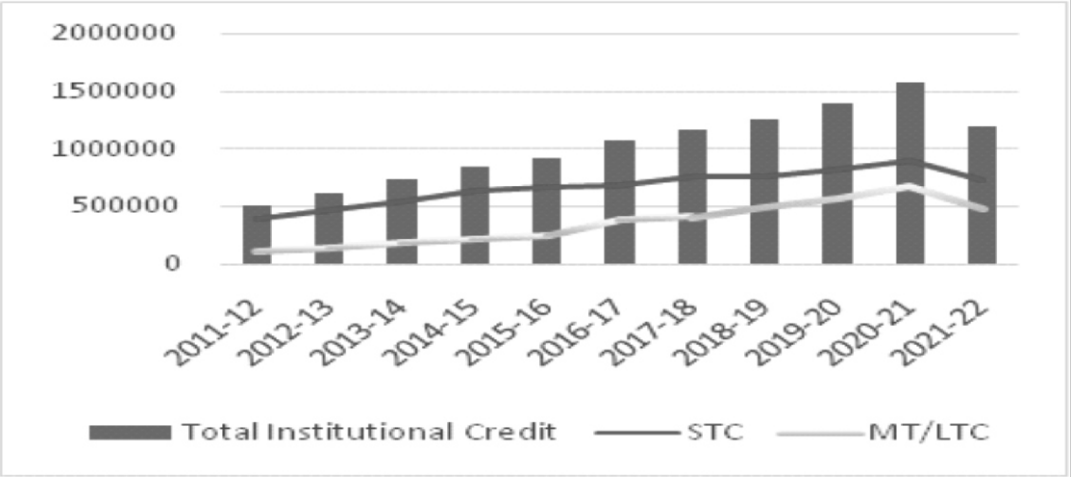


Figure 1 Flow of institutional credit to the agriculture sector.

Table 5 ANOVA Results

Groups	Sum of squares	df	Mean square	F	Sig.
Between Groups	385816826544.061	2	192908413272.030	12.725	0.000
Within Groups	454778482996.182	30	15159282766.539		
Total	840595309540.242	32			

Source: Authors' calculation

As ANOVA results are shown in Table 5, there is a significant difference between the co-operative banks, RRBs and commercial banks' short-term credit to the agriculture sector. The result is evident from the calculated p-value of 0.000, which is lesser than the 0.05 significance level. Thus, one among the co-operative banks, RRBs, and commercial banks' lending amount is different from others. However, which financial institution is lending the highest short-term credit to the sector is not clear from the ANOVA results. Thus, Least Significant Difference (LSD) test is employed in the following section to find the difference among the financial institutions.

Table 6 Post Hoc test Result (Mean Difference in crore Rupees)

(I) Parameters	(J) Parameters	Mean Difference (I-J)	Std. Error	Sig.
Cooperative Banks	RRBs	89497.18182	52499.84029	0.099
	CBs	-171131.18182*	52499.84029	0.003
RRBs	Cooperative Banks	-89497.18182	52499.84029	0.099
	CBs	-260628.36364*	52499.84029	0.000
CBs	Cooperative Banks	171131.18182*	52499.84029	0.003
	RRBs	260628.36364*	52499.84029	0.000

*. The mean difference is significant at the 0.05 level.

The short-term institutional credit by the different financial institutions has been compared through the LSD multiple comparison test in Table 6. The results clearly show that commercial banks are significantly lending the highest amount of short-term credit than the co-operative banks and RRBs. Though co-operative banks lent more short-term credit than the RRBs, it is statistically insignificant. Therefore, co-operative banks and RRBs have to penetrate their short-term lending to the sector as they are easily accessible by the rural population and intendedly established to lend to the sector.

