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Possibility Assessment of Utilization of Crop Residues to Uplift Economic Condition of the Rural Women

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ABSTRACT

Women have the most creative mind to make various products by using various designs. Handmade products are in fashion at present time and by this economic support can be provided to creative women. The present study was conducted in Mathura district to perceive an opportunity for the upliftment of rural women economically in the handicraft sector with the use of crop residues. A total of 75 rural women were selected for the present study and data was collected through a self-prepared interview schedule. The findings of the study depicted a significant difference at <0.01 level in the interest of women related to basketry handicrafts based on their age, occupation,and educational status. Most of the women were involved in labour work and they want to uplift their economic condition through basketry. There is a significant difference found at <0.05 level in skilled women in making basketry handicraft based on their occupation and monthly family income. Handicraft making is one of the major opportunities for those women who are not so educated but yet can support their families economically. Income is a very important factor for rural women to motivate them to nurture their interests which will help to develop their handicraft skills.

Keywords:Basketry handicraft, Crop residues, Interest and skills of women, Tradition, Upliftment

INTRODUCTION

Handicrafts that are made in huge variety are the identification of the rich cultural heritage of India. Nowadays, hand-made products are considered to be a fashion statement and an item of luxury. Indian handicrafts are helping to survive many of the artisans from the very old period. According to Singh and Fatima (2015), Uttar Pradesh has a lot to offer in handicraft sector in India and it provides markets opportunities for economic growth in this sector. Women's creative minds always help in making newly designed products. Earlier, rural women used their time to make handicraft products with plants and crop residues (Rani & Brar, 2017). At present, they can use their skills in handicraft entrepreneurship for empowering themselves. It is a useful tool for rural women for being economically empowered. The most relaxing part is that these types of handicraft products can be prepared at home without having any problems and they can involve to serve their hand in rural craft tradition (Din, 2017). Empowered rural women are also working for rural economic development unknowingly. Handicraft products can be made attractive and innovative when the artisan works with enough skills and interest. The present study focused on the assessment of the interest and skills related to basketry handicraft in Mathura district in Uttar Pradesh, to find out the tradable possibilities of these products for the purpose of earning money.

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OBJECTIVES

- To assess the utilization status of crop residue for making basketry handicraft products.
- To assess the skills and interests of women regarding basketry handicrafts.
- To perceive the tradable possibilities for basketry handicraft products.

MATERIAL AND METHODS

The present study is descriptive in nature. Three villages of Mathura District namely Nauhjheel, Firozpur, Daulatpur were selected purposively. Seventy five rural women were randomly selected by taking equal number (25 women) from each village. A self-made interview schedule was used to fulfil the first and second objective of this study which was divided in two parts. First was for their socio-economic status, their skills for making handicraft products, types of products which they have produced, type of material used, reasons for discontinue products making and their interest to restart making trade-based basketry handicraft products through plant material. Second part included the details related to their interest and skills level. A total of 13 items were used to collect primary data. Scoring was used to analyse the data. For the first part, positive response was scored as 1 and the negative response was scored as 0 and for the second part, 5 point rating scale was used. Secondary data was collected for the third objective by using authentic journals, previous research, articles, etc.

RESULTS AND DISCUSSION

Objective 1: To assess the utilization status of crop residue to making basketry handicraft products.

Basic parameters were included to know the utilization of locally available crop residue for making the basketry products which were prepared by skilled rural women. It is important to know that which types of crop residues can be used in making products in the selected area so that the women can reach easily and get different crop residues.The results obtained are given below:

Table 1. Types and no. of products which were prepared with locally available crop residues by skilled women

S. No.	Name of products	Name of the used crop	The part of crop used	No. of women who prepared products (50)	%	Total no. of products prepared in three months *(average)*
1.	**Basket (Bohiya)**	Wheat crop	Stem (Straw)	42	84.00	4
		Moonj Grass (Saccharum munja)	The outer part of the grass	33	66.00	3
2.	**Hand fan (Beejna)**	Moonj Grass	Stem	39	78.00	5
3.	**Doormat (Aasan)**	Maize	Husk	27	54.00	4
		Rice	Stem	07	14.00	2
4.	**Winnowers (Soop)**	Moonj Grass	Stem	12	24.00	3

The above given table1 revealed different types of basketry products that were prepared in three months by rural women. Most of the products were prepared by using the stems of each crop. The majority of respondents 84.00% were used wheat crop residue for making Bohiya and average of 4 products were prepared by the women in three months whereas 66.00% of women were used the outer part of Moonj grass for making the same product but in

different design and methods and an average of 3 items were prepared in three months. Hand fans were prepared by them which was called Beejna in the local language. Total of 78.00% of respondents have used the stem of Moonj grass which is locally called Barua for making Beejna and an average of 5 items were prepared in three months. Doormats locally called Aasan were prepared by 54.00% of women by using maize husk and 14.00% of women used the stem of rice crop for making doormats. Out of the 50 women, a total of 12 (24.00%) were able to make Winnowers with the use of the stem of Moonj grass (Barua) and average of 3 items were prepared by them in three months. This data shows that the residues of wheat, rice, maize and Moonj grass were used for making handicraft products in selected areas.

Rural women took residue from different places for making basketry handicraft products. As per data most of the women (64.00%) got residues from other farmers' fields without any charges and 36.00% of women got residue from their own farming. So that none of the women were needed to pay for getting crop residue for making handicraft products.

Objective 2: To assess the skills and interests of rural women regarding basketry handicrafts.

This objective will help to know the present status of women related to their interest and skills for making basketry handicrafts domestically. The researcher found skilled and interested women in making handicraft products with the use of crop residue. To fulfil the objective the results obtained are given below:

Table 2: Status of the rural women related to their skills and interest

1.	**Interested/ skilled women in making products**	**No.**	**%**
i.	Only interested	58	77.33
ii.	Only skilled	50	66.66
iii.	Both interested and skilled	33	44.00
2.	**Skilled women status (N= 50)**		
i.	Involved in basketry handicraft at the time of the survey	11	22.00
ii.	Stopped making products	39	78.00
iii.	Interested to restart this handicraft	22	44.00
iv.	Interested to learn new designs and techniques	33	66.00
v.	Do not want restart the activity	17	34.00

The table shows the status of women related to their skills and interest in making basketry handicraft products at the time of the survey which was conducted in November 2019. This data shows that the majority of the women 77.33% were interested in making basketry products and 50 (66.66%) women were skilled and can make products by using 2 or more different techniques and from the total, only 44.00% women were those who were interested and also skilled at the same time. The researcher got more information from skilled women that a total 22.00% of skilled women were involved in making basketry products and on other hand, 78.00% of women stopped their work because of various reasons and 44.00% of women were interested to restart this work. The data also indicated that 66.00% of women were interested to learn new designs and techniques

The maximum number of respondents were skilled to make basketry handicraft products. The respondents who were not skilled but wanted to learn about handicraft making and the uninterested respondents had various reasons for not to involve in this field like their age, eyesight problems, involvement in other works, etc. whereas interested respondents wanted to involve in this field to increase their family income, to spend their time in the development of rural handicraft tradition.

Table 3: Assessment of Interest of women in basketry handicrafts based on their age, occupational status, educational status and monthly family income

		N	%	Mean	SD	t value	*p-value*
Age	Below 30	48	64.00	4.94	6.30		
	Above 30	27	36.00	3.89	4.70	3.30	***<0.01***
Occupational status	Employed	44	58.66	4.88	5.98		
	Unemployed	31	41.33	3.90	5.14	2.99	***<0.01***
Educational status	Below 5th class	43	57.33	4.59	5.93		
	Above 5th class	32	42.66	3.63	5.26	3.12	***<0.01***
Monthly family income	Below 5,000 Rs.	54	72.00	3.83	6.98		
	Above 5,000 Rs.	21	28.00	3.77	3.98	0.12	>0.05

Table 3 indicates the interest of women related to basketry handicrafts based on the above given factors. It shows that the mean value of rural women of age below 30 for their interest was found higher (4.94) in comparison to the women of age above 30 which was 3.89. A significant association was obtained between both categories of age and it has been proved by t=3.30 at <0.01 significant level. Same as employed women were more interested in making basketry handicrafts for which mean score was 4.88 and unemployed women had lesser interest regarding this with mean score 3.90. This difference is cleared by the t value 2.99 which is significant at <0.01 level. Employed women might understand the importance of income so they have shown more interest. The women having below 5th class education were more interested with mean score 4.59 as compared to the women having above 5th class education with mean score 3.63. It has been obtained by the t value 3.12 which is significant at <0.01 level.This craft provides an opportunity to the uneducated or less educated women but education helps more in handicraft production as per a study of Gurung in 2017.

Mean score of the interest of women whose monthly family income was below 5000 Rs. was found to be similar (3.83) with the women whose monthly family income above 5000 Rs. in this regarding (3.77). I cant as >0.05 level. **Gurung (2017)** also stated that women should also develop the feeling to support their family financially by involving in making handicraft products.

Table 4: Status of skills of women for basketry handicraft based on their age, occupational status, educational status and Monthly family income

		N	%	Mean	SD	t value	p value
Age	Below 30	48	64.00	3.69	6.58		
	Above 30	27	36.00	4.07	4.72	1.13	>0.05
Occupational status	Employed	45	60.00	4.19	6.30		
	Unemployed	30	40.00	3.45	5.12	1.77	*<0.05*
Educational status	Below 5th class	43	57.33	3.72	6.26		
	Above 5th class	32	42.66	4.30	5.11	1.53	**>0.05**
Monthly family income	Below 5,000 Rs.	54	72.00	4.6	6.95		
	Above 5,000 Rs.	21	28.00	3.72	4.11	1.70	*<0.05*

Table 4 shows the status of skilled women in basketry handicraft. There is a significant difference found in between employed and unemployed skilled women. Employed women were more skilled with the mean score 4.19 than the unemployed women (3.45) and this difference is resulted as significant (t=1.77, p<0.05 level). Similarly, the mean score of the women whose monthly family income was below ₹5,000 related to their skills was 4.60

and the women whose monthly family income was above ₹5,000 had mean score 3.72. This difference has cleared by t value 1.70 which is significant at <0.05 level. It can be seen that the women who have lesser income were more skilled. Income is the main reason which motivates them to be learned and to work in the handicraft sector. But Gurung (2017) stated that lack of money restricts women not for doing any other enterprise.

On the other hand, there is similarity found in mean value of skilled women of age below 30 (3.69) and the women of age above 30 by 4.07 related to their status of skills.There is no age limitation to learn anything. It is proved by the t value 1.17 which is insignificant at >0.05 level. Similar results have been observed in skilled women whose educational status was below 5^{th} class and the women of above 5^{th} class education. It has been cleared by t= 1.53 which is insignificant at >0.05 level.

Objective 3: To perceive the tradable possibilities for basketry handicraft products.

As the handicraft is Indian culture and tradition and this thing helps to generate interest among women. This handicraft will provide the opportunity to earn money for them. Women have to develop their skills and have to grow their interest in making handicraft products which can be possible with the help of training programs. They would get appropriate profits for them and it can be possible by making these handicrafts successfully tradable.Here are some previous researches which worked on different handicrafts and made its trade possible.

Arif and Nadeem in 2020 mentioned in their study that in Kashmir, with the help of government facilities of training centres artisans were showing keen interest in that field and they produced many types of attractive products which are demandable and fashionable. To provide better marketing reach, the Ministry has launched an E-commerce initiative in Flipkart. This strengthens the existing primary weaver co-operative society by assisting entrepreneurs from the weavers' families in taking up production and supply directly to the customers in a market.

According to Chakraborty (2013) whose research was based on Zari's work in Murshidabad District of West Bengal, some measurements were provided for marketing of handicrafts successful as like:

- There was insufficient selling in the local area so marketing was limited. Effective marketing strategies at the block level connected with district-level headed by a government agency can create a scope to propagate products outside the known boundaries.
- Local government interferences are required for ensuring raw materials at convenient prices for the female employed in situ.
- Workshop and group classes can be held to standardize the craft and to enhance the modern designs and trends going on to facilitate and meet the present demands.
- Financial assistance in form of short-term loans on uncomplicated conditions will be helpful to any handicraft as lack of monetary support restricts proper development. Organizations like Self Help Groups or the formation of cooperative societies will be beneficial for them. It will act as the bridge between government and root-level workers.

Another study by Tripathi and Mohanty (2014) found that in the villages of Jajpur district of Odisha products were prepared in two categories as traditional and modern and attractive and marketable products were produced. The training was provided to make modern items through Some government organizations like ORMAS (Odisha Rural Development and Marketing Society) as well as some NGOs.

The above given data analysed that online marketing is also a good option in modern times for trading and different organizations are also playing a great role in making handicrafts

tradable successfully. It can be said that if an initiative is taken in Mathura district, then basketry handicraft may be successful at some level. They also can produce innovative and unique products which will attract people.

CONCLUSION

Handicraft is one of the major sources of India which helps to increase not only the economic growth but also the fame of our nation globally. But if we see the rural area which was the main centre for the basketry handicraft than they just need the opportunity to develop their skills and knowledge about the market opportunities so that they can prove themselves. The present study analysed that below 5th class of women were more interested than the women who were above 5th class educated. This handicraft can give opportunities to those women who are not so educated. Skills are also affected because of income. The present study analysed that the women below ₹5,000 of monthly family income were more skilled than the women whose income was above ₹5,000. So that low income can also be effective to increase in the interest for better skills and to involve in the basketry handicraft sector. Based on their interest and skills many women can involve in this field but for this, they have to provide some more skills by which they can stand in the present market and it can be said that with more efforts in this basketry handicraft, economic support for skilled women may be possible.

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Panel Data Approach to Estimate Factors Affecting Wheat Yield in Rajasthan

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Abstract

India is the topmost wheat producer in the world after China. Rajasthan state has fifth place in wheat production after U.P., M.P., Punjab, and Haryana.The second most important food crop in India is wheat, next to rice.This study focuses on some factors that may affect the crop's yield. The objective of the paper is to determine the impact of panel data factors for all districts (30) of the state. For this twenty years (1998-2018) data were used for the empirical study. The data have been set according to districts, and proper adjustments have been made because some districts craved out. This study concluded that gross cropped area, fertiliser consumption, energised wells and tube wells play a significant role in enhancing wheat yield across the districts.The investigation revealed that rainfall and cropping intensity role in wheat yield is insignificant. It can be commenced by this study that government policy for subsidised fertiliser and infrastructure development influenced wheat yield significantly.

Key Words: *Factors, Panel Data, Rajasthan, Wheat and Yield*

INTRODUCTION

Wheat is the second most popular cereal in the country, next to rice. Wheat is the staple food of millions of people, particularly in India's northern and north-western regions. Rajasthan (9,871 thousand tonnes) state has fifth place in wheat production after U.P (26,874)., M.P (17,689). Punjab (16,081) and Haryana (1,352) in the year 2015-16 (DES, Ministry of Agriculture, GoI). On the other hand, Punjab(5188 kg/hectare) state has the highest wheat yield, followed by Jharkhand(4925), NCT Delhi (4312) and Rajasthan(3501) in the year 2018-19 (RBI). The state is one of the significant states in wheat production because there is 61 per cent of desert area. Hence, it is the most potential state for agriculture and allied activities. Although the country has had sufficient stock of wheat production in recent years'erratic rainfall, change in climate, and population enhancement over the year can make its production more significant shortly. In India, there are few states where the potentiality of enhancement of wheat has remained consistant while most productive states such as Punjab and Haryana are suffering the problem of the low yield of crops due to the non-rationalise use of fertiliser. Rajasthan state can be a better state in terms of cereals production because it is the state where most of the area is not utilised effectively.

The growth of agriculture products in the country is 28.36%, with exports of USD 29.81 billion in 2020-21, which was USD 23.23 billion in 2019-20. The food security bill was launched in 2007 in India to increase food grain production by 20 million tonnes, rice by 10 million tonnes, wheat by 8 million tonnes and pulses by 2 million tonnes over the following five years. So, India achieved food grain production in 2019-20. The country's foodgrains output stood at a record 297.5 million tonnes. The GDP growth rate of Rajasthan in 2017-18 is about 5per cent which is much better than others in which the agriculture sector is playing an important role.

Literature Review

There are some studies on wheat crops focused on specific regions of the country like

Sindhu and Byerlee (1991, 1992), Sims (1988), Gandhi (1997), Gandhi*etal.* (2004), Nair and Eapen(2011). There are also some studies focused on the food grain of the country for example, the green revolution gives a better growth rate in food grain production (Sharma and Gandhi, 1990).

Concerning factors that can affect wheat yield, few studies have been done in this respect. A yield function for corn is used in the United States with relative fertiliser price, an index of moisture conditions, land acreage dummies estimated by Houck and Gallagher (1976). Sagar (1978) has been attempted to measure the contribution of the three technological factors, viz, high yielding varieties of seed, fertilisers, and irrigation, in the growth of agricultural yield in Rajasthan from 1961-to 74. Byerlee and Traxler(1995) related wheat yield in different regions of the world to the release of varieties of CIMMYT germplasm.Sharma and Gandhi(1990) estimated yield functions for food grains in India using fertilisers, high yielding varieties, irrigation and rainfall as explanatory variables. Gandhi *et al*.(2004) estimated Cobb Douglas functional form of yield in India using fertiliser consumption, high yield varieties area, irrigated area, and rainfall as explanatory variables. Mathur *et al*. (2007) clarified that the agricultural output is significantly and positively dependent on the government's expenditure on agriculture, fertiliser usage, rainfall and population.

Kulshrestha (2017) explained based on data from 1956-57 to 2009-10 using a semi-log model of econometrics that the yield of the wheat became more than triple per hectare in Rajasthan as it was below 1000 kg per hectare before 1968-69. The wheat crop in the state also replaces most of the rabi crops. This study also estimated the growth rate during the period of study in the crop area was 4 percent per year, and the production growth rate was 1.5 per cent. So the growth rate of wheat yield plays a significant role in the state, and irrigated areas contribute a substantial part because the growth rate of the irrigated area was 3 per cent per year.

Gulati (2014) suggests that the enhancement of export cereals free market does help it, but there is a need to impose a tax on input-intensive commodities. Dev (1985) describes the analysis of the performance of all crops in Indian agriculture in the late 1970s compared to the early 1970s and early 1960s. He has revealed some of the high growth, and most of the hostile growth districts in 1962-65 to 1970-73 have shifted to medium growth in agriculture. Thus the evidence of the decline in output in one-fourth of the Indian districts is not valid in the late 1970s. The analysis across agro-climatic regions also indicates similar results.

Further, rich regions are becoming relatively more prosperous in output per capita and per area. On the other hand, though poor areas have not become poorer, they have remained relatively poor in production per area. As a result, the poor regions have become poorer in output per capita.

Methodology

This study is based on secondary data from 1997-98 to 2017-18. The data were collected from Agricultural Statistics of Rajasthan published by the Directorate of Economics & Statistics, Rajasthan.The given formula calculates yield as below;

Yield or Productivity=(production/area) *100 Panel or longitudinal data are repeated measurements at different time points on the same unit. Regressions are helpful for both variations over units, similar to regression on cross-section data and variation overtime.

The model is $y_{it} = \alpha^{i} + X^{1}_{it}\beta + \varepsilon^{it}$

There y_{it}=*Wheat yeild*

X_{it}=*Six factors*

i. Cropping intensity
ii. Rainfall
iii. Gross irrigated area
iv. Fertiliser consumption
v. Road length
vi. Wells and tube wells energised

Where X_{it} are regressors, are random individuals' specific effects, and is an idiosyncratic error. (Cameron and Trivedi, 2009)

This study is based on panel analysis's fixed and random effect model.

In the fixed effect (F.E.) model, the are permitted to correlate with the regressors $X_{it,}$ and in the random effects (RE) model, it is assumed that in purely random, a stronger assumption implies that is uncorrelated with regressors.

Analysis

Panel Data Analysis

Panel data is used to know the impact of various factors on wheat yield. First, we standardise the data due to doing units free to those factors. There are six factors such as cropping intensity (CI), rainfall (R.F.), gross irrigated area (GIA), fertiliser consumption (F.C.), road length (R.L.) and wells and tube wells energised (WE), which influenced wheat yield. Table 1.1shows thestandardised mean of all variables. The standard deviation of the standardised variable is the overall one. The standard deviation between the 30 districts is more diminutive than within the 20 districts, except for wheat yield and cropping intensity.

Table 1.1 Descriptive Statistics of Panel Data

Variable		Mean	Std. Dev.	Min	Max.
Whepty	**Overall** **Between** **Within**	3.82e-10	1 .7005538 .7247273	-2.656747 -1.281551 -2.549459	3.140254 1.323367 3.309487
CI	**Overall** **Between** **Within**	-.0372936	1.12793 .7968937 .8110108	-7.45873 -1.420281 -8.075276	2.619991 1.246279 2.046173
RF	**Overall** **Between** **Within**	-.00127	.9996491 .9260032 .4111204	-.7619951 -.5101203 -3.548786	8.913571 4.784726 4.127576
GIA	**Overall** **Between** **Within**	-.0019107	.9978574 .9833213 .2438292	-.3821353 -.3643791 -2.597948	7.010269 5.107982 1.900376
FC	**Overall** **Between** **Within**	-.0020972	1.000485 .8406448 .5638072	-1258302 -1.219676 -2.416636	10.20252 2.097844 9.097829
RL	**Overall** **Between** **Within**	-.0292279	1.020375 -911282 -48597017	-1.94853 -1.144137 -1.37948	4.521019 2.097844 9.097829
WE	**Overall** **Between** **Within**	-.0018253	1.000165 .9569561 .3432176	-1.095198 -1.030061 -1.20289	4.915514 3.494965 1.609871

Results and Discussion

Many factors influence wheat yield, but this model takes only six factors that our data can estimate. In this model, we have used standardised variables. We have used the Hausman test to identify whether a fixed effect or random effect is applicable for this purpose, as shown in table 1.2. The Hausman test does not discriminate whether it uses the random or fixed effect.In table 1.3 fixed effect model drawn the result based on 600 observations across 30 districts over the twenty years that gross irrigated area, fertiliser consumption, road length and energised well as tube wells are statistically significant, and the F test is also significant. As the result of table 1.4, there is the same result as the random effect model. Only two variables such as cropping intensity and rainfall, are insignificant. In this model wild chi-square test is also statistically significant. It is tested in table 1.5, and we reject the null hypothesis of the presence of homoskedasticity through the modified wald test. To avoid the problems such as heteroskedasticity and autocorrelation,

a robust standard error method need to be used in the panel analysis. Table 1.6 shows a positive impact of gross cropped area, fertiliser consumption, energised wells and tube wells on wheat yield. Although road length impact on wheat yield is negative, we can describe that standardised road length increases by one standard deviation by holding other variables constant. On average, the standardised wheat yield decreases by about 0.32 standardised deviation. The errors are correlated with the regressors in the fixed effect model is -0.70, and the variance not explained by differences across districts is 0.68, also known as intraclass correlation. In the Random effect model, as in table 1.7, there are the same results in terms of impact, but standardised coefficients are different due to random error is not included in regressors. This model can be said better than the fixed effect due to overall R- the square is better.