Table 7 ANOVA Results

Groups	Sum of squares	df	Mean square	F	Sig.
Between Groups	266115155485.273	2	133057577742.636	5.161	0.012
Within Groups	773412498706.909	30	25780416623.564		
Total	1039527654192.182	32			

Source: Authors' calculation

It is apparent from Table 7 that the mean difference of medium-term/long-term credit by the financial institutions significantly differs since the calculated p-value, i.e., 0.012, is less than the 0.05 significance level in the ANOVA results. But which financial

institutions' credits have significantly differed is not clear from the ANOVA results. Therefore, to do multiple comparisons LSD test is conducted as a post hoc analysis. Further analysis showed in table No.8 that on average, commercial banks' medium-term/long-term credit is more than the co-operative banks' and RRBs' credit. Still, the difference is only significant against the RRBs' credit, not of cooperative banks' credit. Therefore, it can also be interpreted that the commercial banks have distributed more medium-term/long-term credit to the agriculture sector than the cooperative banks and RRBs in the reference period.

Table 8 Post Hoc Test Results (Mean Difference in crore Rupees)

(I) Parameters	(J) Parameters	Mean Difference (I-J)	Std. Error	Sig.
Cooperative	RRBs	89371.54545	68464.21311	0.202
Banks	CBs	-129377.36364	68464.21311	0.068
RRBs	Cooperative Banks	-89371.54545	68464.21311	0.202
	CBs	-218748.90909*	68464.21311	0.003
CBs	Cooperative Banks	129377.36364	68464.21311	0.068
	RRBs	218748.90909*	68464.21311	0.003

*. The mean difference is significant at the 0.05 level.

Table 9 Model Summary

Model	Sum of squares	df	Mean of square	F	Sig.
Regression	0.982058	3	0.327353	61.042349	0.000022
Residual	0.037539	7	0.005363		
Total	1.019597	10			

Source: Authors' Calculation

Table 10 Empirical Results

Dependent variable: LOG(AGVA)				
Variables	Coefficient	Std. error	t-Statistic	Prob.
Constant	5.250	3.508	1.496	0.178
LOG(ISTC)	0.450	0.428	1.052	0.328
LOG (IMT/LTC)	0.111	0.235	0.471	0.652
LOG (GEXPA)	0.196	0.079	2.473	0.043
R-Squared	0.963183	Adjusted R-Squared	0.947404	
Durbin-Watson stat	2.017735			

Source: Authors' Calculation.

To determine the presence/absence of autocorrelation in the selected variables Durbin-Watson (DW) statistic was employed. The calculated DW value of 2.017735 (Table No. 5) lies between 1.5 to 2.5; it implies no autocorrelation between the successive observation of the model.

Table 9 presents a calculated F (3,7) = 61.042349 and a p-value of 0.000022, which is lesser than the significance level of 0.05; it

indicates that institutional production (ST) credit, institutional medium term/long term credit to the sector and the government expenditure on the agriculture can play a significant role in bringing the short-run changes in the Indian agricultural output during the reference period. Table No. 5 specified the high value of R-Squared of 0.963183 and Adjusted R-Squared of 0.947404 revealed that the model has a goodness of fit on the estimation. Moreover, adjusted R-Squared 0.963183 illustrates the 96 percent systematic variation in the dependent variable (AGVA) due to changes in the independent variables (ISTC, IMT/LTC and GEXPA). The statistical significance of the overall model confirms that there is a high degree of a linear relationship between the variables. This also implied that all explanatory variables have a combined effect on the explained variable.

As mentioned in Table No.10 estimated regression line of the constant term has a positive intercept value of 5.250 which means that all explanatory variables cause a 5.250 percent change in the agricultural output, given the condition that all things remain equal. The positive sign of the coefficient of institutional production (ST) credit and institutional MT/LT credit shows the existence of a positive relationship between the ISTC, IMT/LT credit and agricultural output (AGVA) in India. This means that other factors remain the same; a percentage increase in ISTC and IMT/LT credit will lead to a 0.450 percent and 0.111 percent increase in the Indian agriculture output respectively. Even though both types of institutional credit positively affect the dependent variable, the effect is statistically insignificant in the reference period. The positive sign of government expenditure on agriculture revealed a positive relationship between GEXPA and the agricultural output in India. This means that a percentage increase in GEXPA will lead to a 0.196 percent increase in the Indian agriculture output., ceteris paribus.