Table 1.2 Hausman Test Result

	Coefficients		Difference	S.E
	Fixed	Random		
CI	.0385023	.0385023	0	0
RF	.0761827	.0761827	0	0
GIA	.590114	.590114	0	0
FC	.1894911	.1894911	0	0
RL	-.3240968	-.3240968	0	0
WE	.7196954	.7196954	0	0

Table 1.3 Result of Fixed Effect Model

	CI	RF	GIA	FC	RL	WE	cons
Coef.	0.04	0.08	0.59	0.19	-0.32	0.72	-0.01
Std. err	0.04	0.07	0.12	0.05	0.07	0.10	0.03
P>[t]	0.29	0.27	0.00	0.00	0.00	0.00	0.85
sigma_u	0.99	sigma_e	0.67	rho	0.68	corr(u_i, xb)	-0.69
F(,564)	21.34	Prob > F	0.00				

Table 1.4 Result of Random Effect Model

	CI	RF	GIA	FC	RL	WE	cons
Coef.	0.05	0.01	0.25	0.26	-0.24	0.46	0.00
Robust Std. Err.	0.04	0.06	0.08	0.05	0.06	0.08	0.11
P>|z|	0.13	0.82	0.00	0.00	0.00	0.00	0.97
sigma_u	0.54	sigma_e	0.67	rho	0.39	corr(u_i, x	0.00
Wald chi2(6)	108.77	Prob > chi2	0.00				

Table 1.5 Test of Heteroskedasticity in Fixed Effect Model

Chi^2 (30)	39616.25
Prob>chi^2	0.00

Table 1.6 Result Fixed Effect Model (Robust)

	CI	RF	GIA	FC	RL	WE	cons
Coef.	0.04	0.08	0.59	0.19	-0.32	0.72	-0.01
Robust Std. Err.	0.03	0.13	0.08	0.08	0.13	0.09	0.00
P>|z|	0.22	0.56	0.00	0.03	0.02	0.00	0.17
sigma_u	0.99	sigma_e	0.67	rho	0.68	corr(u_i,xb)	-0.69
F(6, 29	33.1	Prob > F	0.00				

Conclusion

This study focuses on wheat yield in terms of factors that can be affecting it. No doubt there may lot of factors that affect it, but as far a concern we included six factors in which irrigation facilities and energised wells and tube wells done well in this context and both model whether fixed effect or random effect the role of energised contribute much there variable due to this crop demand timely water facilities. Since the state is water-deficient, traditionally, farmers depend on the bajra crop for food purposes. Still, electricity facilities make towards wheat crop although we cannot ignore the role high yield variety seeds besides that the yield also influenced by electricity and irrigation facilities. Fertiliser consumption also plays a very crucial role in this. The policy of subsidised fertiliser makes farmers use it extensively. Road length is even an indicator of development, and it made accessible transportation facilities. Still, it also has an adverse impact that area of cultivation is going to decrease due to this. As far as a result is concerned across the districts, road length is negative. Cropping intensity shows how much time per year crops are shown. Still, it is not statistically significant because all the crops have influenced cropping intensity, and some crops have been negatively affected because of the wheat crop. The state's farmers grow more wheat in rabi season than other crops due to the government's minimum support price. The role rainfall is crucial in the state however over and under rainfall adversely impact the productivity of the crop. So dependency on the unexpecting behaviour of rain makes significant impacts on wheat yield.

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Molecular Characterisation and Phylogenetic Analysis of Two ildmushroom Species from Pathanapuram in Kerala, India

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(Period of research: March-June 2020)

ABSTRACT

The aim of the study to determine the evolutionary relationship of two wild mushrooms based on 18s rRNA sequences. Genomic DNA was isolated by CTAB method, and 18S ribosomal RNA amplified by PCR using forward ITS4 and reverse ITS5 primers. Sequencing was done and similarity identified with BLAST. The analysis revealed 98.89% similarity with *Earliella scabrosa* 99.20% with *Fomitopsismeliae.*Phylogenetic tree was constructed using Neighbor-Joining tree, Minimum evolution tree methods using MEGAXI software.

Key words: phylogenetic tree, BLAST, evolutionary relationship, molecular markers, fungi

INTRODUCTION

Fungi are eukaryotes with complex cellular organization being heterotrophic in nature is responsible for decomposition and nutrient cycle thereby maintain an ecological balance. Mushrooms are fleshy fruiting bodies that comprise 80-90% water and are excellent source of vitamins and minerals except iron and calcium (Manikandan, 2011). Mushrooms are used as functional foods; their low-calorie protein rich diet are of importance in diet of rural and urban dwellers. Mushrooms are priced vegetable owing to their increasing cultivation and consumption. Classification of fungi and basidiomycetes is based on standard barcoding markers of which the internal transcribed spacers (ITS) region is widely used. ITS markers enable successful molecular characterization and species identification of wild edible mushrooms and are used in the construction of phylogenetic tree (Khaund and Joshi, 2014; Schoch et al., 2012). The polymorphic nature of rDNA (Gardes and Bruns, 1993) enable speciation (Martin et al., 2004), morphological, ecological adaptation. *Earliella scabrosa* is a bracket and puffball type fungus proposed in the global fungal red list is a well distributed species throughout the world. This species of fungus is mainly found on newly fallen trees (Adarsh et al., 2015). This species is known to possess antioxidant activity and anticancer activities and hence used in therapy (Zmitrovich et al., 2017). *Fomitopsis meliae*is a brown rot fungus found on living or dead conifers and hardwoods known to produce endoglucanase. Though known as a saprophytic fungus, the aim of the experiment is to extract the rDNA-ITS fragments of two wild mushrooms species and amplify using ITS4 and ITS5 primers and determination of nucleotide sequence. Sequence was analyzed for similarity using BLAST of NCBI database to identify the species. The FASTA format was used to create Phylogenetic tree. Mushrooms find wide applications in marketing as a source of income, apart from medicinal use, in ceremonies, dyeing wood and natural fibres, as fire starters and gun powder.

MATERIALS AND METHODS

Sample Collection: Both the wild mushrooms found on tree bark were collected from Pathanapuram (9.079944/76.821610) on 16th March 2020. Mushroom with its base intact was removed by hand and stored in a polythene bag under refrigeration until processing.



Figure 1: SAMPLE A



Figure 2: SAMPLE B

ISOLATION OF GENOMIC DNA (Healey *et al.*, 2014)

Material required: Mushroom fruiting bodies

Preparation of CTAB buffer:2 g CTAB (Cetyl trimethyl ammonium bromide); 10 ml 1 M Tris pH 8; 4ml 0.5 M EDTA pH 8 (Ethylenediaminetetraacetic acid di-sodium salt); 28 ml 5M NaCl; 40.0ml H_2O; 1 g PVP 40 (polyvinyl pyrrolidone (vinylpyrrolidine homopolymer) MW 40,000). Adjust all to pH 5.0 with HCl and make up to100ml with H_2O.

Liquid Nitrogen, Absolute Ethanol (ice cold), 70% Ethanol (ice cold), 7.5M Ammonium Acetate, Chloroform:Iso Amyl Alcohol(24:1), Water(sterile), RNase (10μg/ml) 200mg of plant tissue was ground to a fine powder with liquid nitrogen and added approximately 500µl of CTAB buffer. Transferred CTAB/plant extract mixture to a microfugetube and incubated the CTAB/plant extract mixture for about 15 min at 55°C in a water bath. After incubation, the CTAB/ plant extract mixture was centrifuged at 12000 rpm for 5 min and the supernatant transferred to clean microfuge tubes.

To each of the tubes added 250µl of chloroform: isoamyl alcohol (24:1) and mixed the solution byinversion. The tubes were centrifuged at 13000 rpm for 10minutes. The upper aqueous phase (containing the DNA) was transferred to a clean microfuge tube. To the tubes added half volume of chloroform: isoamyl alcohol and mixed well by inversion. After mixing, the tubes were centrifuged at 13000rpm for 10minutes. Then, added 50µl of 7.5 M ammonium acetate followed by 500µl of ice-cold absolute ethanol. The tubes were inverted slowly several times to precipitate the DNA. The tubes were placed at -20˚C for 1hrtoprecipitatethe DNA. The tubes were centrifugedat13000rpmfor 5min and the supernatant decanted. 500µlofice cold 70% ethanol was added and slowly the tube was inverted. The tubes were centrifuged at 13000rpm for 1 min to pellet the DNA. The process was repeated twice. Removed the supernatant and allowed the DNA pellet to air dry (approximately 15 min). Resuspended the DNA in sterile DNase free water(10µlRNaseA can be added to the10ml water prior to dissolving the DNA to remove any RNA in the preparation). After resuspension, the DNA was incubated at 65˚C for 20 min to destroy any DNases that may be present and stored at 4˚C. Further the DNA was stored at–14˚C. Quantitation of DNA was done using spectrophotometer and the quality analysed by gel electrophoresis.

POLYMERASE CHAIN REACTION: Taken PCR buffer (10X), dNTP (100mM), Taq polymerase (5U/µl), deionised water, Primer (Forward ITS4 and reverse ITS510 pmol/µl), template DNA 11ng/µl|), Ethidium bromide (10mg/ml). Materials Required: PCR machine, microcentrifuge, crushed ice, adjustable micropipettes, plastic tips, PCR eppendorf (0.5ml).

FOR REACTION VOLUME: Mixed 2xPCRMasterMix- 12.5 µl;10µM ITS4 - 2.0µl; 10µM; ITS5- 2.0µl; DNA10ng/µl -2.0µl; Deionised water - 6.5µl

PCR CONDITIONS: Initial denaturation for 95˚C 1.00 min followed by Denaturation at 95˚C 0.30 min; Annealing at 53˚C 1.00min; Extension at 68˚C 1.40min; repeat for 30 cycles; Final extension at 68˚C 5min; Finally stored at 4˚C.

PHYLOGENETIC ANALYSIS

Sequence analysis was performed using online tool BLAST of NCBI database. Forward and reverse sequences were assembled, and coding was generated after trimming the low-quality bases. The trimmed genetic sequences were then compared to different 18S rRNA gene of different mushrooms in there reference RNA sequence (18SrRNA) databases of NCBI nucleotide BLAST website to identify the genus of the selected mushroom. The query sequences were constructed to FASTA format and then used to create a Phylogenetic tree using Neighbor Joining and Minimum evolution tree construction methods.

RESULTS AND DISCUSSION

DNAISOLATIONBYCTABMETHOD

Genomic DNA was extracted with the established CTAB method. Briefly cell walls of fungal mycelia were broken down by grinding with glass rods or in the presence of liquid nitrogen. The CTAB extraction buffer was then added, and after incubation, purified with phenol chloroform isoamylalcohol and precipitation with isopropanol were conducted. Finally, the DNA was isolated and performed quantification by spectrophotometry and analyzed quality by agarose gel electrophoresis.

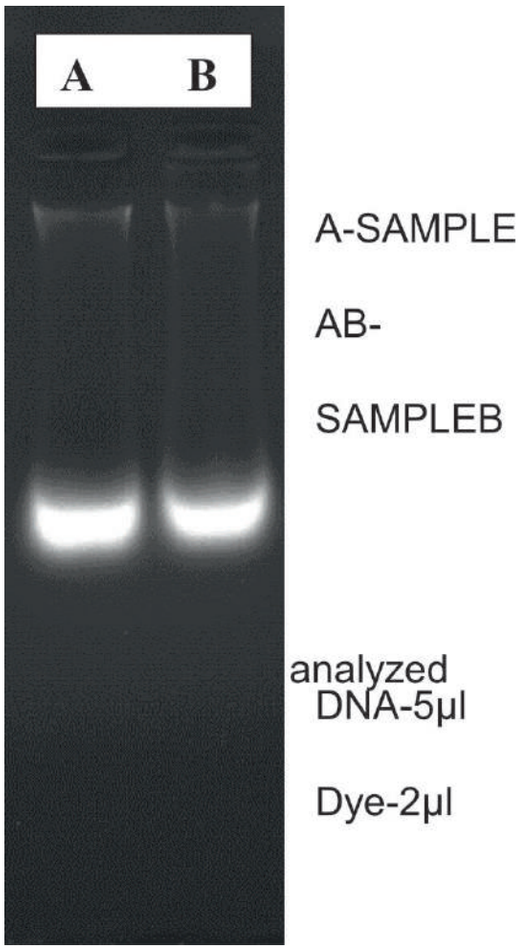


Figure 3: Gel Electrophoretic pattern of DNA of mushroom samples A and B

POLYMERASECHAINREACTION

The genomic DNA of the mushroom was extracted, the rDNA-ITS fragment of the genomic DNA was amplified using ITS4 and ITS5 primer and subjected to nucleotide sequence AA, Ganesh, P.N. (2015). Decomposition of wood by Polypore Fungi

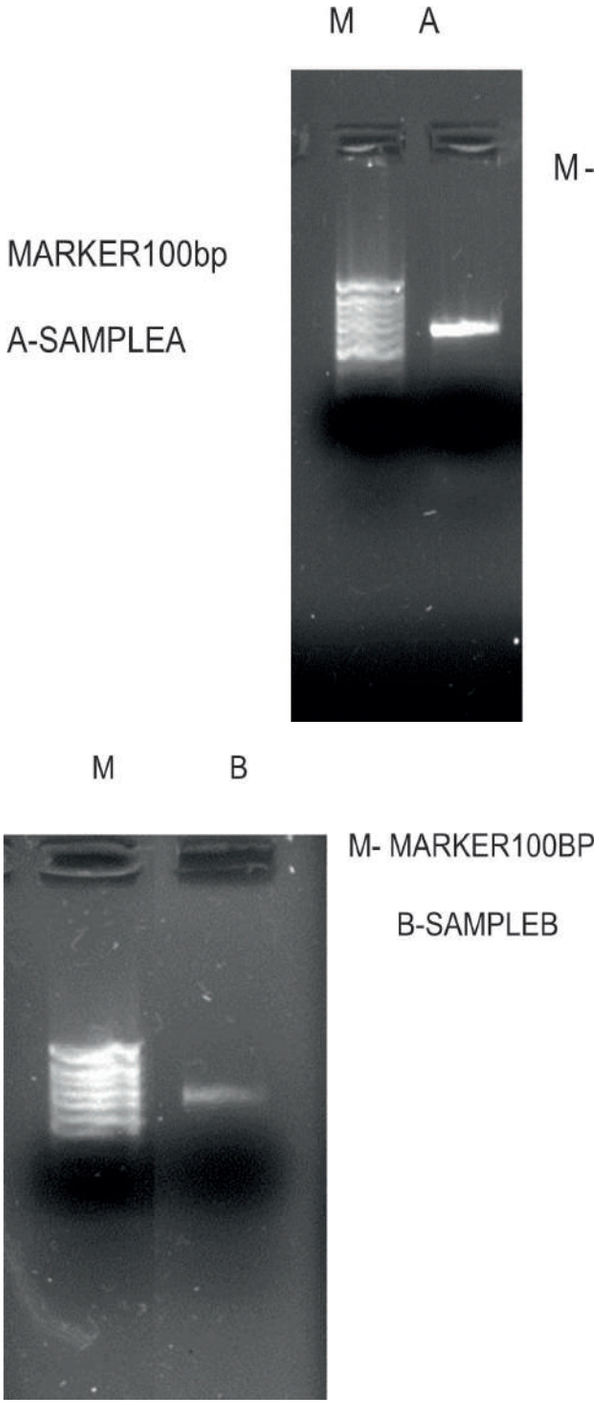


Fig:4: Gel Electrophoretic pattern of PCR amplified 18S rRNA of mushroom samples A and B

CODING

SAMPLE A

GGGACTGGAGTCTACCTGATTTGAGGTCAGAGGTCATAAGAAGCTGTCTCTTGAG
CGAGACGATTAGAAGCCAGCCAAACGCTTCACGGTCGCGGCGCAGATAATTATC
ACACCGAGAGCCGATCCGCAAGGAAACAGGCTAATGCATTTAAGAGGAGCCGAC
TTGCGAGAAGCCAACAAAAACCTCCAAGTCCAAGCCTACAAACCCGCAAAGATTT
GTAGGTTGAGAATTTCATGACACTCAAACAGGCATGCTCCTCGGAATACPSCAAG
GAGCGCAAGGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATT
ACTTATCGCATTTCGCTGCGTTCTTCATCGATGCGAGAGCCAAGAGATCCGTTGC
TGAAAGTTGTATATAGATGCGTTACATCGCAATACACATTCTGATACTTTGTAGTT
GGTAGTAAAACGTAGACCCAGTAACGTCACGGCGTTGAAAGCCGCGGCGCGCC
TGAAGCCTACAGTAAGTGCACAGGGGTAAGAGTGGATGAGCAGGGCGTGCACA
TGCCTCGGAAGGCCAGCTACAACCCGTTTCAAAACTCGTTAATGATCCTTCCGCA
GGTTCACCTACAAAGAGACATTGTTACGACTTTTAATTCCCA

The BLAST annotation shows 98.89% identity with Earliella scabrosa.

SAMPLE B

AGGACGGTACGTACTGATTTGAGGTCAAGGTCAAAGTGATTGTCCTCGAGTAAAA
GGACGATTAGAAGCCGATCCCATTAACATGCTTCACAGCAACGGCGTAGACAAT
TATCACACCGATAGCTGATCCACAAAGGTTCGAGCTAATGCATTCAAGAGGAGC
CGATCACAGACATACCGGCAATAAACCTCCAAGTCCAAGCTCTATTCACAAAAGC
AAATAGAGTTGAGAATTCCATGACACTCAAACAGGCATGCTCCTCGGAATACCAA
GGAGCGCAAGGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCA
CATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCGAGAGCCAAGAGATCCG
TTGCTGAAAGTTGTATTTAAATGCGTTAGACGCAAGAGTGACATTCTTTAAACTGA
AGTAGTTTGTAATAAAACATAGGAAGGCCTACAACCAAAGCAGACACACAGAGTG
CCCACTCCAGCCACAAACCGACCTACAGTGTGTGCACAGGTGTGTGAGGTGGAT
AATGATCAGGGCGTGCACATGCCGCTGACGGCCAGCAACAACCCCTTTCAGAAT
TCATTAATGATCCTTCCGCAGGTTCACCTACGGAAACCTTGTTACGACTTTTAC
TTCCCAA

The BLAST annotation shows 99.20% identity with Fomitopsis meliae.

The PCR products of ITS regions of Earliella scabrosa and Formitopsis meliae are 640 and 660 base pairs respectively in consistent with that of Hussein et al., (2014), Gardes and Bruns (1993) and Maeta et al. (2008) as the fragment size of the DNA target region is 600-800 bp. However according to Blaalid *et al.* (2013) fungal ITS region varies roughly between 450 and 750 base pairs (bp) in length.

PHYLOGENETICTREE

Phylogenetic tree based on a comparison of the 18S rRNA sequences of two mushroom samples and some of their close phylogenetic relatives, the tree was created by the Neighbor Joining, and Minimum Evolution methods.

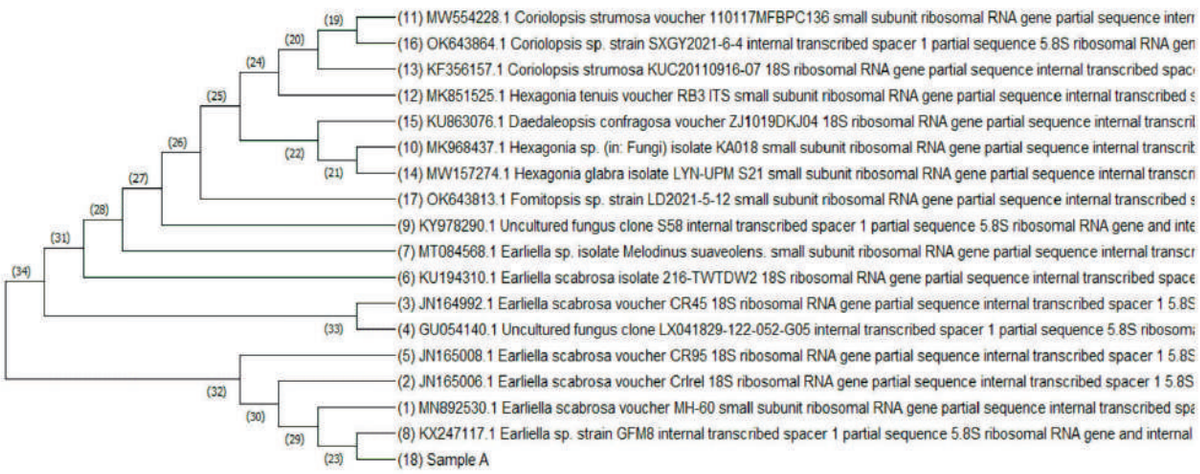


Fig5: Phylogenetic tree of mushroom sample A using Neighbor Joining Method



Fig6: Phylogenetic tree of mushroom sample A using Minimum Evolution Method

PHYLOGENETICTREEOFSAMPLEA

Earliella scabrosa is a fungus of family Polyporaceae, found in the tropical region associated with trees of Acacia, Ficus and dead dying trees. Though known as a saprophytic fungus, in-vitro cultivated Earliella scabrosa showed antifungal activity against certain wood-degrading fungi of rubberwood (Teoh and Don 2013). It is found in rain forests of both eastern and western hemispheres. Some reports have described its antioxidant properties and free radical scavenging ability. Earliella scabrosa contains isocoumarin used in cancer therapy (Zmitrovich et al., 2017).



Fig7: Phylogenetic tree of mushroom sample B using Neighbor Joining Method.



Fig8: Phylogenetic tree of mushroom sampleB using Minimum Evolution tree Method.

PHYLOGENETICTREEOFSAMPLEB

Species of *Fomitopsis* cause brown roton living or dead conifers and hardwoods. The genusis characterized by its sessile to effuse-reflexed, white to pinkish or brown to dark-brown basidiomata, poroid, white to pinkish pores surface with round, angular to irregular pores, dimitic or trimitic hyphal system, presence or absence of cystidia, and sub-globose to cylindrical, hyaline, smooth basidiospores. *Formitopsis meliae* CFA2 brown rot fungi is a potent produce of multi-enzyme cocktail in particular endoglucanase that can be used for enzyme saccharification (Patel et al., 2021). This fungi *Formitopsis meliae* could be grown to check for the production of multi-enzyme cocktail, paves way for further studies. Xylanase and ligno-cellulosic materials can use in saccharification (Lin et al., 2018) and for the production of biofuels (Wu and Shin, 2016).

CONCLUSION

Variation in morphological features might often mislead identification of wild mushrooms. In combination with molecular data, it enables better species identification and characterization. In this study, the ITS markers helped in exact identification of the two species of wild mushrooms. Further study is needed to check for the nutritional, medicinal and industrial use of these two species.

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Women Empowerment and Techonology Diffusion - A Study on Skill Training Programme of Rubber Board of India

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ABSTRACT

The 17 SDGs and 169 targets seek to rebuild a new universal agenda and envision to realise the human rights of all and to achieve gender equality and empowerment of all women (United Nations, 2015). Ending all discrimination against women is indispensable for future because it is already proven that empowering women helps economic growth and development. This paper tries to analyse the impact of skill development training programme offered by Rubber Board of India on women empowerment. 240 women respondents who participated in various technical training session organised by Rubber Board of India has taken as sample. As evidenced by the facts presented in the paper better technology usage has a significant impact on women's empowerment. Hence all the extension services delivered by the Rubber Board have significant positive relationship with technology adoption and women empowerment in natural rubber cultivation in Kerala

Keywords: Women, Natural rubber, Training, Women empowerment, Technology

INTRODUCTION

The agenda for 2030 Sustainable Development Goal is not only to ensure global prosperity but also to addresses the issue of removal of poverty and inequality. There has been an urgent action required to resolve it now and it is essential to set some tactics for Sustainable Development Goals 2030 (SDGs) (Esquivel, 2016; Pogge& Sengupta 2015). An immediate transformative step we necessity to take in order to commence the collective journey without anyone left behind. Among the 17 SDGs we seek to rebuild a new agenda and aims to realise the human rights of all and to realize gender equality and women empowerment (United Nations, 2015). Ending all gender disparity is indispensable for the future because it is already proven that empowering women helps economic growth and development (Dhar, 2019).