CONCLUSION

Despite an increase in efforts by the GoI to increase access to agricultural credit through the formal institutions' disbursements, the growth in agriculture output continues to be stagnant. Timely and sufficiently available agricultural credit makes all activities in the farm possible and leads to an increase in agricultural production. Corresponding to the perception, the study aims to ascertain the impact of institutional credit on the agricultural output in India. The study found that institutional production (ST) credit and MT/LT credit have a positive role in agricultural output performance. The study's outcomes exhibited that the institutional production (ST) credit has a higher impact on AGVA than MT/LT credit, as it has the lion's share in the total institutional credit. The model results of the positive effect of institutional credit, but statistical insignificance demands more allocation of formal sector credit to agriculture in India; simultaneously, the lending institutions have to follow up on whether the given credits are being used for agriculture purposes only. This can be achieved through the active involvement of formal financial institutions in India. Further investigation revealed that government expenditure on agriculture through the various schemes have a positive and significant effect on agricultural output in India. Therefore, it is suggested to come up with new schemes which can revamp the agriculture sector in India.

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Impact of Wheat Grass Products (Juice and Powder) on the Health of Adult Male and Female in Agra District.

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ABSTRACT

The greatest cereal-grass crop in the world is wheat, a member of the genus triticum and the *Gramineae (Poaceae)* family. It is a dense, dry grass with a bright green appearance that resembles hay or straw. Wheat grass contains several vitamins, minerals, amino acids, and essential enzymes that are crucial to human survival. The juice and powder versions of wheat grass have anti-inflammatory and antioxidant effects. Wheat grass juice promotes healthy tissue growth, red cell production, and illness prevention. This study was conducted on 50 respondents (male and female) in Agra District. A multistage stratified random sampling technique was used for selecting respondents for the present study. The finding revealed that majority of the respondents (52%) were in the age group of 25–35 years, liked the taste of wheat grass juice and powder (96%); felt energy all the time after taking wheat grass juice and powder; boosted energy and digestion (22%); and boosted metabolism and prevented the constipation problem and also increased hemoglobin (32%). In the male respondents, before consuming wheat grass products, the average BMI status, blood pressure, and hemoglobin level were 62.0, 120.8/80, 12.12 respectively, and after consuming wheat grass products, the average BMI status, blood pressure, and hemoglobin level were 62.4, 122.5/79.1, 12.21, respectively (P value = 0.05). For the female respondents, the average BMI status, blood pressure, and hemoglobin level were 59.3, 120.8/121.5, and 11.2 respectively before consuming wheat grass products and 59.2, 122.5/121.5, and 11.32 respectively after consuming wheat grass products (P value = 0.05). It indicates that the BMI status, blood pressure, and hemoglobin level were increased in male and female respondents after consuming wheat grass products. The benefits of wheat grass juice and powder on human health and disease prevention are significant.

Keywords : Wheatgrass, Juice, Powder, Hemoglobin, BMI, Blood pressure.

INTRODUCTION

Wheatgrass is an agropyre that belongs to the Triticum aestivum plant family and is high in nutrients. This superfood contains over 100 nutrients, including all vitamins A, C, E, and K, as well as a full range of B-complex vitamins. It also contains 17 essential amino acids and is one of the best sources of chlorophyll. It functions as a natural blood purifier. Wheatgrass contains a wide range of antioxidants, including glutathione and vitamin C, which combat harmful free radicals, prevent oxidative stress, and prevent damage to cells. Wheatgrass juice, which is high in chlorophyll and vitamins, is also beneficial in lowering the risk of heart disease, cancer, arthritis, and neurodegenerative diseases. Currently, natural or organic bioactive substances found in herbs are regarded as an "alternative" kind of treatment. Wheatgrass is one of the herbal

and natural active components that are getting scientific recognition as "functional foods". It is also becoming more widely available and well-liked as a study topic. The mature shoot of Triticumaestivum Linn, a member of the Gramineae family, is wheatgrass (Rana et *al.* 2011). Fortunately, wheatgrass is thought to have a high nutritional phytoactive component content that boosts the effectiveness of alternative medicine. It also possesses anti-inflammatory, antioxidant, immunomodulatory, anticarcinogenic, laxative, and anti-aging effects.

OBJECTIVES

1. To study the effect of wheatgrass products (juice and powder) on health (BMI, blood pressure, and blood hemoglobin level) for the treatment.
2. To compare the health status before and after taking wheatgrass juice and powder.

MATERIALS AND METHODS

The present study was conducted in the urban area of Agra district. This study was conducted with 50 respondents (male and female). A multistage stratified random sampling technique was used for selecting respondents for the present study.

Firstly, the researcher recorded the interviewer's health status before giving wheat grass juice and powder, and then gave wheat grass juice (30 ml) and powder (5 gm) for 15 days and checked the health status. After this, same quantity of wheat grass juice and powder were given to the respondents for 30 days and checked the health status.

Table1 : Distribution of the respondents according to their BMI status on interview or before consumption of wheat grass juice and powder. n=50

Respondents	BMI status (before consumption of wheat grass juice and powder)								Total	
	Underweight		Normal		Overweight		Obesity			
	f	%	f	%	f	%	f	%	f	%
Male	0	0	7	14	3	6	1	2	11	22
Female	1	2	13	26	12	24	13	26	39	78
Total	1	2	20	40	15	30	14	28	50	100

RESULTS AND DISCUSSION

Table 1 highlights the distribution of the respondents according to their BMI status at the interview (before consumption of wheat grass juice and powder). Out of total respondents, the majority of them (40%) were normal, followed by 30 per cent overweight, 28 per cent obese, and very few (2%) were underweight.From the classification of BMI according to Brahmam *et al.* (2005), it could be noted that the obese male and female subjects were in Grade I obesity (25–30).

Table 2 highlights the distribution of the respondents according to their BMI status after taking wheat grass products. Out of total respondents, the majority (58%) had a normal body mass index, followed by 32 per cent with an overweight body mass index, 14 per cent with obesity, and very few (2%) were underweight.

H0: There is no change in body mass index after giving wheat grass products.

H1: Body mass index changes after giving the wheat grass product.

Table2 : Distribution of the respondents according to their BMI status after consumption of wheat grass products (juice and powder). n=50

Respondents	BMI status after taken consumption of wheat grass products *i.e.* juice andpowder (after 30 days)								Total	
	Underweight		Normal		Overweight		Obesity			
	f	%	f	%	f	%	f	%	f	%
Male	0	0	7	14	3	6	1	2	11	22
Female	1	2	12	24	13	26	13	26	39	78
Total	1	2	19	38	16	32	14	24	50	100

Table 3: Difference in BMI before and after giving the wheat grass product among the male and female group . n=50

BMI	Male					Female				
	f	Mean	SD	T Value	P Value	f	Mean	SD	TValue	P Value
Before	11	62.0	5.82	0.07	0.1*	39	59.3	8.72	0.06	0.4*
After		62.4	5.68				59.2	7.83		

* Significant at 5% level

From the above Table 3, it is clear that the P value is < 0.05, therefore the null hypothesis is rejected. It means that the body mass index stabilizes significantly after giving the wheat grass product to the male and female group.

Table 4: Difference in blood pressure (Systolic) status before and after giving the wheat grass product among the selected group of male and female. n=50

Blood Pressure	Male					Female				
(Systolic)	f	Mean	SD	T-P	PValue	f	Mean	SD	T-P	P Value
Before	11	120.8	6.6	0.7	0.8**	39	120.8	6.6	0.4	0.2*
After		122.5	4.5				122.5	4.5		

H0: There is no change in blood pressure level after giving wheat grass products.

H1: Blood pressure level becomes normal after giving the wheat grass product.

In male group, the P value is >0.05; therefore, the alternative hypothesis is accepted. It means that the blood pressure changes after giving the wheat grass product to the group of males. In the female group, the P value is <0.05 , therefore the null hypothesis is rejected. It means that the blood pressure became significantly normal after giving the wheat grass product to the group of males and the blood pressure changed after giving the wheat grass products to the female group.

Table 5: Difference in blood pressure status (diastolic) before and after giving the wheat grass product among the selected group of male and female. n=50

Blood Pressure	Male					Female				
(Diastolic)	f	Mean	SD	T Value	P Value	f	Mean	SD	T-P	P Value
Before	11	80	4.26	0.5	0.1*	39	121.5	8.1	0.3	0.7**
After		79.1	2.88				121.2	6.9		

** Significant at 5% level * Significant at 5% level

H0: There is no change in body mass index after giving wheat grass products.

H1: Body mass index changes after giving the wheat grass product.