Recently, women empowerment spot into the limelight and there has been a bidirectional relationship between development and gender empowerment. In one direction, improving the ability of women to access and control over resources can play a major role in the development and in another direction gender inequality which hinder development (Esther Dufli, 2012). According to World Bank report, improving the ability of women to access the constituents of development in particular education, training, earning opportunities, expansion of choice, building self-confidence, increased access and control over resources,

skill acquisition, etc, should be the focus to achieve the gender equality in present situation. There are several literatures which stated the issues related to the previously mentioned components and it is widely accepted that these strength-oriented approach is crucial for women empowerment especially in developing country like India (Conrad-Joseph WulekaKuuder, Mariam Essel, Rikiatu Husseini, Evelyn KuusozumeYirbekyaa, 2020).

In India there may be "too many goals, too little focus" for the meaningful policy making and has experienced a substantial transformation in all spheres of the economy since independence (Eden, L., & Wagstaff, M. F. , 2021) (Selin, H., 2015). One of the major reflectionthat can be found is the position of women in the society and their participation in social as well as economic assets. Though they have always worked, whether in paid jobs, or in the home, and often in both, but their work is generally unrecognized and under valued (Desai, S. B., Dubey, A., Joshi, B. L., Sen, M., Shariff, A., & Vanneman, R., 2010). The annual report of Ministry of Labour and Employment 2017-18 acknowledged the problem, nothing that encouraging more women to join the workforce is critical to nation's ability to benefit from the so-called demographic dividend (Ministry of Labour & Employment, 2017-18). Women will account for a substantial proportion of the working age population and therefore active participation of women in the labor force is of almost importance to realize the full benefits of demographic dividend and increasing the labor force participation (LFPR) for women is vital for achieving high growth of employment and overall economic growth. A major question that the women are currently addressing is that does their active participation in economic as well as social life are the fundamental rights to allowing them to have control over their society and life and what are the major challenges in women empowerment in present society. In order to address the issue of low female participation rate (LFPR) and WPR (worker population ratio) the government haslaunched various legislation- based schemes and welfare programmes where its emphasis was on female participation and empowerment. In order to improve the condition, the government has announced several women employment programmes like Pradahan Mantri Kausal Vikas Yojana, National Skill Development Mission, SWADHAR greh, and Support to Training and Employment Programme (STEP). This paper examined the effectiveness of skill development training programme offered by Rubber Board of India on women empowerment.

Technology and Women Empowerment

Some prominent theorists especially the Neo-classical and the Marxist feminist approaches explain gender differences with respect to human manpower such as education, training, technology adoption and diffusion etc., which sway the productivity and empowerment level. The importance of theme-based human resource development programmes implemented by the Rubber Board of India was examined by Prince Sebastian (2009).He attempted to assess how training programmes such as seminars, group meetings, annual campaigns, and tappers training affected plantation management, productivity, product quality, technology acceptance, and skill development.A study was undertaken in India that employed a stratified random selection method to find the study region and divided the participants into two groups: smallholders who had attended training programmes and growers who had not. According to the survey, there is a considerable difference in productivity between the two targeted groups, with a 13 percent rise among individuals who participate in Rubber Board's training programmes. Another paper by Sunny Varghese and A. N. Jayaprakash (2006) try to describe the overall performance of the Rubber Plantation Development Scheme during the tenth plan period. They pointed out that the 10th plan's schemes prioritised rubber expansion through new planting, particularly in non-traditional locations, as well as productivity improvement.Other extension activities, such

as training for establishing economic viability of rubber production through promotion of supplementary income generating and its adoption, have also received a boost from the strategy. Finally, the paper attempts to summarise the result level study. A sufficient level of literature review is concentrated on gender related issues and challenges across the world. According to a thorough examination of the literature, there are few studies on the influence of training on women's empowerment, and there is little study on the dissemination of technology and human resource development among women. To fill this vacuum, this study made an attempt to know the effectiveness training on technology adoption and women empowerment

Methodology

Primary data and secondary data were used for the study. Primary data was collected with the help of questionnaire, andit was distributed among 240 women. The sample respondents were those who participated the various technical training session organised by Rubber Board of India and Rubber Producer's Societies (RPS's). The secondary data was collected from Economic survey, Indian Rubber Growers Guide(various Years), Indian Rubber Statistics (2016, 2017 and 2018) etc. The sampling method used in the study was convenient sampling because the sample is taken from a group of people who are easy to contact or to reach. The study used Likert scale questions (N.K Malhotra, 2005).

Exploratory Factor Analysis was applied and it have extracted the emphasized factors, which summarize the most important information in the variable. The numerical numbers that show the magnitude and direction of a factor's influence on a measured variable (F .J Floyd & K.F Widaman, 1995). The aim of this study was to find underlying variables, or factors, that explain the pattern of correlations within a set of observable personal variables that may influence the human resource development of natural rubber growers especially women growers. The purpose of data reduction is to remove redundant (highly correlated) variables from the data file, perhaps replacing the entire data file with a smaller number of uncorrelated variables. In this study, the consequent effects of various training sessions conducted by Rubber Board of India on technological diffusion and human resource development among women rubber growers in India. This tool is ascertained using a measurement instrument under Likert framework consisting of 12 statements. Further, statements were reduced to 8 based on the communalities in the extraction. Four statements were excluded from the analysis frame because of the low extraction values (Communalities with values more than 0.5 may be taken as important as a thumb rule when the sample size is sufficiently large). It is observed that the communalities after deleting four statements show sufficiently large values suggesting that the statements are equally important for the contemplated problem. The aim of this study was to find underlying variables, or factors, that explain the pattern of correlations within a set of observable personal variables that may influence the human resource development of natural rubber growers.

Training

The Rubber Board of India as an apex institution in natural rubber sector has hosted several programmes for the expansion of area under cultivatione specially in rubber small holdings, because the current productivity (1437 kg per ha) is much lower than the potential productivity of more than 3500 kg per ha. Thus, it is essential to provide grassroots level extension activities particularly in far flung areas. It has been successfully carried out by the Board's extension officers commendably over the past several decades. The task of the extension machinery aiming at capacity building and self sufficiency of growers especially small growers in production and productivity is onerous. They deliver a range of extension services and among them the Human Resources Development (HRD)

aspect of extension support of the Rubber Board has a crucial role in improving the productivity of plantations as well as diffusion of technology. HRD programmes are focused on all aspects of agriculture, productivity enhancement, processing, and product manufacturing. A scientific perspective toward agriculture can be instilled in farmers' minds by meeting them individually or by organising seminars, workshops, demonstrations, and campaign programmes. The Rubber Institute offers more than 40 distinct training programmes. In total the participation rate of women is comparatively low when it compared to man.

The following table shows the major heads in which all the programmes has been arranged.

Table No: 1	
Title	**Objective**
Skill Training Programmes	1. Tappers Skill Development Schools (TSS) 2. Tappers' Intensive Skill Improvement Programme (TISP)
Visit Cum Training	*Sasthradarshan* is a one-day visit with training for small growers who want to learn about scientific methods of rubber production, primary processing rubber technology, and related topics while visiting the RRII.
Annual Mass Contact Programme	Weekly tapping, controlled upward tapping, rain guarding, soil protection and water collecting technique, RubSIS, skill enhancement in harvesting to assure cheap cost of production, higher output, and greater profit, intercropping, and other topics are covered in campaign meetings.
Source: Rubber Growers Guide 2019	

Human resources development aspect of the RPD scheme was significant and it aims to bring capacity development among Women small growers. The Kaiser-Meyer-Olkin (KMO) Measure of Sampling Adequacy is depicted in the below table, which has generated a value of .681 which above the generally acceptable minimum of (.50). This reveals that the acceptable cut-off 0.50 for performing the factor analysis.

Table-2 KMO and Bartlett's Test		
Kaiser-Meyer-Olkin Measure of Sampling Adequacy.		.801
Bartlett's Test of Sphericity	Approx. Chi-Square	166.110
	df	28
	Sig.	.000

Source : Primary Data

The results of two tests, the Kaiser-Meyer-Olkin Measure of Sampling Adequacy and Bartlett's Test of Sphericity, are used to determine whether the association between variables is significant. Kaiser-Meyer-Olkin (KMO) measure to varies between 0 and 1, and values closer to 1 are better. Here in this case the values closer of Sampling Adequacy shows the value of test statistics as 0.801 which means factor analysis for selected variable is appropriate. The Bartlett's Test of Sphericity generally test the null hypothesis that the correlation matrix is an identity matrix. In this case the test was significant with chi square value= 166.110, df= 28 and p<.005 and hence the hypothesis is rejected.

Table No: 3 -Total Variance Explained

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	2.198	27.460	27.460	2.197	27.460	27.460	2.175	27.191	27.191
2	1.507	18.862	46.322	1.509	18.862	46.322	1.530	19.131	46.322
3	0.964	12.031	58.354						
4	0.844	10.562	68.916						
5	0.732	9.194	78.107						
6	0.661	8.264	86.376						
7	0.594	7.453	93.832						
8	0.491	6.169	100.000						

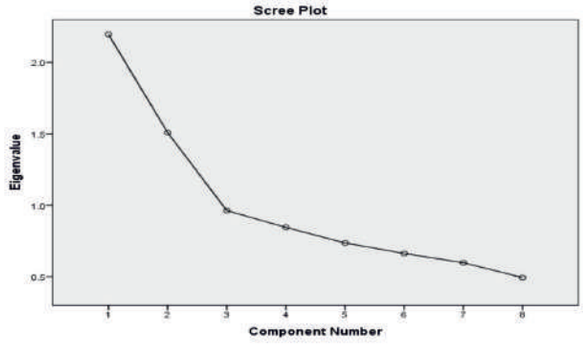
Extraction Method: Principal Component Analysis.

Source: Primary Data

The above table 3 it abscrred that 46.322% variation in the responses on 8 variables can be reduced to 2 different factors using the standard procedure to consider those factors having Eigen values greater than 1.

The screen plot is almost parallel to X axis after the second item and overall 8 statements comes under these two factors. In the below table, the variables having high loading are indicated. These variables are collected and organised based on their loadings.

Figure No: 1



Source: Primary Data

Table No:4-Rotated Component Matrixa

	Component	
	Effectiveness of Training	Challenges
Technology adoption	.716	
Training on agromanagement practices	.702	
Field Training	.700	
Training on additional Income Generation	.590	
Training on usage of new clones	.569	
Difficult to adopt the sugessions		.778
Arrangement of training		.701

Source: Primary Data

In the above table No: 4,the variables having high loadings are indicated. These variables are collected and organised based on their loadings. Based on the common thread seen among the statements in each group, appropriate names were suggested after discussion with experts. Thus the information contained in the responses may imply the information contained in factors named as, effectiveness of training and challenges of different training programmes conducted by Rubber Board of India.

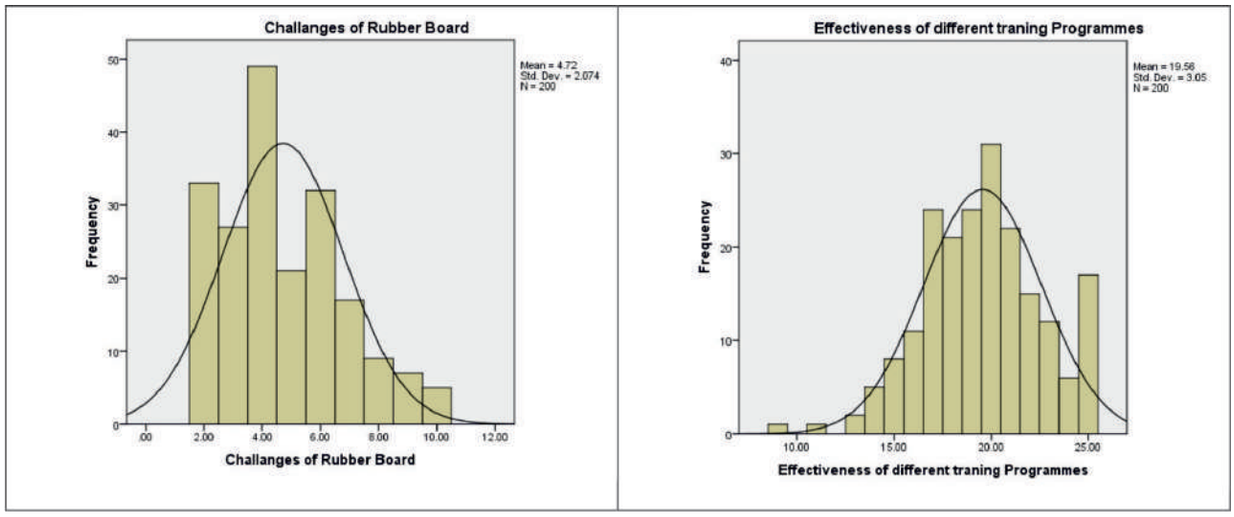
In the statistical framework a measure of normality is essential to analyse the distribution of data. This assumption can be examined by the distribution of scores at an item level and distribution of scores within combination of two items. The below figure represents that, both

are normally distributed and centred around.

Table 5: Statistics		
	Effectiveness of different training Programmes	**Challenges of Rubber Board**
Mean	19.5550	4.7200
Std. Deviation	3.05016	2.07440
Minimum	9.00	2.00
Maximum	25.00	10.00
Source: Primary Data		

Two factors were thus generated by following the procedures explained in the initial paragraph of this paper/rescarch. Table no: 5 explains about the basic statistics of all the two factors such as effectiveness of different training programmes and challenges of Rubber training sessions

Figure No: 2



In the above figure 2, we can see that all statements related to the effectiveness of various skill development training programmes among women. These were grouped in two, the former explains the effectiveness of programmes in technological diffusion among women and the later explains the challenges of the execution of training programme.

- The first set of five statements related to the benefits of training, which showed a high mean score value of 19.55 and the SD was 3.05.
- The next set components related to the present challenges and problems in training programme. It involves three statements, the mean value given was 4.72 which was relatively low and the SD was 2.07.

Various training programmes conducted by the Rubber Board has highest significant relationship on technology adoption among women natural rubber growers in Kerala. It evident from the data provided above that better adoption of technology to a large extent influences the women empowerment. Hence all the extension services delivered by the Rubber Board have significant positive relationship with technology adoption and women empowerment in natural rubber cultivation in Kerala. Besides, in the case of technology adoption, a long way towards the achievement of self-sufficiency among women rubber growers ?

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Screening of Phytotoxin Producing Fungi for the Mycoherbicidal Management of Weeds of Leguminous Crop

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Abstract

Pulses are the most important food crops after cereals, constitute the major source of dietary protein. India is the world's largest producer and the largest consumer of leguminous crop. Green gram and pea are the major legume crops and today prominently figures in the crop priority of the farmers in Madhya Pradesh contributing to a major percentage to the total legume production in the country. Looking to the potential the objectives is to develop and exploit indigenous strains of fungal pathogens as mycoherbicides to manage some of problematic weeds of leguminous crop. During the periodic survey of various leguminous fields, it was observed that leaf spot, leaf blight, die back, petiole rot etc. diseased were associated with leguminous weeds. A total of 40 fungal isolates were selected from various diseased parts. The initial symptoms of Phytotoxic effect was distinct epinasty followed by necrosis, blackening and stiffness of the stem ultimately causing death of the plants. *Alternaria petroselini* (FCLW#23) was found in maximum frequency and hence, it would be the best fungal candidate for biological management.

Keywords: Legume, weeds, Bio-control, fungal pathogens, Phytotoxicity.

Introduction

Legume crops are the second most important group of food plants and the major source of protein in the predominately vegetarian diet of the people of India. Madhya Pradesh has distinction of being the world's single largest producer of legumes/pulses, having an area approximately 20- 24 million hectares under legumes. Pests especially weeds have increasingly become a major threat to sustainability of legume crops. Weeds are ubiquitous and continually changing pests in agriculture. They claim their own share of soil fertility and productivity at the cost of crop yield. They impose severe allelopathic effects on crops. Conventional methods of weed control have failed due to one or other reasons while microbial management either directly or their secondary metabolites have received significant momentum as evidenced by commercialization of several products as mycoherbicides or biorationals. In the present investigation was undertaken with the objectives to develop and exploit some indigenous strains of fungal pathogens as bio-herbicides to manage some of the problematic weeds of leguminous crop. (Pandey 2001, 1999)

MATERIALS AND METHODS

Survey and collection:

A systematic, periodical and thorough survey of the weedy leguminous crop fields especially in different localities of Mahakaushal Region was undertaken. The main localities surveyed were Jabalpur, Gadarwara, Kareli, Narsinghpur, Pipariya etc. In the Green gram and Pea season monthly survey of the fields were carried out which helped in the understanding of the pattern of vegetative growth as well as different stages in the development of disease. The infected plant parts viz. leaves, stems, roots and rhizospheric soil of some weed samples collected during survey were brought to the laboratory and were used for the isolation of the fungal pathogens.

Isolation of fungi from infected leaves and stems:

Infected portion of the weed were cut into small pieces (2 mm). They were then surface sterilized with 1 % sodium hypochlorite (NaOCl) solution for about three minutes, followed by rinsing with sterile water to remove any possible contamination (Chopada *et al*., 2014). These were then transferred in presterilized petri dishes containing potato-dextrose agar medium (Walker, 1981; Agarwal and Hasija, 1986). This was incubated for 3-4 days at 26°C in BOD (Agrawal and Hasija, 1986). All the emergent fungi were isolated and pure cultures were obtained (Marak *et al*., 2014).

Isolation of Rhizospheric Fungi

The isolation of fungi from soil samples of weeds (*Commelina benghalensis*, *Cyperus rotundus*, *Euphorbia heterophylla*, *Echinochloa crus-galli*) of leguminous crop (green gram and pea) was done by using following methods:

Warcup's Soil Plate Method

A pinch of finely grounded oven dried soil (0.5-1.0 gm) was taken and transferred aseptically in presterilized petri-dishes. The soil aggregates were crushed and spread after adding 2-3 drops of sterilized distilled water. Approximately, 15 ml of melted and cooled (45°C) PDA medium was then poured into petri dishes. The petri dishes were gently rotated to assure even distribution of soil particles. These petri dishes were incubated for 5 to 7 days at 28±1°C in BOD incubator and then transferred to PDA slants as earlier mentioned.

Dilution Plate Methods

10 grams of soil samples were dissolved in 90 ml of sterile distilled water and thoroughly shaken to obtain the dilution 1:10 (10-1) from this 10-1 dilution a serial dilution of 1:100 (10-2) and 1:1000 (10-3) were made in sterile distilled water. 1 ml of each sample dilution was pipetted aseptically into sterilized petri dishes and approximately 15 ml of melted and cooled (45°C) PDA medium was poured into petri dishes. The petri dishes were gently rotated to assure even distribution of soil suspension and kept in a BOD incubator at 28± 1°C for 3-4 days and when fungal colonies developed in plates, it was transferred to slants (Agarwal and Hasija, 1986; Chouhan *et.al*., 2019).

Purification and maintenance of fungal culture

The fungal species isolated earlier were purified by sub culturing techniques (Agarwal and Hasija, 1986). The purified cultures of the 40 isolated fungi were multiplied on PDA plates. The stock cultures of the microorganism were maintained on PDA slants.

Identification of the fungi

Identification of fungi was done with the help of taxonomic experts, manuals, monographs, papers of various workers and available literature (Subramaniam, 1971; Ellis, 1971; Barnet and Hunter, 1972 and Arx, 1974; Sutton, 1980 Chouhan *et.al*., 2019)

Pathogenicity and Selection of Virulent Strain

All the isolated fungi were grown on PDA liquid media and incubated in BOD for 21 days. Development of the secondary metabolites secreted by different fungi using broth media. The media was inoculated incubated and the cell free culture filtrates (CFCF) were obtained.

Phytotoxicity Test for Selection of Potential Strain

(a) Seed germination bioassay (Plate bioassay)

The petridish were covered with filter paper supported by a thin layer of adsorbent cotton. Twenty five seeds of different weeds of leguminous crop (*Commelina benghalensis*, *Cyperus rotundus*, *Euphorbia heterophylla*, *Echinochloa crus-galli*) were taken in the petriplates which were incubated in seed germinator at 26 ± 2°C, 75 ± 15% relative humidity. These seeds were treated with 25, 50, 75 and 100 concentrations of CFCF. Three replicates for each treatment including control were maintained and observed daily. (Chouhan *et.al*., 2019)

(b) Shoot cut bioassay:

Shoots of healthy weed plant samples were cut under water and kept in sterilized test tube having Hogland's solution and placed in growth chamber under controlled conditions. . The shoots were then placed in Hoagland's solution in the test tubes and were sprayed the CFCF with an automizer until wetness. Control plants were sprayed with sterile distilled. These plants were then kept in environment growth chamber at 28°C and 70% relative humidity. The disease severity was rated by visual observation at an interval of 24 hours till 7 days.

(c) Detached leaf Bioassay:

Selected weeds leaves were used for detached leaf bioassay. The healthy leaf of weed were surface sterilized with 0.2% NaOCl and were incubated in a sterilized moist filter paper in 9 cm Petri plate using cotton and filter paper under continuous fluorescent light at 25±1°C. The effect was observed after 24, 48 and 72 hours at room temperature. In all bioassay sterilized unmetabolised growth medium was taken as control and the sterilized distilled water was taken as control.

(d) Whole plant bioassay:

Seeds of different weeds were surface sterilized with 0. 05% NaOCl solution, scarified 15 min in concentrated H_2SO_4 and then rinsed with distilled water. Seeds were sown in plastic pots containing a mixture of pre sterilized sand, soil and peat (1: 1: 1) and grown under 26±2°C, 75±15% relative humidity for a period of two weeks. These were then sprayed evenly with 25, 50, 75 and 100% concentrations of CFCF. The experiment had three test sets and one control set. These were kept in plant growth chamber (Yorko) and observed daily for disease severity. (Chouhan *et.al*., 2019)

RESULTS

During a periodical survey of various leguminous fields, it was observed that various plant diseases viz. leaf spot, leaf blight, die back, petiole rot, root rot, etc were found in selected weed plants of leguminous crop. A total of 40 fungal sp. were isolated from various diseases plant parts of weeds.

Screening of phytotoxic metabolites produced by fungal strains was done by Detached Leaf Bioassay, Shoot Cut Bioassay, Seed Germination Bioassay and Whole Plant Bioassay against the four major weeds of leguminous crop, namely, *Echinochloa Crus galli, Euphorbia heterophylla*, *Cyperus rotundus* and *Commelina benghalensis***.** On the basis of primary screening of 40 isolates 5 most potential isolates were selected for secondary screening. They were *Aspergillus flavus*, *Fusarium oxysporium*, *Curvularia lunata*, *Alternaria petroselini* and *Phoma herbarum*. These were again screened as earlier and *Alternaria petroselini* was found to be the most potential and induced maximum phytotoxic damage after 24-48 hours post treatment (hpt).

Alternaria petroselini (FCLW#23) was responsible for controlling the selected four weeds of leguminous crop caused extensive damage. *Alternaria petroselini* (FCLW#23) induced significant toxicity even at lower concentration against all the four selected weeds. Biological control using plant pathogens has shown substantial impact on the weed. The herbicidal potential of a fungal strain may be assayed by bioassay that is highly sensitive and qualitative and many of these are synthesized by fungal strains. Figure No – 2 clearly indicate that the results of whole plant bioassay by CFCF of *Alternaria petroselini*.