In the male group, the P value <0.05 , we may reject our null hypothesis. It means that the blood pressure changed after giving the wheat grass products to the female group.Inthe female group, it is clear that the P value is >0.05; therefore, we may accept our alternative hypothesis. It means that the blood pressure changes after giving the wheat grass product to the group of females.

The present result are supported with results of Mathur *et.al.* (2017) that wheatgrass can be used for detoxifying the body and lowering blood pressure due to its high content of chlorophyll.

Table 6: Difference in hemoglobin levels before and after giving the wheat grass product among the selected group of male and female. n=50

Hb level	Male					Female				
	f	Mean	SD	TValue	PValue	f	Mean	SD	TValue	PValue
Before	11	12.12	0.90	0.26	0.03*	39	11.2	0.83	1.0	0*
After		12.21	0.87				11.32	0.82		

* Significant at 5% level

H0: There is no change in hemoglobin level after giving wheat grass products.

H1: The hemoglobin level increased after giving the wheat grass product.

In the male and female group, the P value is <0.05 , thus the null hypothesis is rejected. It means that the hemoglobin level increased significantly after giving the wheat grass product to the group of male and female group. Similar results are reported by Chauhan (2014). He found that wheat grass juice can also be used to detoxify an organism, improve blood by increasing hemoglobin levels, or boost immunity levels. Mathur *et al.* (2017) found the similar result that blood hemoglobin levels are significantly influenced by wheat grass juice, which also aids in the treatment of anemia. None of the patients reported any negative effects from the juice. No side effect of juice was complained about by any of the subjects.

Table7 shows the distribution of respondents based on their consumption of wheat grass products. All the respondents reported that wheat grass products helped in improving

Table 7: Distribution of the respondents according to perceived benefits of wheat grass products.
n=50

Benefit	Respondent							
	Male				Female			
	Yes		No		Yes		No	
	f	%	f	%	f	%	f	%
Increase haemoglobin	8	16	3	6	24	48	15	30
Help in digestion	11	22	0	0	39	78	0	0
Provide energy	11	22	0	0	39	78	0	0
Boost metabolism	8	16	3	6	23	46	16	32
Help in constipation	9	18	2	4	7	14	16	32
Help in weight loss	0	0	0	0	1	2	0	0

digestion and providing energy. Majority of the respondents reported that it is beneficial in increasing hemoglobin (64%), and boosting metabolism (62%). ThammanaMukthi et al. (2017) found similar result that wheat grass juice helps in improving digestion and is an effective skin cleanser. Wheatgrass juice removes heavy metals and is useful to treat various blood disorders.

CONCLUSION

It can be concluded that wheat grass products such as juice and powder provide many holistic benefits and also help in maintaining blood pressure, BMI, and hemoglobin levels. Thus, it can be said that wheatgrass products play an important role in human life and prevent health problems like ulcerative colitis, cancer, diabetes, obesity, skin problems, high blood pressure, etc.

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Gujarat Agricultural Universities Research Journal

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The Gujarat Agricultural Universities Research Journal is published quarterly. The following types of manuscripts are considered for publication on meeting the standard of the journal.

1.1 ARTICLES ON ORIGINAL RESEARCH COMPLETED, not exceeding 4000 words (up to 15 typed pages, including references, tables, etc.) should be exclusive for the journal. They should present a connected picture of the investigation and should not be split into parts. A complete information of Ph.D. thesis should preferably be given in one article.

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1.5 Author should note: (a) period (years) of conducting the experiment must be indicated, (b) article should preferably be submitted soon after completion of experiment. (c) articles on genetics and plant breeding and on plant crops should be based on data of minimum two years. (d) contribution involving a former or present student must clarify that it is not based/based on complete M.Sc. thesis, or complete or a part of the Ph.D. thesis, indicating its year of submission and (e) Article Certificate must be signed by all the authors and must contain subscription numbers of authors.

2.1 TITLE should be short, specific and informative. It should be phrased to identify the content of the article and include the nature of the study and the technical approach, essential for key word indexing and information retrieval.

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4.5 The tables should fit in the normal lay-out of the A-4 page only. All weights and measurement must be in SI (metric) unit. Tables and illustrations (up to 20% of text) should not reproduce the same data.

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