Table 1 (i): Fungal strains isolated from leguminous weeds

S.No.	Fungus Isolate from Leguminous fields	Isolate No.	Symptoms
1	*Fusarium oxysporium*	FCLW#01	Lamina
2	*Fusarium moniliforme*	FCLW#02	Lamina, Petiole
3	*Aspergillus* sp.	FCLW#03	Rhizosphere
4	*Aspergillus niger*	FCLW#04	Leaf

S.No.	Fungus Isolate from Leguminous fields	Isolate No.	Symptoms
5	*Cephalosporium* sp.	FCLW#05	Leaf, Petiole
6	*Colletotrichum dematium*	FCLW#06	Lamina, Stem
7	*Curvularia* sp.	FCLW#07	Petiole
8	*Curvularia lunata*	FCLW#08	Stem
9	*Aspergillus candidus*	FCLW#09	Rhizosphere
10	*Aspergillus flavus*	FCLW#10	Leaf
11	*Fusarium orthoceras*	FCLW#11	Lamina
12	*Fusarium* sp.	FCLW#12	Rhizosphere
13	*Septoria* sp.	FCLW#13	Stem
14	*Trichoderma viride*	FCLW#14	Stem
15	*Phoma* sp.	FCLW#15	Seed
16	*Phoma herbarum*	FCLW#16	Seed
17	*Rhizopus* sp.	FCLW#17	Rhizosphere
18	*Trichoderma* sp.	FCLW#18	Leaf
19	*Aspergillus clavatus*	FCLW#19	Petiole
20	*Aspergillus sulphureus*	FCLW#20	Stem
21	*Aspergillus* sp.	FCLW#21	Rhizosphere
22	*Alternaria* sp.	FCLW#22	Leaf
23	*Alternaria petroselini*	FCLW#23	Leaf
24	*Cephalosporium gregatum*	FCLW#24	Leaf
25	*Cercospora* sp.	FCLW#25	Petiole
26	*Cercospora sojina*	FCLW#26	Seed
27	*Alternaria alternata*	FCLW#27	Leaf
28	*Pythium ultimum*	FCLW#28	Seed
29	*Sclerotinia sclerotiorum*	FCLW#29	Leaf
30	*Rhizotonia leguminicola*	FCLW#30	Rhizosphere
31	*Cladosporium* sp.	FCLW#31	Stem
32	*Mucor* sp.	FCLW#32	Leaf, Petiole
33	*Mucor hiemalis*	FCLW#33	Seed
34	*Glomerella glycine*	FCLW#34	Rhizosphere
35	*Colletotrichum truncatum*	FCLW#35	Lamina
36	*Penicillum* sp.	FCLW#36	Leaf
37	*Phoma sorghina*	FCLW#37	Seed
38	*Drechslera indica*	FCLW#38	Leaf
39	*Drechslera* sp.	FCLW#39	Stem
40	*Helminthosporium* sp.	FCLW#40	Lamina, Stem

FCLW – Fungal Culture Leguminous Weed

Table 1 (ii): Phytotoxicity of CFCF of fungal strains on leguminous weed.

S.No.	Fungus Isolate from Leguminous fields	Isolate No.	Phytotoxic damage (in%)												
			PPB				SCB				WPB				Overall rating
			CB	CR	EH	EC	CB	CR	EH	EC	CB	CR	EH	EC	
1	*Fusarium oxysporium*	FCLW#01	58	62	60	66	60	69	79	80	70	73	75	80	5
2	*Fusarium moniliforme*	FCLW#02	40	31	42	38	39	44	39	46	32	31	39	41	3
3	*Aspergillus* sp.	FCLW#03	11	10	19	18	14	13	13	19	20	11	12	17	1
4	*Aspergillus niger*	FCLW#04	17	12	20	21	18	16	15	13	11	10	14	13	1

S.No.	Fungus Isolate from Leguminous fields	Isolate No.	Phytotoxic damage (in%)												Overall rating
			PPB				SCB				WPB				
			CB	CR	EH	EC	CB	CR	EH	EC	CB	CR	EH	EC	
5	*Cephalosporium* sp.	FCLW#05	44	50	61	48	61	55	51	53	46	55	63	48	4
6	*Colletotrichum dematium*	FCLW#06	10	13	15	20	11	12	17	18	12	13	19	21	1
7	*Curvularia* sp.	FCLW#07	61	60	69	71	58	61	69	57	57	52	70	69	4
8	*Curvularia lunata*	FCLW#08	74	80	70	78	70	75	80	82	80	75	70	78	5
9	*Aspergillus candidus*	FCLW#09	72	67	68	71	70	61	68	59	69	65	70	62	4
10	*Aspergillus flavus*	FCLW#10	65	78	70	74	80	73	83	85	83	85	80	81	5
11	*Fusarium orthoceras*	FCLW#11	39	40	48	51	50	55	38	43	42	57	47	44	3
12	*Fusarium* sp.	FCLW#12	22	39	29	28	32	33	25	34	38	27	29	30	2
13	*Septoria* sp.	FCLW#13	38	48	41	39	38	34	35	36	42	40	39	38	3
14	*Trichoderma viride*	FCLW#14	44	30	45	50	51	48	47	44	39	43	50	51	4
15	*Phoma* sp.	FCLW#15	50	55	52	52	49	69	70	40	68	61	65	66	4
16	*Phoma herbarum*	FCLW#16	65	70	75	72	76	80	80	70	81	75	85	80	5
17	*Rhizopus* sp.	FCLW#17	68	67	61	60	57	59	60	59	58	70	71	68	4
18	*Trichoderma* sp.	FCLW#18	67	66	70	72	59	62	63	68	65	72	65	70	4
19	*Aspergillus clavatus*	FCLW#19	55	59	57	49	61	55	57	56	54	53	60	61	3
20	*Aspergillus sulphureus*	FCLW#20	61	67	68	69	73	69	70	68	55	59	60	63	4
21	*Aspergillus* sp.	FCLW#21	11	23	24	19	22	33	31	39	35	37	38	29	2
22	*Alternaria* sp.	FCLW#22	39	40	38	31	34	33	40	42	44	38	33	42	3
23	*Alternaria petroselini*	FCLW#23	80	74	81	79	76	84	70	76	84	78	80	79	5
24	*Cephalosporium gregatum*	FCLW#24	69	60	72	69	67	75	72	70	69	76	77	68	4
25	*Cercospora* sp.	FCLW#25	69	70	40	68	61	50	55	52	52	68	61	50	4
26	*Cercospora sojina*	FCLW#26	55	60	61	56	58	62	65	55	61	62	59	57	3
27	*Alternaria alternata*	FCLW#27	13	20	18	16	12	15	20	22	20	15	16	29	1
28	*Pythium ultimum*	FCLW#28	68	59	69	65	70	62	69	63	48	75	68	61	4
29	*Sclerotinia sclerotiorum*	FCLW#29	52	52	49	69	70	40	68	61	65	62	69	63	4
30	*Rhizotonia leguminicola*	FCLW#30	65	55	61	62	59	57	40	49	51	48	53	59	3
31	*Cladosporium* sp.	FCLW#31	21	22	28	19	24	13	22	27	26	20	34	30	2
32	*Mucor* sp.	FCLW#32	18	17	12	16	21	25	25	19	24	28	22	14	1
33	*Mucor hiemalis*	FCLW#33	70	61	68	59	69	65	72	69	67	75	72	70	4
34	*Glomerella glycine*	FCLW#34	61	60	68	72	70	75	77	63	67	67	59	65	4
35	*Colletotrichum truncatum*	FCLW#35	13	12	19	18	11	14	18	24	17	16	17	15	1
36	*Penicillum* sp.	FCLW#36	75	72	70	69	76	77	68	55	63	67	69	67	4
37	*Phoma sorghina*	FCLW#37	21	10	19	22	15	18	17	20	24	26	18	17	1
38	*Drechslera indica*	FCLW#38	51	53	46	55	63	48	75	72	69	65	72	69	4
39	*Drechslera* sp.	FCLW#39	22	33	31	39	35	37	38	29	31	39	35	38	2
40	*Helminthosporium* sp.	FCLW#40	72	69	63	48	75	68	55	63	67	75	72	70	4

FCLW = Fungal culture leguminous weed

PPB = Petri plate bioassay

SCB = Shoot cut bioassay

WPB = Whole plant bioassay

CB - *Commelina benghalensis*

CR - *Cyperus rotundus*

EH - *Euphorbia heterophylla*

EC - *Echinochloa crus-galli*

Table 1 (iii): Secondary screening of selected fungal strains against leguminous weeds.

S. No.	Culture No.	Name of the fungus	Concentration of CFCF	Phytotoxic damage (%)			
				CB	CR	EH	EC
1.	(FCLW#23)	*Alternaria petroselini*	Control	0	0	0	0
			25%	60	65	68	70
			50%	70	75	78	78
			75%	83	80	82	85
			100%	92	90	95	95
2.	(FCLW#10)	*Aspergillus flavus*	Control	0	0	0	0
			25%	50	55	52	55
			50%	58	59	65	62
			75%	65	68	70	70
			100%	70	72	75	85
3.	(FCLW#08)	*Curvularia lunata*	Control	0	0	0	0
			25%	35	46	45	56
			50%	42	55	58	60
			75%	52	62	65	68
			100%	68	72	70	71
4.	(FCLW#01)	*Fusarium oxysporium*	Control	0	0	0	0
			25%	41	60	60	55
			50%	50	68	65	60
			75%	58	70	70	65
			100%	75	82	80	78
5.	(FCLW#16)	*Phoma herbarum*	Control	0	0	0	0
			25%	54	58	60	65
			50%	63	70	70	72
			75%	74	75	77	78
			100%	80	82	84	83

CB - *Commelina benghalensis*
CR - *Cyperus rotundus*
EH - *Euphorbia heterophylla*
EC - *Echinochloa crus-galli*

CONCLUSION:

Studies conducted indicate and all observations show the frequency and phyto-toxicity of *Alternaria petroselini* (FCLW#23) found to be most virulent strain and exhibited potential to be exploited as natural herbicides for some of problematic weeds of leguminous crop.



Fig No 1 – Microscopic photograph of *A. petrosolini*



Fig No 2 – Filtration process of Cell Free Culture Filtrate (CFCF) of *Alternaria petroselini* (FCLW#23)

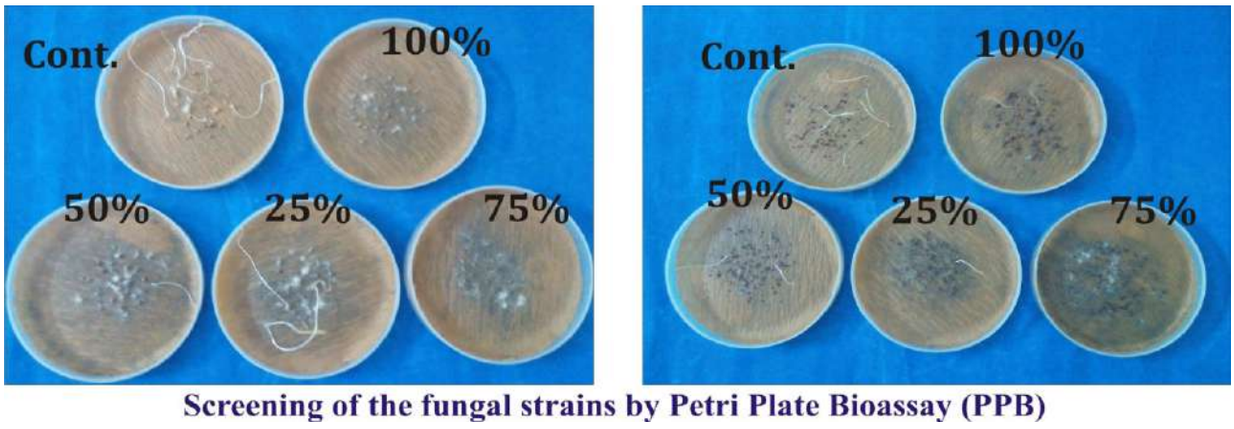


Fig No 3 - Results of Petri Plate Bioassay by developed Cell Free Culture Filtrate (CFCF) of *Alternaria petroselini* (FCLW#23)



Fig No 4 - Results of Whole Plant Bioassay by developed Cell Free Culture Filtrate (CFCF) of *Alternaria petroselini* (FCLW#23)

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Development and Evaluation of Multigrain Flour for Traditional Recipes

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Abstract

In the past few years, the people have shown more attention towards eating the multigrain food products made from multigrain flour. Eating multigrain flour recipes helps in prevention and better management of different types of chronic diseases. Multigrain flour is the rich source of protein, fiber, vitamins, minerals and energy. Not just nutrient rich it's a tasty, digestive, affordable, locally available and energetic ingredient used for making the various value-added recipes. Therefore the major objective of the study was to Develop and Evaluate the Multigrain flour for making various Indian traditional recipes. Total three traditional recipes were prepared from 14 multigrain flour treatments. Sensory evaluation of these recipes was done by using composite rating scale. Data obtained during study were statistically analyzed using completely randomized design(CRD). Shelf life of the flour was up to 3 months. All the 14 treatments were orgenoleptically acceptable. However, the developed multigrain flour consisted of wheat flour (65%) and bajara, amaranth, oats, soyabean and ragi (each grain 5%) along with blackgram dhal flour (10%) was highly acceptable, also improved the taste and nutritional quality of the traditional Indian recipes especially puri and paratha. Rheologically, the dough also showed good extensibility which is desired quality for all these recipes. It was also accepted for making chapatti.

Key words: Multigrain flour, sensory evaluation, traditional recipes, shelf life, nutrients

INTRODUCTION:

- Multi-grain flour is a combination of different flours including cereals and pulses. Pulse is the important food crop due to the high protein and essential amino acid content. Pulses are 20-25% protein by weight. The digestibility of protein is quite high. Due to lack of methionine, consuming it with grains which are deficient in lysine provides a complete diet of protein. It also provides complete carbohydrates, several vitamins, iron, magnesium, phosphorus and zinc. Ragi provide high amount of amino acid methionine, calcium, vitamins and fiber. Legumes are a rich source of protein and form a major component of a vegetarian diet. Now a days demand of multigrain food have increased in the market as people are more aware regarding the facts that multigrain food helps in reducing and managing chronics diseases (Niu M, Hou G G, KindelspireJ, et al. (2017) and Zhang N, Xiu L, Min W H, et al (2014). Some researcher also have found that consuming multigrain food is linked with the decreasing risks in cardiovascular diseases and metabolic risk factors Ahn Y, Park S, Kwack H, *et al.* (2013), and Anderson J W (2003). In view of the facts regarding nutritional quality of cereal, pulses and oil seeds, combination of different ratio of various ingredients (ICMR, 2002) were mixed to develop acceptable multigrainflour. Therefore, along with wheat and bajara, soybean, ragi, oat, black gram dal flour and amaranth was added in the flour.

OBJECTIVES:

1. To develop and standardize the multigrain flour from locally available food grains
2. Toe valuate the multigrain flour for preparation of Indian recipes, i.e. Chapati, Puri, Paratha and its sensorial quality
3. To assess the nutritional profile of multigrain flour
4. To analyze the multigrain flour for its shelf life
5. To measure rheological properties of the developed multigrain flour.

MATERIALS AND METHODS

Grains selected for the study viz. wheat, bajara, amaranth, oat, soybean, ragi, black gram dhal, were purchased from the local market in Sardarkrushinagar, Gujarat. Grains were selected on the basis of nutritional profile (Gopalan et al.1989.), price and local availability.Multigrain flours were prepared by substituting the wheat flour with all other grain flours as shown in Table 1**.** Total three recipes were prepared namely Chapati, Paratha and Puri.Sensory evaluation was carried out using composite rating scale. Consumer acceptance was also carried out to check the acceptability of the multigrain flour for making and eating qualities of chapati, puri and paratha. More than 25 respondents were provided the best rated multigrain flour and were asked to prepare the chapati, puriand paratha at home and they were asked to rate the quality of each product after testing on 9 point hedonic scale rating. The prepared composite flours were stored in airtight glass bottles and shelf life was evaluated at an interval of 7 day for 3 months.The moisture, fat, protein, crude fiber, mineral (iron & Calcium) content of best rated composite flour was estimated by standard methods using reference book published by NIN. All the different samples of flour were kneaded into smooth dough which then was pressed and rested in the Kieffer Dough and Extensibility Rig's Dough Press attachment for 40 min. The dough so obtained was ten cut into thin strips using the same rig's Cutting Attachment. The strips so obtained were loaded onto the test platform for extensibility test. The Kieffer rig was affixed on the machine base. It was ensured that the hook probe was covered with the plastic sleeve to prevent it from shearing through the sample. The hook probe was lowered to just above the upper surface of the spring loaded clamp and the strip of dough/ gluten was placed onto the grooved region of the sample plate and, holding down the spring clamp lever was loaded. The plate was then inserted into the rig and handle was released slowly. Completely Randomized Design (CRD) was used for data analysis.

RESULTS AND DISCUSSION

The study was conducted in 2015 to 2019 at ASPEE College of Home Science and Nutrition, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar. There are very few data available on the standardization of multigrain flour. Keeping this in view, Multigrain Flours were prepared by mixing locally available ingredients namely wheat, bajara, amaranth, oat, soybean, ragi and black gram dhal in different proportion.

Preparation of Multigrain Flour: The proportion of each ingredient was different in all the 14 developed flours as stated in Table 1.

Quality parameters of flour: Percent absorption and Quality of dough made up from multigrain flour was also evaluated and presented in Table 2. The results obtained revealed that $T_{1,}$ i.e. wheat flour absorbed slightly higher water, pale yellow in colour and it was non sticky on touch where as other treatments contained different grains in different proportion, i.e multigrain flour absorbed 65 to 84% of water, dull white to dark brownish in colour and slight sticky to very sticky on touch. This was may be due to the color pigments of the different grains and different binding capacity.

Shelf-life / Storage study of Multigrain Flour: The shelf life of a product can be defined as the time phase in which the product is safe and has acceptable qualities to be purchased and consumed. The shelf life study was done for period of 3 months. The results indicated that T_{12} where ragi and wheat were main ingredients smells rancid from the 35th day of storage. There was no significant change found in all other 13 treatments. Therefore, it can be concluded that the flour is safe for consumption for up to 3 months under standard storage conditions. (Table 3)

Preparation of recipes: Three Indian recipes, i.e. chapati, puri and paratha were prepared from this Multigrain flour (Figure 1) and comparison was made between each type of treatments to evaluate which level of proportion is highly acceptable and sensory evaluation was carried out.

Sensory Evaluation: Sensory evaluation of three standardized *recipes viz.* chapati, puri and paratha was carried out using Composite Rating Scale. The same dough was used to prepare chapati, puri and paratha. These were coded as T_1 to T_{14}.Semi trained panel members were enrolled to rate various attributes of each food preparations. Seven attributes were included for composite rating scale. All three recipes prepared from Multigrain flour were found to be orgenoleptically acceptable. However, the acceptable level of flour varied in different food preparation and different treatments of one food recipe. The mean scores for various sensory traits for the recipe chapati are mentioned in Table 4.

As per the Chakrabarti-Bell et al. 2013 *Chapatis* are unleavened flat breads and a staple food made in every Indian homes. *Atta* flour is widely used to make *chapatis* and is obtained by grinding wheat in stone *chakkis*. Supplementing *atta* with various other grain and legumes flours increases protein and mineral content and therefore increases the nutritional content of *chapatis*. Grains differ in their nutritional composition; some are richer in one nutrient and others in different nutrients. Therefore different grains and legumes can be selected based on their nutrient composition and combined to produce more nutritious flours to get more wholesome and balanced food. Since *wheat* is the staple food, *chapatis* with multigrain flour can be a healthier option without changing the food habits and are also being eaten at least twice a day by a majority of the population in India. Multigrain flour brings significant differences in the various sensory attributes of chapati. A typical wheaty aroma and creamish white in color was desirable for any homemade chapati which was found in control chapati, but in multigrain flour chapati, it became bland in taste and dark in color. As expected, overall acceptability of the chapatis was found to be highest for T1 i.e. control chapati. However, the T2 and T3 chapati were nearer to the overall acceptability scores of chapati form control.

Table 5 depict the mean scores of the attributes of the recipes puri. Here, T_{14} gained maximum score followed by T_{13} and T_{11}.

In the recipe paratha (Table 6), T_{13} scored highest followed by T_1 and T_2. No significant differences were found between treatments of both the recipe puri and paratha. This may be due to the use of different cooking process like roasting, deep fat fraying and shallow fat fraying in chapati, puri and paratha respectively. As fat improves the textural quality of same mouth feels which hinder to differentiate the sensory attribute of treatments. Similar course grain products were prepared by Baljeet et al. (2010), Desai et al. (2009) and Kumar et al. (2013).

Consumer acceptance test: Consumers scored the acceptability of multigrain flour sample using 9 point hedonic rating scale that varied from 'like extremely' to 'dislike extremely'. The targeted three multigrain products were chapatti, puri and paratha. The results indicated that the highest scored treatment was T_{13} among all of the 14 multigrain flour treatments. Multigrain flour paratha received the highest mean score i.e. 7.76 followed by multigrain

puri (7.41) and multigrain chapati (6.94). (Table 7 , Figure 2) Based on consumer acceptance analysis, it was found that all the multigrain products scored like moderately to like slightly; however there is a need for larger consumer acceptance to increase the consumption of multigrain products at household levels.

In the present study, T13 was found to be the highest rated which contained higher percent of black gram dhal. Incorporation of black gram dhal at 10% level in the blend gives the appropriate taste and other attributes of the recipes and thus it is recommended.

Nutritional Profile: Nutrient contents of all fourteen treatments was assessed and presented in Table 8. Results of nutritional profile showed that moisture content of T_1 was 12.20% whereas, in T_{13} it was found 11.74%. Protein content was highest in multigrain flour i.e. T_{13} (14.59%) as compared to T_1 (11.8%). Data also depicts that 1.20% and 1.95% fat present in T_1 and T_{13} respectively. Fiber content of T_{13} was found to be higher (1.95%) whereas T_1 has 1.2% fiber. In T_1, iron and calcium content was 4.90 mg and 48.0 mg respectively, where as in $T_{13,}$ iron content was 5.42 mg and calcium content was 105.9 mg.(Table 8)

Rheological properties of the dough/flour: The dough was prepared and the extensibility and resistance of the dough was measured. Once the test commences, the hook then proceeds to centrally extend the dough sample until its elastic limit (at the maximum force) is exceeded and the dough separates. At this point the distance was noted and used as an indication of dough extensibility. The results showed that T_9, T_{10} and T_{12} showed the resistance to extension in similar range, which is drastically different from that of other treatments. Thus, It can be concluded that these 3 flour samples will not extend much and thus the use of these flour samples may be different as compared to the rest of the lot.(Table 9)

Conclusion:

The developed multigrain flour consisted of wheat flour (65%) and bajara, amaranth, oats, soyabean and ragi (each grain 5%) along with blackgram dhal flour (10%) was highly acceptable, also improved the taste and nutritional quality of the traditional Indian recipes especially puri and paratha. Rheologically, the dough also showed good extensibility which is desired quality for all these recipes. It was also accepted for making roti/chapati. Further, nutritional profile showed that It was rich it the nutrient such as protein, calcium and iron and fiber than normal wheat flour. It has a long shelf life and safe for consumption till 3 months period under standard storage conditions. Thus, it is recommended that the developed multigrain flour can be used for Indian recipes for enhancing the nutrients.

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DATA TABLES AND FIGURES

Table 1: Proportion of different ingredients used to develop multigrain flour.

Treatment No.	Wheat	Bajara	Ama-ranth	Oat	Soy-bean	Ragi	Black gram dhal	Total
	(g)	(g)	(g)	(g)	(g)	(g)	(g)	(g)
T_1	100	0	0	0	0	0	0	100
T_2	70	**5**	5	5	5	5	5	100
T_3	65	**10**	5	5	5	5	5	100
T_4	60	**15**	**5**	5	5	5	5	100
T_5	65	5	**10**	5	5	5	5	100
T_6	60	5	**15**	**5**	5	5	5	100
T_7	65	5	5	**10**	5	5	5	100
T_8	60	5	5	**15**	**5**	5	5	100
T_9	65	5	5	5	**10**	5	5	100
T_{10}	60	5	5	5	**15**	**5**	5	100
T_{11}	65	5	5	5	5	**10**	5	100
T_{12}	60	5	5	5	5	**15**	**5**	100
T_{13}	65	5	5	5	5	5	**10**	100
T_{14}	60	5	5	5	5	5	**15**	100

Figure.1: Method of preparation of multigrain flours

Step 1: Cleaning and sun drying of grains
Step 2: Milling the grain separately
Step 3: Mixing different flours to develop Multigrain flour
Step 4: Mixing thoroughly (Avoid sieving to retain the fibre content)
Step 5: Packaging of Multigrain flours

Table 2: Quality of dough made up from multigrain flour

Treatment	% H_2O absorption (ml)	Colour of dough	Nature of dough
T_1	86	Yellowish	Non sticky
T_2	84	Creamish white	Slight sticky
T_3	78	Light creamish white	Sticky
T_4	77	Light brownish white	Very sticky
T_5	75	Dull white	Sticky
T_6	76	Dull white	Non sticky
T_7	74	Dull white	Slight sticky
T_8	72	Dull white	Very sticky
T_9	70	Brownish white	Sticky
T_{10}	70	Brownish white	Sticky
T_{11}	73	Brownish white	Sticky
T_{12}	74	Dark Brownish white	Slight sticky
T_{13}	71	Brownish white	Very sticky
T_{14}	65	Brownish white	Very sticky

Table 3: Shelf life study of multigrain flour

Storage Period	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13	T14
Day 0	8.90	8.90	8.90	8.80	9.00	9.00^{a}	9.00^{a}	9.00^{a}	9.00^{a}	9.00	9.00^{a}	8.90^{a}	9.00	9.00^{a}
Day 7	9.00	9.00	9.00	9.00	9.00	9.00^{a}	9.00^{a}	9.00^{a}	9.00^{a}	9.00	9.00^{a}	9.00^{a}	9.00	9.00^{a}
Day 14	8.90	8.80	9.00	8.90	8.80	8.90^{a}	8.90^{a}	8.90^{ab}	9.00^{a}	8.90	8.80^{b}	9.00^{a}	8.90	9.00^{a}
Day 21	8.70	8.80	8.80	8.80	9.00	8.60^{ab}	8.60^{b}	8.70^{abc}	9.00^{a}	9.00	9.00^{a}	8.60^{a}	9.00	9.00^{a}
Day 28	9.00	9.00	9.00	9.00	9.00	9.00^{a}	9.00^{a}	9.00^{a}	9.00^{a}	9.00	9.00^{a}	9.00^{a}	9.00	9.00^{a}
Day 35	8.80	8.80	9.00	9.00	9.00	9.00^{a}	9.00^{a}	9.00^{a}	9.00^{a}	9.00	9.00^{a}	8.70^{a}	9.00	9.00^{a}
Day 42	9.00	9.00	9.00	9.00	9.00	9.00^{a}	9.00^{a}	9.00^{a}	9.00^{a}	9.00	9.00^{a}	2.90^{b}	9.00	9.00^{a}
Day 49	8.50	8.80	8.90	8.60	8.70	8.80^{ab}	8.90^{a}	8.90^{ab}	9.00^{a}	9.00	9.00^{a}	1.80^{c}	9.00	9.00^{a}
Day 56	8.80	9.00	9.00	9.00	9.00	8.30^{b}	9.00^{a}	9.00^{a}	9.00^{a}	9.00	9.00^{a}	1.40^{cd}	9.00	9.00^{a}
Day 63	8.70	8.90	8.90	8.80	9.00	5.40^{c}	9.00^{a}	9.00^{a}	9.00^{a}	9.00	9.00^{a}	1.00^{d}	9.00	9.00^{a}
Day 70	8.80	8.50	9.00	8.90	8.80	4.80^{d}	8.80^{ab}	8.50^{c}	9.00^{a}	8.90	8.80^{b}	1.20^{d}	8.90	8.80^{b}
Day 77	8.90	8.90	8.80	8.90	9.00	4.60^{d}	8.90^{a}	8.90^{ab}	8.80^{b}	8.90	9.00^{a}	1.20^{d}	8.90	9.00^{a}
Day 84	8.80	8.60	9.00	8.70	8.80	4.90^{cd}	8.80^{ab}	8.60^{bc}	9.00^{a}	8.70	8.80^{b}	1.00^{d}	8.70	8.80^{b}
Day 90	9.00	8.90	8.80	9.00	9.00	5.00^{cd}	9.00^{a}	8.90^{ab}	8.80^{b}	9.00	9.00^{a}	1.00^{d}	9.00	9.00^{a}
SE(d)	0.16	0.17	0.10	0.15	0.12	0.26	0.12	0.15	0.07	0.10	0.08	0.27	0.10	0.07
LSD at 5%	NS	NS	NS	NS	NS	0.513	0.23	0.30	0.14	NS	0.17	0.54	NS	0.14
CV(%)	4.25	4.38	2.73	4.01	2.97	7.78	2.88	3.83	1.78	2.59	2.18	13.29	2.59	1.78

Table 4: Mean scores for the sensory attributes of the recipe chapati (Mean ± SEM) n=16)

Treatment	Sensory attributes							Aver-age total score
	Colour and Appearance (10)	Aroma (10)	Texture (10)	Taste (20)	After taste (10)	Mouth-feel (20)	Overall acceptability (20)	
T_1	8.63 ± 0.13^{a}	8.38 ± 0.16^{a}	8.59 ± 0.12^{a}	17.84 ± 0.17^{a}	8.31 ± 0.20^{a}	17.72 ± 0.22^{a}	18.13 ± 0.16^{a}	87.60
T_2	7.84 ± 0.13^{bcd}	7.84 ± 0.19^{ab}	7.84 ± 0.19^{bc}	17.06 ± .27^{ab}	8.00 ± 0.18^{ab}	17.19 ± 0.19^{ab}	17.31 ± 0.15^{ab}	83.08
T_3	7.50 ± 0.22^{bcde}	7.56 ± 0.18^{bcd}	7.81 ± 0.20^{bc}	16.81 ±0.29^{abc}	7.81 ± 0.13^{abc}	16.84 ± 0.30^{abc}	16.78 ± 0.31^{bc}	81.51
T_4	7.31 ± 0.24^{cde}	7.38 ± 0.22^{bcdef}	7.31 ± 0.22^{cde}	16.88 ± 0.26^{abc}	7.66 ± 0.19^{abcde}	16.13 ± 0.27^{abc}	16.75 ± 0.34^{bc}	79.42
T_5	7.19 ± 0.23^{ef}	7.03 ± 0.32^{defg}	7.25 ± 0.28^{cdef}	16.44 ± 0.61^{bcd}	7.44 ± 0.29^{bcdef}	16.25 ± 0.54^{bcde}	16.19 ± 0.49^{cd}	77.79
T_6	5.13 ± 0.26^{b}	5.75 ± 0.30^{h}	5.31 ± 0.30^{g}	13.59 ± 0.89^{f}	6.00 ± 0.30^{a}	13.88 ± 0.91^{f}	13.06 ± 0.84^{e}	62.72
T_7	7.59 ± 0.2^{bcde}	7.31 ± 0.25^{bcdef}	7.38 ± 0.20^{bcde}	15.84 ± 0.42^{bcde}	7.31 ± 0.20^{cdef}	16.25 ± 0.35^{bcde}	16.56 ± 0.34^{bc}	78.27
T_8	6.31 ± 0.25^{g}	6.63 ± 0.22^{g}	6.78 ± 0.29^{ef}	14.69 ± 0.57ef	6.81 ± 0.28^{f}	15.00 ± 0.60^{ef}	15.25 ± 0.48^{d}	71.47
T_9	7.22 ± 0.19^{def}	7.13 ± 0.18^{cdefg}	7.41 ± 0.17^{bcde}	15.59 ± 0.51^{cde}	7.00 ± 0.22^{ef}	15.91 ± 0.51^{cde}	16.00 ± 0.43^{cd}	76.26
T_{10}	7.94 ± 0.28^{bc}	7.50 ± 0.20^{bcde}	8.00 ± 0.24^{ab}	16.16 ± 0.41^{bcd}	7.69 ± 0.25^{abcd}	16.75 ± 0.41^{abc}	16.68 ± 0.40^{bc}	80.72
T_{11}	6.59 ± 0.26^{fg}	6.75 ± 0.23^{fg}	6.63 ± 0.32^{f}	15.25 ± 0.51^{de}	7.16 ± 0.22^{cdef}	15.31 ± 0.48^{de}	15.69 ± 0.51^{cd}	73.38
T_{12}	6.59 ± 0.26^{fg}	6.88 ± 0.85^{efg}	7.13 ± 0.15^{def}	16.19 ± 0.36^{bcd}	7.13 ± 0.27^{def}	15.88 ± 0.38^{cde}	15.94 ± 0.30^{cd}	75.74
T_{13}	8.00 ± 0.20^{ab}	7.44 ± 0.29^{bcde}	7.84 ± 0.23^{bc}	16.25 ± 0.49^{bcd}	7.50 ± 0.31^{bcde}	16.38 ± 0.36^{bcd}	16.84 ± 0.42^{bc}	80.25
T_{14}	7.94 ± 0.21^{bc}	7.69 ± 0.25^{bc}	7.72 ± 0.25^{bcd}	16.53 ± 0.44^{abcd}	7.50 ± 0.30^{bcde}	16.31 ± 0.38bed	16.72 ± 0.56^{bc}	79.86
SE(d)	0.319*	0.323*	0.325*	0.673*	0.348*	0.648*	0.627*	-
LSD at 5%	0.628	0.637	0.639	1.326	0.685	1.278	1.236	-
CV (%)	12.41	12.64	12.48	11.83	13.33	11.32	10.89	-

Table 5: Mean scores for the sensory attributes of the recipe puri (Mean ± SEM) (n=10)

Treatment	Sensory attributes							Aver-age total score
	Colour and Appearance (10)	Aroma (10)	Texture (10)	Taste (20)	After taste (10)	Mouth-feel (20)	Overall acceptabi-lity (20)	
T_1	7.40 ± 0.64	7.10 ± 0.61	7.20 ± 0.61	14.10 ± 0.96	6.70 ± 0.65	14.00 ± 1.21	14.30 ± 1.09	70.8
T_2	7.40 ± 0.56	7.10 ± 0.43	7.30 ± 0.56	15.10 ± 1.07	7.10 ± .48	14.20 ± 1.20	14.90 ± 1.03	73.1
T_3	7.10 ± 0.52	7.10 ± 0.38	7.00 ± 0.62	14.40 ± 1.39	7.00 ± 0.52	14.20 ± 1.02	15.50 ± 0.82	72.3
T_4	7.50 ± 0.56	7.20 ± 0.44	7.30 ± 0.56	15.00 ± 1.31	7.20 ± 0.42	14.90 ± 1.07	14.90 ± 1.01	74
T_5	7.40 ± 0.43	6.70 ± 0.51	7.20 ± 0.50	14.90 ± 1.31	6.60 ± 0.52	14.10 ± 0.99	14.40 ± 0.96	71.3

Treatment	Sensory attributes							Average total score
	Colour and Appearance (10)	Aroma (10)	Texture (10)	Taste (20)	After taste (10)	Mouth-feel (20)	Overall acceptability (20)	
T_6	7.20 ± 0.39	7.10 ± 0.38	8.00 ± 0.42	14.30 ± 1.20	7.30 ± 0.42	13.70 ± 1.10	15.50 ± 0.86	73.1
T_7	7.80 ± 0.35	7.40 ± 0.37	8.00 ± 0.27	14.90 ± 1.13	7.40 ± 0.34	13.80 ± 0.84	15.70 ± 0.80	75
T_8	7.60 ± 0.48	7.10 ± 0.53	7.90 ± 0.38	14.50 ± 1.23	6.80 ± 0.55	14.80 ± 0.81	15.40 ± 0.82	74.1
T_9	7.80 ± 0.42	7.50 ± 0.28	7.80 ± 0.39	15.10 ± 0.99	7.60 ± 0.40	15.00 ± 0.88	16.10 ± 0.75	76.9
T_{10}	7.80 ± 0.36	7.40 ± 0.37	7.80 ± 0.42	15.40 ± 0.88	7.80 ± 0.42	15.20 ± 0.68	16.20 ± 0.59	77.6
T_{11}	7.80 ± 0.44	7.50 ± 0.26	8.00 ± 0.40	15.90 ± 0.88	7.90 ± 0.28	15.50 ± 0.68	16.70 ± 0.58	79.3
T_{12}	6.80 ± 0.47	7.00 ± 0.41	7.50 ± 0.46	15.00 ± 0.88	7.50 ± 0.45	14.60 ± 0.67	15.70 ± 0.60	74.1
T_{13}	8.00 ± 0.50	7.60 ± 0.48	8.20 ±0.50	16.20 ± 0.68	8.00 ± 0.39	15.60 ± 0.60	16.80 ± 0.53	80.4
T_{14}	8.40 ± 0.45	7.80 ± 0.40	8.50 ± 0.30	16.60 ± 0.77	8.00 ± 0.38	15.80 ± 0.66	16.90 ± 0.54	82
SE(d)	0.672	0.599	0.658	1.484	0.642	1.287	1.138	-
LSD at 5%	NS	NS	NS	NS	NS	NS	NS	-
CV (%)	19.82	18.48	19.13	22.08	19.54	19.69	16.30	-

Table 6: Mean scores for the sensory attributes of the recipe paratha (Mean ± SEM) (n=9)

Treatment	Sensory attributes							Average total score
	Colour and Appearance (10)	Aroma (10)	Texture (10)	Taste (20)	After taste (10)	Mouth-feel (20)	Overall acceptability (20)	
T_1	8.14 ± 0.34	7.56 ± 0.38	7.78 ± 0.32	16.11 ± 0.86	7.00 ± 0.60	15.44 ± 0.10	16.33 ± 0.76	78.36
T_2	7.89 ± 0.42	7.62 ± 0.32	7.63 ± 0.33	16.22 ± 0.70	7.51 ± 0.26	15.44 ± 0.82	16.22 ± 0.60	78.13
T_3	7.78 ± 0.28	7.56 ± 0.24	7.56 ± 0.29	15.44 ± 0.69	7.22 ± 0.40	14.89 ± 0.68	15.89 ± 0.51	76.34
T_4	7.89 ± 0.26	7.22 ± 0.40	7.33 ± 0.29	15.78 ± 0.64	7.22 ± 0.36	15.11 ± 0.66	15.89 ± 0.46	76.44
T_5	7.67 ± 0.37	7.11 ± 0.46	6.89 ± 0.39	15.44 ± 0.53	7.00 ± 0.37	15.00 ± 0.58	16.00 ± 0.41	75.11
T_6	7.67 ± 0.41	7.11 ± 0.48	7.11 ± 0.46	15.11 ± 0.63	7.00 ± 0.24	14.78 ± 0.59	15.67 ± 0.65	74.45
T_7	7.78 ± 0.36	7.56 ± 0.34	7.33 ± 0.37	15.33 ± 0.41	7.33 ± 0.29	14.56 ± 0.63	15.78 ± 0.36	75.67
T_8	7.78 ± 0.40	7.56 ± 0.34	7.33 ± 0.33	15.44 ± 0.44	7.22 ± 0.32	15.00 ± 0.60	15.67 ± 0.53	76
T_9	7.78 ± 0.28	7.56 ± 0.29	7.44 ± 0.38	15.56 ± 0.65	7.33 ± 0.37	15.00 ± 0.69	15.67 ± 0.44	76.34
T_{10}	7.78 ± 0.27	7.44 ± 0.34	7.44 ± 0.41	15.67 ± 0.60	7.44 ± 0.38	14.67 ± 0.76	16.00 ± 0.58	76.44
T_{11}	7.22 ± 0.47	7.33 ± 0.29	7.11 ± 0.48	15.22 ± 0.70	7.44 ± 0.41	15.22 ± 0.68	16.00 ± 0.58	75.54

Treat-ment	Sensory attributes							Aver-age total score
	Colour and Appearance (10)	Aroma (10)	Texture (10)	Taste (20)	After taste (10)	Mouth-feel (20)	Overall acceptabi-lity (20)	
T_{12}	6.78 ± 0.36	7.00 ± 0.33	7.11 ± 0.31	14.56 ± 0.58	7.00 ±0.33	14.33 ± 0.58	15.67 ± 0.58	72.45
T_{13}	8.22 ± 0.28	7.57 ± 0.38	7.89 ± 0.37	16.00 ± 0.55	7.56 ± 0.24	15.51 ± 0.72	16.41 ± 0.51	79.11
T_{14}	7.89 ± 0.35	7.22 ± 0.40	7.89 ± 0.26	14.89 ± 0.66	7.44 ± 0.29	14.78 ± 0.70	16.33 ± 0.50	76.44
SE(d)	0.499	0.512	0.513	0.887	0.509	0.990	0.766	-
LSD at 5%	NS	NS	NS	NS	NS	NS	NS	-
CV (%)	13.64	14.77	14.75	12.21	14.91	14.05	10.19	-

Table 7: Mean scores of products of recommended Multigrain flour treatment (T13) for consumer acceptability test through hedonic rating scale (n=17)

Treatment Name	Treatment of 'Quality'	Rank of Treatment
Chapati	6.94	3
Paratha	7.76	1
Puri	7.41	2
General Mean	7.37	.
CV(%)	14.67	.
SE(d)	0.371	.
LSD at 5%	NS	.

Figure 2: Quality graph for products for consumer acceptabilityw

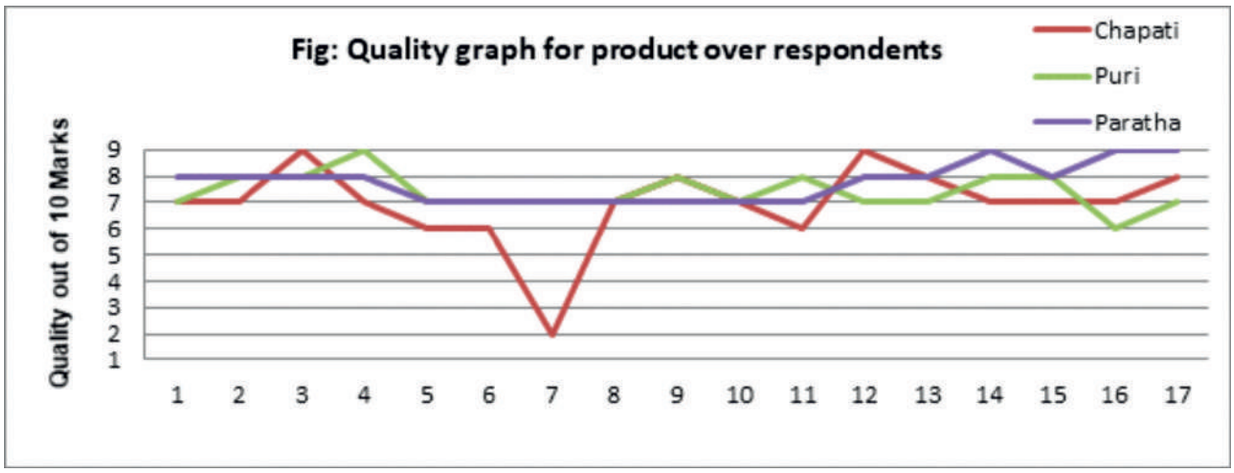


Table 8: Nutrient contents of Multigrain flour

Treatment	Moisture (%)	Fat (%)	Protein (%)	Crude Fiber (%)	Iron (mg/100g)	Calcium (mg/100g)	Ash (%)
T1	12.20^{a}	1.70^{g}	11.80^{f}	1.20^{f}	4.90^{e}	48.00^{i}	1.50^{g}
T2	11.80^{bcd}	3.03^{f}	13.98^{d}	1.97^{de}	5.48^{cd}	100.60^{h}	1.94^{f}
T3	11.81^{bcd}	3.19^{e}	13.97^{de}	1.97^{de}	5.63^{bc}	100.33^{h}	1.98^{ef}
T4	11.82^{bc}	3.36^{d}	13.96^{de}	2.39^{b}	5.79^{b}	100.00^{h}	2.02^{def}

Treatment	Moisture (%)	Fat (%)	Protein (%)	Crude Fiber (%)	Iron (mg/100g)	Calcium (mg/100g)	Ash (%)
T5	11.69^{def}	3.04^{f}	14.13^{d}	2.39^{b}	5.78^{b}	123.00^{c}	2.02^{def}
T6	11.58^{f}	3.05^{f}	14.27^{cd}	2.81^{a}	6.09^{a}	146.80^{a}	2.10^{bc}
T7	11.58^{f}	3.32^{d}	14.07^{d}	2.08^{cde}	5.42^{d}	101.70^{h}	1.95^{ef}
T8	11.73^{cde}	3.62^{c}	14.16^{cd}	2.20^{bcd}	5.37^{d}	100.83^{h}	1.97^{ef}
T9	11.65^{ef}	3.92^{b}	15.58^{b}	2.09^{cde}	5.75^{b}	110.20^{f}	2.09^{bcd}
T10	11.39^{g}	4.81^{a}	17.12^{a}	2.22^{bc}	6.03^{a}	119.83^{d}	2.25^{a}
T11	11.85^{bc}	3.01^{f}	17.12^{a}	2.09^{cde}	5.43^{d}	115.42^{e}	2.02^{def}
T12	11.89^{b}	2.99^{f}	13.53^{e}	2.21^{bc}	5.38^{d}	130.20^{b}	2.06^{bcd}
T13	11.74^{cde}	3.01^{f}	14.59^{c}	1.95^{e}	5.42^{d}	105.91^{g}	2.02^{cde}
T14	11.67^{ef}	3.00^{f}	15.20^{b}	1.94^{e}	5.37^{d}	111.22^{f}	2.11^{b}
SE(d)	0.061	0.037	0.221	0.114	0.084	1.453	0.038
LSD at 5%	0.1252	0.0759	0.4517	0.233	0.1727	2.9757	0.0781
CV(%)	0.64	1.41	1.86	6.61	1.86	1.65	2.33

Table 9: Rheological Properties of Multigrain Flour (Multigrain Flour Extensibility Test using the Kieffer Dough and Gluten Extensibility Rig)

Treat-ments	Resistance to Extension (g)	Extensibility (mm)
	Force 1	Distance 1
T1	404.97	-3.64
T2	537.67	-5.14
T3	282.74	-6.3
T4	520.12	-8.04
T5	520.56	-8.7
T6	532.92	-7.44
T7	699.93	-8.82
T8	564.3	-6.96
T9	282.3	-9.27
T10	306.29	-8.76
T11	528.31	-8.18
T12	264.96	-7.78
T13	526.99	-6.98
T14	527.14	-4.35

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Isolation of Plant Growth Promoting Bacteria from Fermented *Panchagavya* and their Effect on *Vigna Radiata*

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Abstract

Panchagavya is a mixture of five cow products viz. cow dung, urine, milk, curd and ghee containing many beneficial microorganisms mentioned in ancient Indian scripts. Two bacterial cultures (PG-7 and PG-34) isolated from fermented panchagavya were studied for gibberellic acid (GA)production and phosphorus (P) solubilization, respectively. Initially, PG-7 produced 0.81 mg/ml GA and was increased to 5.70 mg/ml after media optimization. PG-34 was able to solubilize considerable amount of tricalcium phosphate (213.36 µg/ml). The effect of pure cultures (PG-7, PG-34) and mixed culture (PG-7 plusPG-34) on *Vigna radiata* was studied in the pots. The application of pure as well as mixed culture showed the significant positive effect on the growth of *Vigna radiata*. The mixed culture increased about 2.07, 2.16, 1.50, 7.50, 2.50, 2.72 times fresh weight, dry weight, shoot length, root hair numbers, leaf numbers and leafarea,respectively on 20thday.The bacterial isolates (PG-7, PG-34) offermented panchagavya have a potential to be used as biofertilizer.

Keywords: Bacteria, Biofertilizer,Fermented panchagavya, *Vigna radiata*.

INTRODUCTION:

Panchagavya (PG) means mixture of five cow products viz. dung, urine, milk,curd and ghee. Microbial fermentation of the panchagavya resulted in a rich repertoire of compounds and microbes that are beneficial for plant growth. NMR metabolomics of panchagavya, showed several primary metabolites such as plant growth regulators consisting of indole acetate; gibberellic acids; vitamins and co-factors; nucleobases; that make up many of the biochemical pathways (Ukale, etal. , 2016). Microbes obtained from panchagavya have been said to possess the property of being able to produce phytohormones (Amalraj, et al., 2013).

Biofertilizers are living microorganisms that increase the growth as well as development of plant by amassing the accessibility of mineral nutrients, solubilizing phosphorus, and phytohormonesproduction (Mushtaq, et al., 2021). Individual or mixed cultures microorganism inoculant, have been proved to significantly increase crop production at a very low cost when compared to chemical fertilizers. Plant growth promoting bacteria (PGPB) alleviates the effect of biotic and abiotic stress factors directly or indirectly. PGPB have some properties such as phosphate solubilization, ACC deaminase production and phytohormone production. PGPB are colonized at/near the plant root, their wave of efficacy hits the doorstep of major functional aspects of the entire plant (Mondal, 2020).

The production of such growth regulators by microorganisms delivers several benefits to the host plant with the facilitation of root system expansion, which increases the absorption of water and nutrients and improves plant survival (Verma, et al., 2017). Phytohormone comprise cell wall and cuticle thickening, structural changes of roots, stems, and leaves.Stem elongation is stimulated by gibberellic acid (GA). Gibberellins are plant hormones affecting germination, stem elongation, flowering, abolition of dormancy, leaf

and fruit senescence, and enzymatic stimulation (Singh, et al., 2020).

Soil is a foremost source of nutrients required by plants for growth. The three chief nutrients are nitrogen (N), phosphorus (P) and potassium (K). Phosphorous is the essential elements that are necessary for plant development and growth; it makes up about 0.2% of a plant's dry weight. Phosphorousis second only to nitrogen among mineral nutrients, most commonly limiting the crops growth (Azziz, et al., 2012).On average, the phosporous content of soil is about 0.05% (w/w); however, only 0.1% of this phosphorus is available for plant use. Phosphate solubilizing microorganisms (PSMs) increases the availability of insoluble soil phosphorus for plant use. PSMs secrete organic acids and phosphatases which convert insoluble phosphates into soluble phosphate which can be utilized by plants (Hii, et al., 2020).

In order to scrutinize plant, soil and microbial interactions, pot experiments are normally used as an imperative tool for exploring plant responses to treatment manipulations. The pot study is a preliminary experiment that provides us evidence of the impact our test bacteria on the experimental crop. In the present study, *Vigna radiata* (mung bean) crop was used for studying the effect of bacteria isolated from fermented panchagavya on their growth.

MATERIAL AND METHODS

Bacterial isolates

We have reported the bacteria showing plant growth promoting activities isolated from fermented panchagavya (Gohil, et al., 2020). Out of these, two bacterial isolates, viz. PG-7and PG-34 were selected for further study. PG-7 was studied for GA production and PG-34 for phosphorus solubilization and their effect on the growth of *Vigna radiata* was tested.

Maintenance and preservation of isolates

The pure cultures of bacterial isolates were grown on Nutrient agar medium. They were preserved by monthly transfer onto Nutrient agar slants and were kept in the refrigerator at 4°C.

Inoculum preparation

For inoculum preparation, 20.0ml of sterile Nutrient broth was inoculated with freshly grown cultures and incubated at 30°C,120 rpm on an orbital shaker for 24 h. Bacterial suspension having a cell density ~10^8cells/ml was used to analyze the optimized parameter. The prepared culture suspension was used as 1% (v/v) inoculum.

Furthermore, the cells were separated by centrifugation at 5000 rpm for 15 minutes and the supernatant was removed. Cell pellet was resuspended in sterile normal saline to obtain a cell density ~10^8cells/ml and it was used for pot treatment.

Plant growth promoting traits of PG-7and PG-34

Gibberellic acid production by PG-7

To study the effect of various carbon sources on the production of gibberellic acid, 100 ml Nutrient broth having pH 7.0 was supplemented with different carbon sources viz. dextrose, lactose and fructose and the concentration of these sugars was kept 2% (w/v). The flask was inoculated with inoculum of PG-7 and incubated at 30±2℃, at 150 rpm on an orbital shaker.

To investigate the effect of nitrogen source, peptone was replaced by $NaNO_3$, urea, and glycine at 0.5% (w/v) in 100 ml Nutrient broth. It was inoculated with PG-7and incubated at 30± 2℃, 150 rpm on an orbital shaker.

For optimum GA production, 100 mlnutrient brothin 250 ml flask was supplemented with 1% (w/v) dextrose and 0.2 % (w/v) $NaNO_3$. The medium was inoculated with 1% (v/v) PG-7and incubated at 30± 2 ℃, 150 rpm for 72 h on shaker.

Gibberellic acid estimation (Graham and Thomas, 1961) : 2.0 ml broth was taken

aseptically in an Eppendorf tube, and centrifuged at 5000 rpm for 10 minutes. 1.0 mlsupernatant and 1.0 ml ethyl acetate was mixed and shook vigorously. Ethyl acetate layer was allowed to separate and carefully transferred in a 50 mlglass beaker, the ethyl acetate extraction was repeated thrice. The ethyl acetate extract was allowed to evaporate at room temperature. The residue was dissolved in 2.0 mlmethanol and collected in screw cap tubes. To this 1.0ml of DNPH (2,4-Dinitrophenylhydrazine) was added and incubated at 100°C for 5 minutes. After cooling, 5.0 ml alcoholic potassium hydroxide (10%, w/v) and 15.0 ml sterile distilled water was added. Red wine color developed was measured at 430nm. Standard calibration curve of GA (0.8 mg/ml) was prepared in methanol.

Phosphate solubilization by PG-34

Solubilization of different phosphorus sources like Aluminum phosphate (AP), Zinc phosphate (ZP) and Tricalcium phosphate (TCP) was studied using Pikovskaya's broth. The phosphate solubilized by PG-34 was estimated by Stannous chloride method (King, 1932).In three 250 ml flaskscontain100ml sterile Pikovskaya's broth (pH 7.0±0.2),1.0 gm% different phosphate sources (AP, ZP and TCP) were added. Each flask was inoculated with 1% (v/v) inoculum and incubated at 25°C, 150 rpm on a shaker. Phosphate solubilization was tested after five and ten days.

2.0ml sample was centrifuged at 10,000 rpm for 15-20 minutes to remove cells. From this, 0.1 ml supernatant was taken and 0.9 ml double distilled water and 1.0 ml of chloromolybdic acid was added. Contents in test tubes were diluted to 4.0 ml by adding double distilled water. To this, 25.0 µL chlorostannous acid reagent was mixed till blue color was developed. Final volume was made up to 5.0 ml with double distilled water as quickly as possible. Absorbance was taken at 600nm. Total amount of phosphate solubilized was calculated against the standard curve of K_2HPO_4 (100 µg/ml).

Soil sample

The 12.0 kg soil sample was collected in sterile plastic bag from a depth of 4.0 inches from the central library garden area of Gujarat University and transported to the laboratory for pot experiment.

Seeds

*Vigna radiata*seeds were collected from the local market Ahmedabad for pot experiments. Seeds were disinfected by washing them with tap water to remove all the dirt and dust followed by washing twice with distilled water to remove any remaining tap water. The seeds were treated with 0.1% $HgCl_2$ solution for 3 minutes. Seeds were cleaned with 70% ethanol 3 times followed by rinsing them with sterile distilled water 5 times.

Pot study

The pot study was conducted in plastic pots with a height of 14.0 cm and a diameter 6.0 cm between the month of February and March 2020 in Ahmedabad, when the average temperature was 35±2°C.

For the pot preparation, garden soil was autoclaved twice for 20 minutes at 121°C. Each pot was filled with approximately 300 grams sterile soil having a neutral pH. *Vigna radiata*(V) seeds were surface sterilized and watered slightly after transplanting at a depth 0.5cm.

Pots were prepared and named as control, PG-7, PG-34 and mixed culture, respectively. A mixed culture was prepared by mixing the bacterial suspensions of all the two isolates (PG-7and PG-34). To check the culture effect on *Vigna radiata*growth, 5.0 ml bacterial suspension were given on 3rd and 10th day after sowing (DAS). Control plants were treated with sterile distilled water.

The plants from each pot were harvested on the 10th and 20th day to studythe morphological characteristics (fresh weight, dry weight, shoot length, root length, root number, root hair number, leaf number and leaf area).

RESULTS AND DISCUSSION

Gibberellic acid production by PG-7

Gibberellic acid is a plant growth regulator produced by bacteria and fungi that helps in the plants growth. The exploration of various plant growth promoting substances produced by the bacteria makes them an effective biofertilizer. PG-7 was able to produce 0.8mg/ml gibberellic acid after 48 h in Nutrient broth (Figure 1 A).PG-7 bacteria isolated from fermented panchagavya having good capacity to produce gibberellic acid within 48 h, which is as good as fungal

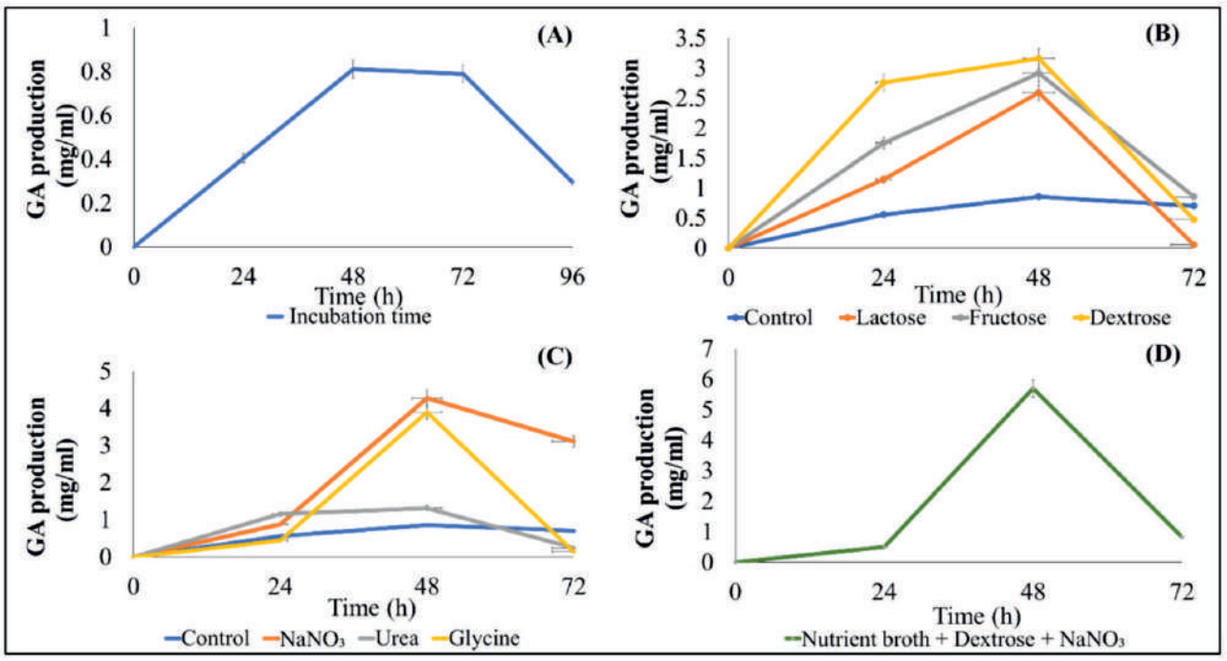


Figure 1 Optimization of gibberellic acid production by PG-7 (A) GA production with time (B) Effect of carbon sources on GA production (C) Effect of nitrogensources on GA production (D) Gibberellic acid production using optimized medium.

production.The gibberellic acid production is primarily reported by various fungi and were used for commercial production.

In comparison to bacteria, fungi require more time to grow and produce GA. *Pseudomonas stutzerii* solated from rhizospheric soil produced 0.03 mg/ml GA, after 72 h (Patel & Saraf 2017). Rhizospheric isolate *Pseudomonas monteilii* produce0.09 mg/ml G Aafter 48h (Pandya & Desai 2014). Panchgavya isolate PG-7 produced significant GA in compare to above isolates. *Fusarium moniliforme* isolated from soil generated 0.4 mg/ml GA at 144 h (Sleem, 2013). PG-7 has produced about double gibberellic acid than the above fungi within 48 h.

Gibberellic acid production was optimized by adding carbon and nitrogensources in the nutrient medium. Medium optimization for GA production can decrease the production costs on large scale.The effect of different sugars on GA production was studied and it boosted the production up to four times. The GA production was increased to 3.1 mg/mlupon addition of dextrose (2% w/v), whereas fructose and lactose showed 2.9 mg/ml and 2.5 mg/ml GA productionafter 48 h, respectively(Figure 1 B). *G. fujikuroi* produced 0.3 mg/ml GA after 168 h in a basal medium supplemented with glucose (Lale & Gadre 2010). Ammonium mineral salt broth supplemented with 0.5% (w/v) Na-succinate as a carbon source showed 0.12 mg/ml GA production by *M. Oryzae* (Siddikee, et al., 2010).About 0.08 mg/ml Gibberellic acid production was obtained with 1% glucose by rhizospheric bacterial isolate K8 (Desai, 2017).

The nitrogen sources $NaNO_3$, Urea, and Glycine

were added to the nutrient broth as a substitute of peptone and 4.2, 1.3, and 3.8 mg/ml GA was produced, respectively after 48 h. The addition of $NaNO_3$ and glycine resulted to about 5 times increase in GA production, whereas urea gave double production. The medium containing $NaNO_3$ as the nitrogen source produced the highest (4.2 mg/ml) GA at 30°C after 48 h (Figure 1 C).

The nitrogen source plays an important role in GA production, 0.29 mg/ml GA with NH_4NO_3 and 0.15 mg/ml GA was produced with glycine (Shukla et al., 2005). Similar results were obtained by Karakoc and Aksoez (2004) and addition of $NaNO_3$increased the GA production (0.14 mg/ml). Desai (2017) investigated the effect of nitrogen supplementation on GA production by *Azotobacter* spp. and found that nutrient broth supplemented with 0.5% ammonium chloride produced 0.07 mg/ml GA.

Optimized medium supplemented with dextrose and $NaNO_3$ showed 5.6 mg/ml GA production after 48 h in shake flask conditions (Figure 1 D). The optimized medium showed 7 times increase in GA production.

Phosphate solubilization by PG-34

Phosphate solubilizing bacteria improve the phosphates availability by solubilizing them and making them available to the plant. PG-34 was capable of solubilizing 216 µg/mlinsoluble inorganic phosphorus after 10 daysin Pikovskay's broth (Figure 2 A). *Bacillus cereus* able to solubilize 159µg/ml phosphate after 10 days (Salwan, et al. 2021). PG-34 was able to solubilize phosphate sources along with pH drop from 7.0 to 4.5 after 10 days. The pH of medium shifted from neutral to acidic due to organic acid production.PS Mswas able to solubilize different inorganic phosphatessources in liquid media and it was also associated with pH reduction (Lacava, et al., 2021).

PG-34 was found to solubilizemore TCP (213.36 µg/ml) than ZP (7.7µg/ml) and AP (2.04 µg/ml) after 10 days (Figure 2 B). PG-34 solubilized maximum ZP (76µg/ml) after 5days, thereafter no significant increase of available phosphorus was observed. PG-34 showed the maximum solubilization of TCP, moderate solubilization of ZP and least solubilization of AP (Figure 2 C).

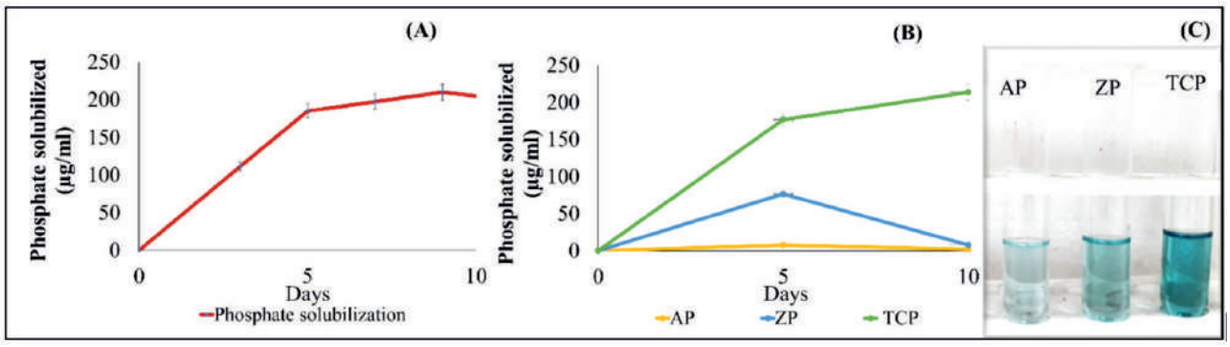


Figure 2 Phosphate solubilization by PG-34 (A) Phosphate solubilization with time (B) Solubilization of different phosphorus sources C) Detection of solubilized aluminium phosphate (AP), zinc phosphate (ZP) and tricalcium phosphate (TCP) on 10th day.

Similarly, Sagervanshi , *et al*., (2012) studied the solubilization of phosphorus sources (AP, ZP, TCP) and reported TCP as the best phosphate source. Phosphate solubilization was 4.73 mg/ml with single super phosphate and 2.89 mg/ml by TCP by *Piriformospora indica* (Seshagiri & Tallapragada 2016). Phosphate solubilization capability varies with different microorganisms. PG-34 may be useful in resolving calcareous soils by increasing the accessible plant phosphate levels.PSBs are present in fermented panchagavya and have higher capacity for phosphate solubilization makes them useful for liquid biofertilizer.

Pot study

In the primary test, isolates were tested for their beneficial role in producing phytohormone and releasing phosphorus in the broth medium. Their biological contribution to improve the mung bean plant was investigated.The effect of pure culture PG-7, PG-34 and mixed culture was tested and they showed significant increase in plant growth compared to control. Variation in the efficiency was apparent and mixed culture performed better than the pure cultures.

Plant growth promotion activity of PG-7 and PG-34 on *Vigna radiata*

The impact of PG-7 (gibberellic acid-producing) and PG-34 (phosphate-solubilizing bacteria) on *Vigna radiata*in pots was studied. Pure cultures (PG-7 and PG-34) and mixed culture effect on the growth of mung plant over control is presented inTable 1. On the 20th day, an improvement in root length, shoot length root hairs and leaf numbers were observed. Leaves were wider and lengthyin treated plants compared to untreated plants. Plant inoculated with PG-7showed significant increase in the fresh weight, dry weight shoot length, root length, root hair number, leaf number and leaf area by 1.97, 2.0, 1.45, 1.03, 6.0, 2.0and 2.7 times, respectively compared to the untreated plant. Similarly, plant inoculated with PG-34 showed significant enhancement in the fresh weight, dry weight, shoot length, root length,root hair number, leaf number and leaf area by 1.71, 1.66, 1.3, 1.08, 4.5, 2.5 and 2.4 times, respectively over control. The plant inoculated with mixed culture showed considerable growth in the fresh weight, dry weight, shoot length, root length, root hair number, leaf number and leaf area by 2.07, 2.16, 1.50, 1.41, 7.50, 2.50 and 2.72 times compared to thecontrol after 20th day.

The difference in growth promotion effects between fresh and dry weights between control and treated might be attributed to PGPB capacity to provide more water holding capacity to plant. Gibberellic acidstimulates seed germination, shoot growth, vegetative to flowering, determines sex expression and grain development along with an interaction of different environmental factors (Gupta & Chakrabarty 2013). PSM increases the bioavailability of soil insoluble phosphorus for plant use. PSMs solubilize and mineralize insoluble organic phosphorus. Therefore, soil or crop inoculation with PSM is a promising strategy for improving plant phosphorus absorption and thus reducing the use of chemical fertilizers that have a negative impact on the environment (Alori et al., 2012). Application of PGPB improved vegetative growth characteristics, notably in leaf area, when compared to control plants(Abdelaal et al., 2021).Our results are in general agreement with numerous studies reported for beneficial effect of the PGPB on plant growth promotion.

Vegetative parameters	Treatments							
	Control		PG-7		PG-34		Mixed culture	
Day	10	20	10	20	10	20	10	20
Fresh weight (g)	0.21	0.38	0.35	0.75	0.38	0.65	0.26	0.79
Dry weight (g)	0.03	0.06	0.08	0.12	0.06	0.10	0.05	0.13
Shoot length (cm)	9.5	10.0	13.0	14.5	12.0	13.0	13.0	15.0
Root length (cm)	1.9	6.0	5.0	6.2	1.0	6.5	2.4	8.5
Root number (No.s)	1	1	1	1	1	1	1	1
Root hair number (No.s)	0	2	1	12	2	9	2	15
Leaf number (No.s)	2	2	2	4	2	5	2	5
Leaf area (cm^2)	2.40	2.50	5.25	6.75	5.60	6.00	5.25	6.80

Table 1 Effect of PG-7, PG-34 and mixed culture on growth of Vigna radiata.

Sangeetha & Thevanathan(2010) stated that panchagavya application encouraged the lateral rootsdevelopment, leaves, leaflets and lamina growth compared to control in experimental plants (*Vigna radiata, Vigna mungo, Arachis hypogea*and *Cicer arietinum*). Phosphate solubilizing bacterial inoculums to assess their ability to promote tomato (*Lycopersicon esculentum*) seedling and plant growth in in-vitro and greenhouse experiment respectively, and reported that isolates significantly enhanced all parameters (shoot length, root length, shoot and root dry weight) compared to control (Pathak et al., 2017). Positive effects of biofertilizers on improving crop growth might be due to increased synthesis of plant growth promoting substances.

CONCLUSION

The present study demonstrates that fermented panchagavya contains potential bacteria with plant growth promoting abilities like gibberellic acid productionand phosphate solubilization. Application of isolatesPG-7 and PG-34, in pure and mixed culture significantly increased the plant growth compared to control. It showed considerable improvements in the fresh weight, shoot length, root length, root hair number, leaf number and leaf areaof *Vigna radiata.* Since most soils in the world are deficient in plant-available nutrients, the use of bioinoculants would increase the nutrients availability and increase the soil fertility. This will reduce the use of chemical fertilizers and encourage the sustainable agriculture. The bacterial isolates of fermented panchagavya are potential candidates to be used as bioinoculants and biofertilizers.

CONFLICT OF INTEREST

The authors declares that there is no conflict of interest.

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Estimation of Correlation and Path coefficient analysis for yield and its component traits in tomato

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Abstract

An experiment was conducted at agriculture research farm, C.C.R. (PG) College Muzaffarnagar, U.P. to study the path and correlation which are very important association among yield and related component traits. Days to first flowering have significant genotypic and phenotypic correlation with number of primary branch (0.214, 0.189) and plant height (cm) (0.190, 0.150). Similarly, Days to first flowering, number of primary branches and number of fruits/plant show affirmative effect on fruit yield at genotypic and phenotypic levels. Further fruit yield can be improved by making the selection of desirable traits in important progenies of further crop improvement programmes.

Keyword: Tomato, Genotypic Correlation, Phenotypic Correlation and Path Analysis

INTRODUCTION:

Tomato (*Lycopersicon esculantum* Mill.) is a member of the family solanaceae and considered avaluable vegetable crop worldwide (He *et al*., 2003). Vegetables are important part of our diet as provides energy and are also is a rich source of minerals (Iron and phosphorus), vitamins (Vitamin A and C), organic acid, essential amino acids, dietary fibres, etc. Further tomato of fruit provides total sugar (3-4%), total solids (4-7%), ascorbic acid (15-30 mg/100g), Titratable acidity (7.5-10 mg/100ml) and fruit weight of lycopene (20-50mg/100g). Tomato and its products lower risk of developing digestive tract and prostate ford (Giovannucci *et al.,* 2002).

Path analysis and Correlation coefficient are key determinants to objective the association between the yield and related components. For the plant breeders positively correlated with the yield are important for selecting desirable genotype (Feyzian *et al.*2009). All the changes in the components need not, however be expressed by changes in the yield. This may be due to degrees of correlations (positive and negative) among yield and its other components or own among the components themselves. In the present study correlation coefficients and path analysis relevance of direct and indirect association of all characters with the final yield and its components have been studied.

Material and Methods:

The study was conducted during year 2020-21 at Agriculture Research Farm, C.C.R. (PG) College Muzaffarnagar, U.P. The material for the present study comprised of ten parents of tomato, namely EC-620406, EC-163605, EC-165700, EC-631364, EC-164563, EC-521067, EC-528360, EC-145057, Floradade, and Arka Meghali and forty five hybrids were produced through half diallel mating in Randomized Block Design with three replications. All the recommended package of practices was adopted for raising a healthy crop. Five plants were randomly selected from each treatment for observations. Analysis of covariance for all combinations was for to study correlations. Phenotypic/genotypic correlations were calculated by the formulae recommended by Al-Jibouri *et al*. (1958). Path analysis was calculated as per the procedure outlined by Wright (1921), and Dewey and Lu (1959). Phenotypic and genotypic correlations and path coefficient analysis were analyzed using R software (Version 4.1.1). Using Lavaan Package (Rosseel, 2012).

Result and Discussion:

In the present investigation the results revealed Table 1 and 2 that the a significant and positive correlation of days to first flowering at genotypic level was observed with days to 50% flowering (0.951), no. of primary branches/plant (0.215), plant height (0.191) and no. of fruits/cluster (0.178). Days to 50% flowering exhibited significant positive genotypic correlation with no. of primary branches/plant (0.297), number of fruits/cluster (0.267), total yield/plant (0.195), no. of secondary branches/plant (0.174). No of primary branches per plant at genotypic level was observed with no secondary branches (0.533), fruit diameter (0.436), days to 50% flowering (0.297) and days to first flowering (0.215). Plant height at genotypic level was observed with no. of secondary branches per plant, days to first

flowering, days to 50% flowering and pericarp thickness with values of 0.223, o.159, 0.191, and 0.155, respectively. Average fruit weight at genotypic level was observed with total yield per plant (0.574), TSS (0.565), pericarp thickness (0.533), fruit diameter (0.513) and fruit length (0.482). Pericarp thickness at genotypic level was observed with fruit length (0.657), average fruit weight (0.533), TSS (0.451), fruit diameter (0.209), T.A (0.197) and plant height (0.156). Ascorbic acid at genotypic level was observed with days to 50% fruiting (0.293) and no. of fruits per cluster (0.151). Total yield per plant at genotypic level was observed with average fruit weight (0.574), no. of fruits per plants (0.462) TSS (0.413), T.A (0.340) and no. of fruits per cluster (0.259). The results are in line with the previous reports (Singh *et al*. 2018, Mishra *et al*. 2019, Kumar *et al*. 2020, Maurya *et al.* 2020 and Akhter *et al.* 2021).

A significant and positive correlation of days to first flowering at phenotypic level was observed with days to 50% flowering (0.849), number of primary branches/plant (0.190), number of fruits/cluster (0.154) and plant height (0.151). Days to 50% flowering exhibited significant positive phenotypic correlation with no. of primary branches/plant (0.194), no. of fruits/cluster (0.169) and days to first flowering (0.849). No. of primary branches/plant reveled significant positive phenotypic correlation with no. of secondary branches/plant (0.489), fruit diameter (mm) (0.305), days to first flowering (0.190), days to 50% flowering (0.194). Fruit diameter showed significant positive genotypic correlation with average fruit weight (0.418), fruit length (0.290), TSS (0.181), total yield/ plant (0.169), titratable acidity (0.168), pericarp thickness (0.165), number of primary branches/ plant (0.305). Average fruit weight showed significant positive phenotypic correlation with total yield/plant (0.553), pericarp thickness (mm) (0.479), TSS (0.473), titratable acidity (0.214), fruit length (mm) (0.463) and fruit diameter (mm) (0.418). Total yield/plant (g) showed significant positive phenotypic correlation with average fruit weight (g) (0.553), no of fruit/plant (0.452), TSS (0.344), plant height (cm) (0.334), titratable acidity (0.328), number of fruits/cluster (0.193) and fruit diameter (0.169). The results are in line with the previous reports (Mishra *et al.* 2019 and Kumar *et al.* 2020).

The path coefficient is considered an important method for estimating the direct or indirect effect cause of inter/intra association. The similarly genotypic path coefficient analysis was also studied in Table 3 and Figure (1 & 2). The direct effect on total yield recorded to be the highest by average fruit weight (1.120) followed by no. of fruit/plant (1.006), days to first flowering (0.902), plant height (0.195), total yield/plant (0.164), fruit diameter (0.118), T.A. (0.095), pericarp thickness (0.074), ascorbic acid (0.054), no. of primary branches (0.045) and fruit length (0.044). While indirect effect at genotypic level days to first flowering had positive (indirect) effect on total yield via average

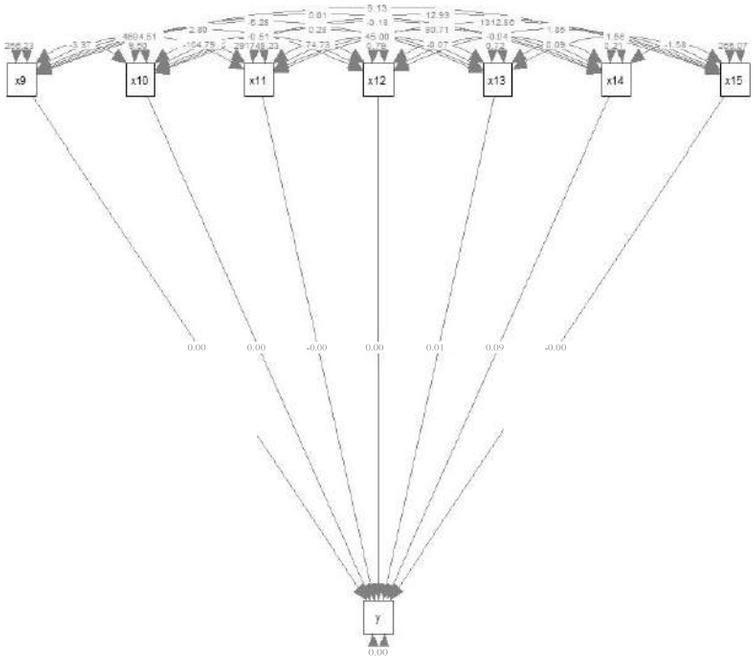
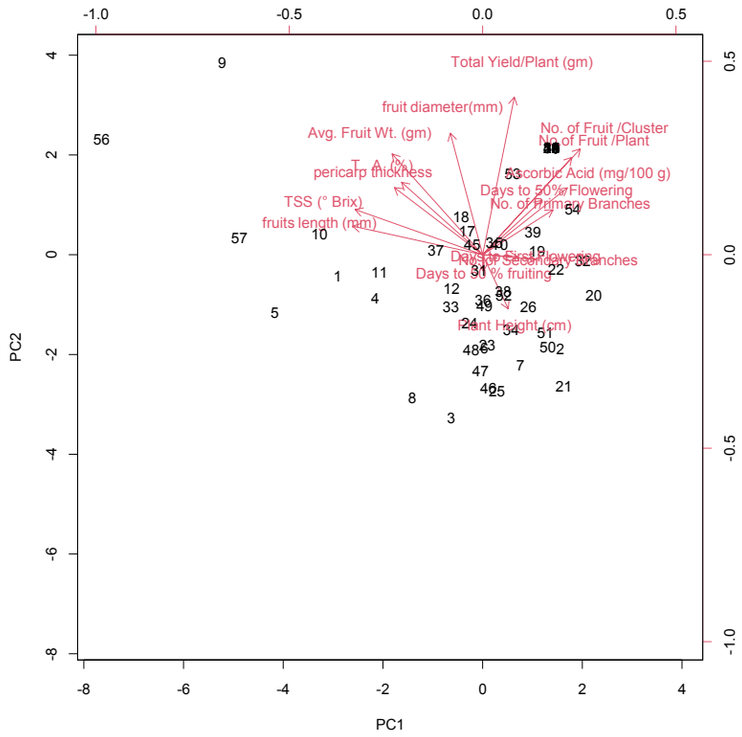


Figure 1. Path coefficient diagram and effect relationships among quantitative traits and total yield.

fruit weight (0.109), no. of fruit/plant (0.090), TSS (0.056), plant height (0.038), days to 50% fruiting(0.017), no. of primary branches (0.010) fruit diameter (0.007). Days to 50% fruiting had positive indirect effect on total yield via days to 50% flowering (0.194), TSS (0.060), plant height (0.023), no. of secondary branches (0.017), ascorbic acid (0.016), pericarp thickness (0.013). No. of secondary branches per plant had indirect effect on total yield via no. of fruit/ plant (0.172), days to first flowering (0.116), days to 50% fruiting (0.058), plant height (0.044), TSS (0.033), no. of primary branches (0.024) and Fruit diameter (0.018). Fruit length had positive indirect effect on fruit yield via average fruit weight (0.539), days to 50% flowering (0.176), pericarp thickness (0.049), fruit diameter (0.041), T.A. (0.032), days to 50% fruiting (0.015), no. of secondary branches (0.009) and fruit/cluster (0.007). Pericarp thickness(mm) had positive (indirect) effect on total yield via average fruit weight (0.597), plant height (0.031), fruit length (0.029), fruit diameter (0.025), T.A. (0.019), no. of primary branches per plant (0.014), ascorbic acid (0.003). Ascorbic acid had positive (indirect) effect on total yield via days to 50% flowering (0.148), TSS (0.132), no. of fruit/plant (0.035), no. of secondary branches per plant (0.006). The finding of these characters is supported by Jogi *et al.* (2018), Namdev and Dongre (2019), Kumar *et al.* (2020) and Singh *et al.* (2021).

Phenotypic path analysis direct and indirect effect on different traits present in table 4. The direct effect on total yield recorded to be highest by average fruit weight (0.800) followed by no. of fruit/plant (0.757), ascorbic acid (0.245), total yield/plant (0.137), TSS (0.085), no. of primary branches (0.054), days to first flowering (0.039), T.A. (0.038), plant height (0.022) and fruit length (0.021).While (indirect) effect at phenotypic level days to first flowering had positive (indirect) effect on total yield via average fruit weight (0.069), number of fruit/plant (0.063) and days to 50% fruiting (0.010). Days to 50% flowering had positive (indirect) effect on total yield via no. of fruit/plant (0.088), average fruit weight (0.073), days to first flowering (0.033), days to 50% fruiting (0.015), no. of primary branches (0.011) and plant height (0.003) show positive (indirect) effect on total yield recorded. Similarly, plant height (cm) also had positive (indirect) effect on total yield via days to first flowering (0.007), no. of fruit/cluster (0.005) and fruit diameter (0.003). Fruit diameter (mm) had positive (indirect) effect on total yield per plant via average fruit weight (0.335), TSS (0.016), no. of primary branches (0.017), days to 50% fruiting (0.009), T.A. (0.007), fruit length (0.006), number of fruit/ cluster (0.003) and first flowering (0.002) show positive (indirect) effect on total yield recorded. Pericarp thickness (mm) show positive (indirect) effect on total yield via average fruit weight

Figure 2: Principal Component Analysis (PCA) Biplot of tomato.



(0.384), TSS (0.028), fruit length (0.013), T.A. (0.010), ascorbic acid (0.008), plant length (0.004) and days to first flowering (0.002) show positive (indirect) effect on total yield recorded. Total Soluble Solid had positive (indirect) effect on total yield recorded by average fruit weight (0.379), titratable acidity (0.024), days to 50% fruiting (0.012), no. of fruits/cluster (0.009), no. of secondary branches (0.005) and days to 50% flowering (0.001) positive (indirect) effect on total yield. Ascorbic acid show positive (indirect) effect on total yield via number of fruit/plant (0.023), no. of secondary branches (0.003), days to 50% flowering (0.002). Titratable acidity show positive (indirect) effect on total yield via no. of fruit/plant (0.173), average fruit weight (0.170), TSS (0.052), days to 50% fruiting (0.009), fruit length (0.007), no. of secondary branches (0.002), no. of fruit/cluster (0.001) and days to 50% flowering (0.001), which fall in line with same previous results (Namdev and Dongre 2019, Kumar *et al.*2020 and Akhter *et al.*2021).

From present investigation it may be concluded that, no. of fruit / cluster, no. of fruits/plant, and fruit diameter and weight were the major yield contributing characters which showed positive and significant association with total yield /plant and also exhibited high direct effect on total yield /plant. Therefore, due there is a need to study characters in for selection which would help in isolating high yielding genotypes from highly segregating population to improve yield potential of tomato.

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Study on horticultural traits and incidence of physiological disorders of indigenous seedling mango germplasm in central and sub-montane zones of Punjab

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Abstract

A Survey on horticultural traits and incidence of physiological disorders of seedling mango germplasm was carried out in central and sub-montane zones of Punjab, during February to October 2012 (on year) and February to October 2013 (off year). The superior genotypes of seedling mangoes to peculiar character were selected on the basis of information provided by tree owners, local inhabitants and officials from Department of Horticulture, Punjab. Wide variability was recorded for eating quality in fruits at the time of ripening indicates that the fruit storage life in different evaluated seedling mango germplasm varied from 3 to 5 days. The time of fruit maturity also differed amongst the genetic resources under evaluation. The seedling mango germplasm collected in central and sub-montane zone of Punjab were irregular, alternate and regular bearers. A wide range of variability was observed in fruit yield of collected germplasm. Thus, there is tremendous possibility of such material to act as source for further propagation as well as breeding material for hybridization to evolve new varieties with desirable traits.

Key words: *Mangifera indica, fruit quality, yield, variability,* Punjab; India introduction

INTRODUCTION:

Mango ! belongs to genus *Mangifera* under family Anacardiaceae and order Sapindales comprising of 69 species (Kostermans and Bompard, 1993), which are distributed throughout the world. Mango peel and pulp contain carotenoid pigments, vitamin C, polyphenols, omega-3 poly unsaturated fatty acids, proteins and carbohydrates along with minerals like calcium, phosphorus and iron. Fruit is utilized at all stages of its development. Young and unripe fruits are liked because of their acidic taste and are used for culinary purposes as well as for pickle, chutney and amchoor (mango powder) making. Ripe fruits are utilized in preparing squash, jam, jelly, custard powder, mango leather (Amb papad) and mango toffee. Its wood is used for making furniture, packing boxes, match boxes and also as a source of fuel. It is the national fruit of India, Pakistan and the Philippines and the national tree of Bangladesh. The heavy canopy of the seedling mango is a source of shelter and shade for both animals and humans and also good as a wind break against strong winds. No other fruit can be put to so many diversified uses in the form of processed product as the mango. Therefore, it is rightly acknowledged as the king of fruits in India. It enjoys the same popularity in the tropics as apple in the temperate regions.

Mango is grown commercially in India, Indonesia, Bangladesh, Philippines, Malaysia, Sri Lanka, Thailand, Pakistan, Egypt, Sudan, South Africa, West Indies, Hawai, Brazil and USA (Florida) etc. In India it is successfully growing in the states of Uttar Pradesh, Andhra Pradesh,

Bihar, West Bengal, Kerala, Karnataka, Punjab and Haryana. The mango is now cultivated mostly in frost-free tropical and warm subtropical climates, almost half of the world's mangoes are cultivated in India alone.

India is the largest producer of choicest table varieties of mango in the world and wide variability has been found in fruit shape, size and taste but commercial importance is given to nearly about 30 mango cultivars in the country. But still, mango industry lags far behind in the world due to low productivity, higher incidence of insect pests, diseases and physiological disorders etc. Mango is cross pollinated fruit crop and almost majority of mango cultivars under cultivation in the world are selections from open pollinated seedling populations.

MATERIAL AND METHOD The present study was conducted in central and sub-montane zones of Punjab consecutively for three years during February to October 2012 (on year) and February to October 2013 (off year) and February to October 2014 (on year). More than hundred genotypes were selected on the basis of information provided by tree owners, local inhabitants and officials from Department of Horticulture, Punjab. Out of which twenty five superior genotype of seedling Mangoes to peculiar character were selected. The selected genotypes were evaluated with the help of IPGRI, Rome 'Mango Descriptor' for various vegetative, floral, fruit maturity, disorders and storage characters etc. Thirty fruits/sample were randomly selected from all the sides of the tree

Yield/tree: The yield/tree was recorded at optimum maturity in kg for estimating yield of a particular tree, mango fruits were harvested and weighed on a physical balance and registered according to their providing marking.

Fruit maturity period: The time of maturity was dependent upon various external characters like appearance of waxy coating, dots on the fruits and relative size of the fruit, change in colour and firmness. the natural fall of mature fruit is considered to be the indication of fruit maturity in mango. All the fruit samples for physic-chemical analysis were taken at optimum maturity. The maturity period for the evaluated types was recorded and classified as :(a) Early (b) Medium (c) Late

Fruit storage life: It was recorded number of days of storage of ripe fruits under ambient conditions after harvest.

Eating quality: The combined assessment of flavour/acidity, sweetness, aroma and astringency of TSS, acidity and sugars to be made and classified as :(a) Poor (b) Good (c) Very good (d) Excellent

Bearing behaviour: The regularity of bearing was assessed during the two successive years of the research study. However the knowledge about regularity of bearing in the past was also taken from the owner and employees of the orchards and are classified as: (a) Irregular (b) Regular (c) Alternate

Horticultural traits and incidence of physiological disorders

Results with respect to various horticultural traits such as eating quality, fruit storage life, time of fruit maturity, bearing behaviour and yield per tree are documented in Table – 1. The genotypes AA-7, AA-9, AA-42 and ABG-1 had excellent taste, flavour and aroma. Genotypes AA-4, AA-16, AA-29 and AA-39 had very good and rest of the genotypes having good taste, flavour and aroma. These results lend support from findings of earlier research workers Iyer and Subramanyam (1993) and Desai *et al*. (2000). The highest keeping quality was recorded in genotypes AA-3, AA-8, AA-39 and AR-1 in which fruit remained sound condition for 5 days. Variation in fruit maturity in different seedling mango germplasm might be due to inherent genetic variation. The genotypes AA-1, AA-2, AA-3, AA-8 and AA-19 mature during 1st week of July and considered as early maturing genotypes. Late maturing genotypes which matured during 3rd and 4th week

of July were AA-7, AA-9, AA-39, AA-42, ABG-1, AGKB-64, AP-1, AUG-1 and AR-1. These findings are corroborated well with experimental outcomes of Boshra *et al*. (2007), Sharma *et al*. (2007) and Guirado *et al*. (2009). Most of the seedling mango germplasm evaluated in the central and sub-mountain zones of Punjab were found to be irregular and/or alternative bearer in nature. However, genotypes AA-101, ABG-1 and AGKB-64 showed tendency towards regular bearing. The results regarding bearing habit are in good tune with the research findings of Saha (1972) and Singh (1988). The genotype AKS-1 ranked first with yield (1400.20 kg/tree), followed by AA-39 (497.84 kg/tree), AA-29 (397.75 kg/tree) AA-19 (385.96 kg/tree), respectively, were high yielding genotypes. The minimum yield (79.50 kg/tree) was found in genotype GRB-1. The findings obtained in the present study lends support from the previous reports of Sanjay and Singh (2004) and Rao and Rao (2007), who also found high range of variation in yield per tree. The variation in the yield of evaluated seedling mango genotypes in the current investigation might be attributed to difference in growing conditions, genetic variations and cultural practices that ultimately influenced the size of the plant.

Fruits from evaluated germplasm were tested for incidence of disorders like malformation, black tip and spongy tissue. Considerable variation had been noticed with respect to occurrence of mango malformation; however none of the genotypes apparently exhibited the incidence of black tip and spongy tissue (Table – 2). The least occurrence of malformation (4.10%) was observed in genotype AR-1, it was followed by the genotypes AUG-1 (5.3%). Genotypes AP-1, AA-16, AKS-1 and AA-39 were also found comparatively less prone to mango malformation and considered as lightly affected genotypes. The variation in genotype susceptibility to mango malformation might be due to genetic makeup, season, climate and tree age. This result of the present study is in good confirmation with the earlier findings of Dhar and Gupta (1979), who recorded the incidence of malformation as low as 3.2% in mango under U.P condition. High range of variations in the occurrence of mango malformation also had been documented from investigations of Singh and Mandal (1999), Pinto *et al*. (2000) and Prakash *et al*. (2006).

CONCLUSION

From the overall outcomes of the present investigation it is evident that the germplasm with desirable traits can be proved to be good genetic material for the utilization in future breeding programmes for improving the mango varieties in Punjab. Genotypes AKS-1 along with AA-39, AA-29 and AA-19 were considered as high yielding genotypes. Amongst the evaluated germplasm AA-101, ABG-1 and AGKB-64 showed tendency towards regular bearing, where as genotypes AR-1 and AUG-1 showed the maximum resistance against mango malformation. Hence, genotypes such as AKS-1, GJB-1, GQ-1, AA-19, AA-29, AA-39, AA-42, AA-101, ABG-1, AGKB-64, AR-1 and AUG-1 can be included for future systematic breeding as well as hybridization programme of mango to inculcate and/or concentrate favourable attributes among the improved progenies.

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Table 1: Horticultural traits of evaluated genotypes.

Selection number	Collector code	Eating quality	Fruit storage life (Days)	Time of fruit maturity	Bearing behaviour	Yield/ tree (kg)
1	AP- 1	Good	3.00	4th week of July	Irregular	158.20
2	AKCA - 14	Good	5.00	2nd week of July	Alternate	149.30
3	GJB -1	Good	3.00	2nd week of July	Irregular	189.10
4	AA -101	Good	4.00	2nd week of July	Regular	179.20
5	AA-15	Good	4.00	2nd week of July	Irregular	297.10
6	AA-4	Very Good	4.00	2nd week of July	Irregular	349.50
7	AA -16	Very Good	3.00	2nd week of July	Irregular	291.40
8	AA -42	Excellent	5.00	4th week of July	Irregular	247.30
9	GRB-1	Good	3.00	2nd week of July	Irregular	79.50
10	AA-3	Good	5.00	1sr week of July	Irregular	345.15
11	GQ-3	Good	4.00	2nd week of July	Irregular	179.20
12	AA-20	Good	4.00	2nd week of July	Irregular	296.10
13	AUG.1	Good	3.00	4th week of July	Alternate	195.20
14	AA-9	Excellent	4.00	3rd week of July	Irregular	238.72
15	ABG-1	Excellent	4.00	3rd week of July	Regular	168.40
16	AGKB-64	Good	4.00	4th week of July	Regular	169.10
17	AA-29	Very Good	3.00	2nd week of July	Irregular	397.75
18	AA-39	Very Good	5.00	3rd week of July	Irregular	497.84
19	AA-2	Good	4.00	1st week of July	Irregular	349.41
20	AR-1	Good	5.00	4th week of July	Irregular	128.20
21	AA-8	Good	5.00	1st week of July	Irregular	287.76

Selection number	Collector code	Eating quality	Fruit storage life (Days)	Time of fruit maturity	Bearing behaviour	Yield/ tree (kg)
22	AA-1	Good	4.00	1st week of July	Irregular	285.88
23	AA-7	Excellent	4.00	3rd week of July	Irregular	270.92
24	AKS-1	Good	4.00	2nd week of July	Alternate	1400.20
25	AA-19	Good	3.00	1st week of July	Irregular	385.96
Range		**--**	**3.00-5.00**	**--**	**--**	**79.50-1400.20**
Mean		**--**	**3.96**	**--**	**--**	**301.41**
S. Em. (±)		**--**	**0.077**	**--**	**--**	**3.899**
C.D. at 5%		**--**	**0.156**	**--**	**--**	**7.840**

Table 2: Incidence of physiological disorders and mango malformation.

Selection number	Collector code	Malformation (%)	Black tip of mango	Spongy tissue
1	AP- 1	6.20	Absent	Absent
2	AKCA – 14	17.20	Absent	Absent
3	GJB -1	27.10	Absent	Absent
4	AA -101	11.20	Absent	Absent
5	AA-15	10.10	Absent	Absent
6	AA-4	13.40	Absent	Absent
7	AA -16	6.30	Absent	Absent
8	AA -42	9.10	Absent	Absent
9	GRB-1	25.20	Absent	Absent
10	AA-3	12.30	Absent	Absent
11	GQ-3	22.30	Absent	Absent
12	AA-20	9.40	Absent	Absent
13	AUG.1	5.30	Absent	Absent
14	AA-9	9.30	Absent	Absent
15	ABG-1	14.10	Absent	Absent
16	AGKB-64	18.10	Absent	Absent
17	AA-29	8.50	Absent	Absent
18	AA-39	8.20	Absent	Absent
19	AA-2	14.30	Absent	Absent
20	AR-1	4.10	Absent	Absent
21	AA-8	15.10	Absent	Absent
22	AA-1	16.40	Absent	Absent
23	AA-7	14.50	Absent	Absent
24	AKS-1	7.60	Absent	Absent
25	AA-19	8.30	Absent	Absent
Range		**4.10-27.10**	**--**	**--**
Mean		**12.54**	**--**	**--**
S. Em. (±)		**0.046**	**--**	**--**
C.D. at 5%		**0.093**	**--**	**--**

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Screening of plant growth promoting attributes and extracelluar hydrolytic enzyme production by soil dwelling fungi

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Abstract

Plant growth promoting (PGP) activities are symbiotic relationship of plant and microorganisms where microbes produce bioactive molecules which help in plant growth, and in return plant gives nutrition and habitat. In the present study, 25 fungal cultures were isolated from agricultural soil. The screening for PGP and extracellular enzyme production revealed two fungal cultures MA2 and TV1 as promising candidates. The fungal isolates were identified by morphological and cultural characteristics. The molecular level identification showed the isolate MA2 as *Aspergillus ochraceus* and TV1 as *Trichoderma yunnanens.* The *in vitro* screening of PGP activity by both the fungal isolates demonstrated positive results for the production of siderophore, ammonia, indole acetic acid (IAA) and gibberellic acid. The isolates were found to be incapable of phosphate solubilization. The extracellular enzyme production by fungal isolates was carried out on solid media incorporated with specific substrates. The isolates displayed amylase, lipase, protease, cellulase and chitinase production capacity.Therefore, the fungal isolates *Aspergillus ochraceus* and *Trichoderma yunnanens* can be explored as potential candidates for the preparation of plant growth promoting and enzyme formulations for agriculture and industrial application.

Key words: Screening, plant growth promotion, *Aspergillus ochraceus*, *Trichoderma yunnanens*, extracelluar enzymes

INTRODUCTION:

The microorganisms are considered as an integral part of agriculture and industry as the preparation of several economic products is possible due to diverse metabolic activities of the microorganisms. Fungi represent an important soil dwelling microorganisms owing to their dynamic growth requirements and excellent capacity to adopt with adverse or unfavorable conditions.They produce an array of extracellular enzymes which mediate breakdown and decomposition of complex molecules into simpler one, and thereby enhancing soil quality and fertility (Zifcakova *et al*., 2016). Based on their roles, soil fungi can be classified as biocontrol agents, ecosystem regulators, and decomposing and transforming agents (Frac *et al*., 2018). The interaction of fungi with plants can be beneficial (mycorrhizal symbiosis) or harmful (fungal diseases). For nutrient acquisition, both symbiotic and most pathogenic fungi penetrate their host plants without breaking the cellular plasma membrane (Chanclud and Morel, 2016).

Successful plant growth is the result of a favorable combination of several abiotic factors and microbial interactions. Rhizosphere, the region around the plant root, is comparatively rich in nutrients; around 40% of plant photosynthesis products are released from the roots and accumulate in this region (Bais *et al*., 2006). The rhizosphere is the site where a large number of diverse groups of microorganisms show intense metabolic activity. Moreover, it provides an ideal habitat for the microbial populations exhibiting positive, neutral or detrimental effects on plant growth. Plant growth promoting fungi (PGPF) are a heterogenous group of nonpathogenic fungi (true fungi and oomycetes) that are associated with plant roots and provoke enhancement in plant growth or health (Bent, 2006). Mostly, the PGPF includes nonsymbiotic

and saprotrophic free living fungi present in rhizosphere soil or on the plant root surface. Plant growth promoting and biocontrol activities of several members of genera *Aspergillus*, *Fusarium*, *Penicillium*, *Phoma* and *Trichoderma* are frequently reported (Brazhnikova et al., 2021). Arbuscular mycorrhizal fungi (AMF) belonging to *Glomeromycotina* phylum have been considered as one of the most important components of the soil ecosystem, as they possess several beneficial characteristics for plant growth in abiotic and biotic stress conditions (Wagg et al., 2019; Yu et al., 2022). *Aspergillus* species among the rhizosphere organisms produce a variety of secondary metabolites which promote plant growth and protect against several soil borne plant pathogens (Akhtar and Siddiqui 2006). *Aspergillus fumigates* (Pradhan and Sukla, 2006) and *Aspergillus* sp. (Rashid *et al.*, 2004) are known for their capability to solubilize insoluble phosphate and make it available to plants. Another candidate is the *Trichoderma* spp., which are free-living fungus commonly found in soil, rhizosphere and foliar environments. This fungus has an excellent plant growth promoting attributes as well as induction of systemic resistance in plants (Yedidia et al. 1999). Different species of *Trichoderma* are widely utilized as biocontrol agents in agriculture. Apart from that, fungi are also known for the production of certain compounds having similarity with plant hormones, such as auxins, gibberellic acids, cytokinins, jasmonic acid, ethylene, salicylic acid and abscisic acid. The role of these hormones in overall plant growth and development as well as in triggering important plant signaling events during biotic and abiotic stresses is well documented (Chanclud and Morel, 2016). The extracellular enzymes produced by fungi have an important role in nutrient acquisition by the plants and defense against plant pathogens.

Present study deals with the isolation and identification of soil dwelling fungi. The *in vitro* screening of plant growth promoting attributes as well as extracellular enzymes production by two fungal isolates *Aspergillus ochraceus* and *Trichoderma yunnanens* is included in this study.

2. Material and Methods:

2.1 Collection of soil sample and isolation of fungal cultures

The soil samples were collected from agricultural farms (21˚59'11.9"N 70˚46'32.7"E) located in Gondal city, District Rajkot, Gujarat. All the soil samples were collected in sterile plastic bags and transferred to the laboratory for further processing. The soil samples were serially diluted with sterile distilled water and plated on potato dextrose agar (PDA) plates with the help of sterile glass spreader. The plates were incubated at 28± 2° C for 4-5 days. The growth of fungi was observed in terms of characteristic colony morphology. The pure cultures of fungi were transferred to PDA slants and stored at 4° C.

2.2 Identification of fungal isolates

The fungal isolates were identified by colony morphology, texture, color and reverse pigmentation. The microscopic analysis was done for the identification of mycelia type and spore arrangements. The molecular level identification was performed by 18S rRNA ITS gene sequencing from GeneXplore Diagnostics & Research Center Pvt. Ltd., Ahmedabad, Gujrat.

2.3 Screening of plant growth promoting attributes of fungal isolates

2.3.1 Phosphate solubilization

Phosphate solubilization capacity of fungal isolates were screened on the Pikovskaya's medium containing (g/l): glucose, 10; yeast extract, 0.5; $Ca_3(PO4)$, 5; $(NH_4)_2SO_4$, 0.5; KCl, 0.2; $MgSO_4 \cdot 7H_2O$, 0.1; $MnSO_4 \cdot H_2O$, 0.001; $FeSO_4 \cdot 7H_2O$, 0.001; agar-agar, 15; pH, 7.0±0.2. The isolates were spot inoculated on the medium and incubate at 28±2 °C for seven days. Clear zone around fungal growth indicated phosphate solubilization.

2.3.2 Siderophore production

Blue agar CAS (chrome azurol S) assay was carried out to test siderophore production as described by Schwyne and Neilands (1987). The basic principle of this test is that when a strong ligand L (siderophore) is added to a colored dye-Fe^{+3} complex, the color changes due to free dye release as the ligand- Fe^{+3} complex is formed. The fungal isolates were spot inoculated on a PDA plate containing CAS and $FeCl_3$. The plates were incubated at 28±2°C for seven days. Orange halo around fungal growth indicated siderophore production.

2.3.3 Ammonia production:

The fungal isolates were inoculated into the test tubes containing 2% peptone water and incubated at 28± 2°C for 3 days. After incubation, 0.5 ml Nessler's reagent was added into each tube. Development of brown to yellow color indicated positive test for ammonia production.

2.3.4 Indole acetic acid (IAA) production:

The potato dextrose broth supplemented with L-Tryptophan (1 µg/ml) was inoculated with fungal isolates and incubated at 28± 2°C for three days. After incubation, the tubes were centrifuged at 10,000 rpm for 10 min. The clear supernatant (1 ml) was mixed with 2 ml of Salkowski's reagent and incubated for 15 min at room temperature. Development of pink color indicated IAA production by the isolates.

2.3.5 Gibberellic acid production:

The screening of gibberellic acid production was carried out using peptone-glucose Czepak's medium. The fungal isolates were inoculated into the medium and incubated at 28±2°C for seven days. The culture broth was centrifuged at 10,000 rpm for 10 min and clear supernatant was separated. The pH of the supernatant was adjusted to 2.5 using 1 M HCl and the extraction of gibberellic acid was done by adding equal volume of ethyl acetate to the culture supernatant. After 1 h, the aqueous phase was discarded and an ethyl acetate layer was collected and evaporated at 65°C in a rotary evaporator. The residues were dissolved in a small volume of acetone and spotted on the TLC plate. The mobile phase used was isopropanol-ammonia-water (10:1:1). The plates were sprayed with developing reagent containing 50 mg $FeCl_3$ and 3% H_2SO_4 in methanol, and incubated at 80°C for 10 min. The TLC plates were observed under UV light. Greenish fluorescence indicated the presence of gibberellic acid.

2.4 Screening of extracellular hydrolytic enzymes production

2.4.1 Amylase production:

The production of amylase by the fungal isolates was evaluated by streaking the isolates on nutrient agar plates containing 2% starch. All the plates were incubated at 28± 2°C for five days. After the incubation, the plates were flooded with gram's iodine solution. The formation of clear zones around fungal growth indicated amylase production.

2.4.2 Cellulase production:

The fungal isolates were streaked on a minimal agar medium containing 5% carboxymethyl cellulose (CMC). The plates were incubated at 28± 2°C for five days. Growth of the isolates indicated hydrolysis of CMC by the production of cellulase.

2.4.3 Chitinase production:

The screening of fungal isolates for chitinase production was performed on the solid medium containing 2% chitin and 0.5% peptone. The fungal isolates were streaked on the medium and incubated at 28± 2°C for five days. After incubation, the formation of clear zones around fungal growth indicated the production of chitinase.

2.4.4 Lipase production:

The lipase production capacity of the fungal isolates was checked on tributyrin agar plate. The fungal isolates were streaked on the plates and incubated at 28± 2°C for five days. After incubation, the formation of clear zones around fungal growth indicated the production of lipase by the isolates.

2.4.5 Protease production:

The production of protease by the fungal isolates was screened on solid medium containing 3% skimmed milk powder and 1% glucose. The isolates were inoculated on the plates and incubated at 28± 2°C for five days. The formation of a clear zone around fungal growth was indicated as a positive result for protease production.

3. Results and Discussion:

3.1 Isolation and identification of fungal isolates:

The serial dilution of soil samples and standard plate method resulted in isolation of 25 fungal cultures. The growth of the isolates on PDA indicated formation of white mycelium during the initial phase. The colored spores developed in later stages of fungal growth. The growth of the isolate MA2 was granular mycelial mat with brown colored pigmentation (Fig. 1A). The microscopic observation of the isolate MA2 revealed septate mycelia containing fungal

nuclei. The fruiting body contained a large spherical vesicle. The phialides were arranged on the vesicle in the circular pattern. The conidiospores were produced from the phialides in the form of long chains (Fig. 1B). The growth of the isolates TV1 was in the form of white mycelial mat on the PDA plate. The formation of conidiospores was evident in the form of loose and fluffy greenish colored growth with distinct concentric rings (Fig. 1A). Under microscope, the septate mycelium with lateral branches was observed. These branches were further divided at an angle of 90° to form secondary branches. These branches contained clusters of phialides. The phialides were enlarged in the middle and the tip containing conidiospores (Fig. 1B). The 18S rRNA sequence was used to carry out BLAST with the database of NCBI GenBank. Based on the maximum identity score, first fifteen sequences were selected and aligned using multiple sequence alignment software programs. The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 1.19439419 (MA2) and 1.08478630 (TV1) is shown (Next to the branches). The evolutionary distances were computed using the p-distance method and are in the units of the number of base differences per site. The analysis involved 16 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 428 (MA2) and 485 (TV1) positions in the final dataset. Evolutionary analysis was conducted in MEGA7. Based on molecular data the isolates MA2 and TV1 were identified as *Aspergillus ochraceus* and *Trichoderma yunnanens* respectively (Fig. 2).

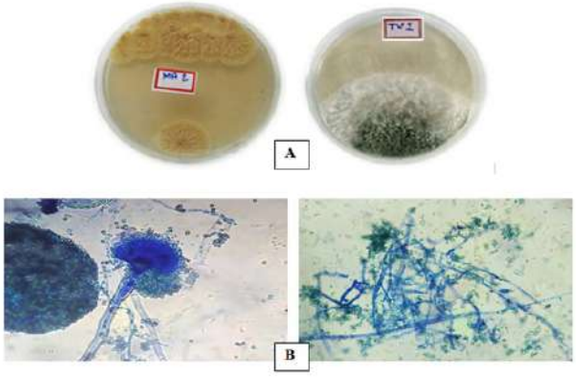


Figure 1: Growth and morphological characteristics of fungal isolates MA2 and TV1 on PDA medium (A), and typical characteristics of fungal isolates MA2 and TV1 under 40X objective of microscope (B)

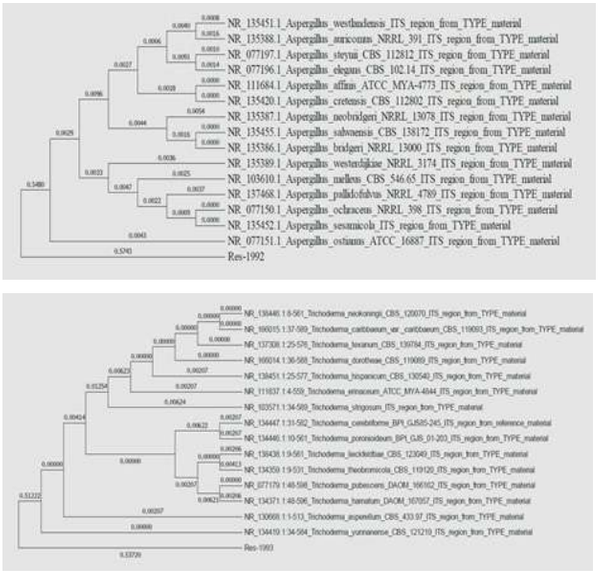


Figure 2: Phylogenetic analysis of ITS gene sequence displaying the place of fungal isolates MA2 (A) and TV1 (B) and its relationship among other species of related genera

3.2 Evaluation of plant growth promoting activities of fungal isolates

The isolates *Aspergillus ochraceus* and *Trichoderma yunnanens* exhibited orange halo around their growth indicating siderophore production. However, the isolate *Aspergillus ochraceus* showed an excellent siderophore production capacity on CAS agar plate in terms of larger size halo (Fig. 3A). Siderophores are low molecular weight compounds produced from microorganisms and some plants. They offer an excellent iron chelating capacity; their production is the part of strategy to obtain iron from the environment containing low amounts of available iron (Ferreira *et al.*, 2019). The biosurfactant producing fungus *Xylaria regalis* was able to produce siderophores which belonged to hydroxamate-type (Adnan *et al.,* 2018). In another study plant growth promoting endophytic fungus *Asprgillus fumigatus* TS1 exhibited siderophore production on CAS agar plate (Bilal *et al*., 2018).

The screening of IAA production was carried out by mixing culture supernatents with Salkowski's reagent. Positive result was indicated by the formation of pink. Both the fungal

isolates *Aspergillus ochraceus* and *Trichoderma yunnanens* exhibited IAA production capacity (Fig. 3B). It is well known that IAA regulates several physiological processes in the plant such as cell growth, tissue differentiation and tactic response. Therefore, IAA production by our fungal isolates adds an advantage of promoting plant growth. The IAA production capacity was found in *Xylaria regalis* (Adnan *et al*., 2018), *Aspergillus flavus* CSH1 (Asaf *et al*., 2018) and *Asprgillus fumigatus* TS1and *Fusarium proliferatum* BRL1 (Bilal *et al*., 2018).

The TLC plates observed under UV light exhibited greenish fluorescence in both the fungal isolates indicating gibberellic acid production capacity (Fig. 3C). Similar to IAA, gibberellic acid also has several developmental and regulatory roles in the plant such as germination of seed and development of seedling, growth of stem and leaf, floral initiation, and flower and fruit growth (Davies 2010; Pharis and King 1985). Moreover, it also helps in the plant root growth and root hair development, inhibition of floral bud differentiation, vegetative and reproductive bud dormancy as well as delay of senescence (Bottini and Luna 1993; Fulchieri *et al*. 1993; Reinoso *et al*. 2002; Tanimoto 1987). Two endophytic fungi, *Asprgillus fumigatus* TS1 and *Fusarium proliferatum* BRL1 isolated from the roots of *Oxalis corniculata* exhibited gibberellic acid production capacity (Bilal *et al.*, 2018). The fungal culture *Aspergillus niger* CSR3 isolated from *Cannabis* sativa exhibited multiple PGP traits like production of siderophores, indole acetic acid (IAA), gibberellins, and phosphate solubilization (Lubna *et al*., 2018).

The culture supernatents of both fungal isolates *Aspergillus ochraceus* and *Trichoderma yunnanens* reacts with Nessler's reagent and produced brownish yellow color indicating ammonia production. The production of ammonia is one of the important features of PGPR microorganisms for enhanced plant growth. In general, ammonia produced by such organisms serve as nitrogen source, which has enhancing effect on plant root and shoot elongation, and biomass production (Marquese *et al*., 2010).

The ability of fungal isolates to solubilize phosphate was evaluated on Pikovskaya's agar medium. In our study, none of the two fungal isolates showed phosphate solubilization as there was no evidence of a clear zone around their growth. The organic acids produced by fungi are responsible for the solubilization of calcium phosphate and liberation of phosphate, which is further utilized for the fungal growth. Solubilization and mineralization are main microbial mechanisms of releasing phosphate from both organic and inorganic phosphate pools in the soil (Bhattacharya and Jha, 2012). The fungi belonging to genera *Aspergillus* and *Penicillium* are principal microorganisms to carry out phosphate solubilization (Nelofer *et al*., 2016). Pradhan and Sukla (2006) reported that *Aspergillus fumigatus* and *Penicillium* sp. isolated from the rice field soil had good phosphate solubilization potential.

The rhizospheric fungi are least explored for their PGP potential as compared to rhizobacteria. Moreover, these fungi have no stringent host specificity, and therefore it provides an added advantage of selection and preparation of fungal inoculants for diverse crops. Therefore, our study was focused on isolation of free-living rhizospheric fungi and *in vitro* screening for their PGP potential. The role of soil fungi is not limited to PGP activity; they play vital roles in nutrient mineralization, biogeochemical cycling of the elements, decomposition of a vast variety of complex organic materials into humus, and thereby improvement of soil fertility and productivity. Organic acids produced by a number of fungi are responsible for mineral mobilization, and thus accelerates mineral uptake process by plant roots (Imran *et al*., 2021).

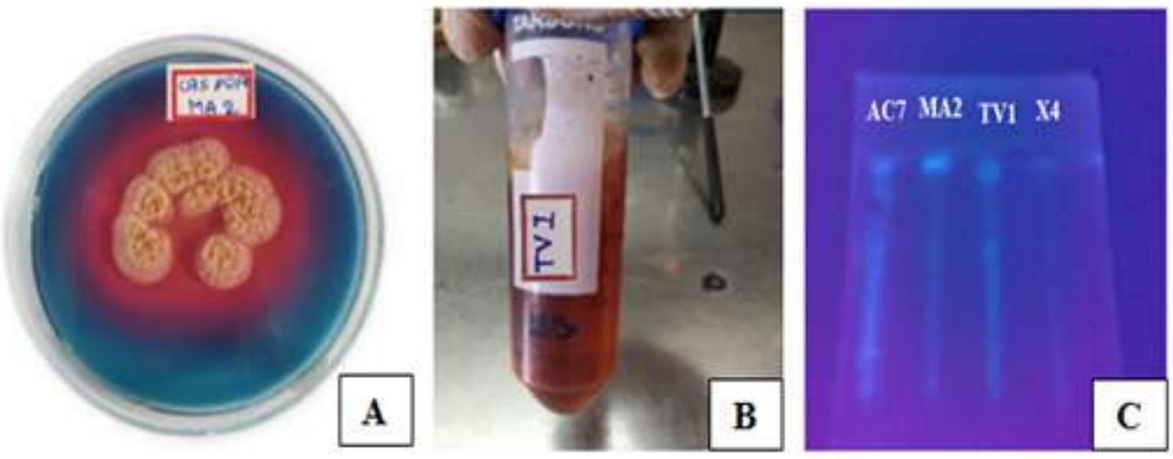


Figure 3: Plant growth promoting activities by fungal isolates: Orange halo around the growth of MA2 (*Aspergillus ochraceus)* indicating siderophore production (A), isolate TV1 (*Trichoderma yunnanens*) showing positive result for indole acetic acid (IAA) production (B) greenish fluorescence under UV light showing positive result for gibberellic acid production by both the fungal isolates (C)

3.3 Screening of extracellular hydrolytic enzymes production by fungal isolates

The production of amylase enzymes was indicated by formation of clear zones around fungal growth as a result of hydrolysis of starch. The amylase enzyme production capacity was observed with both the fungal isolates *Aspergillus ochraceus* and *Trichoderma yunnanens* (Fig. 4A). Fungal amylase is responsible for hydrolysis of starch into sugars, which can be taken up by fungi for their growth. In accordance with our findings, *Aspergillus fumigates* isolated from soil in Eastern Nigeria showed amylase production on solid media (Nwagu and Okolo 2011).

The growth and formation of clear halo on tributyrin agar plate was the indication of lipase enzyme production. In our study, the fungal isolates *Aspergillus ochraceus* and *Trichoderma yunnanens* produced lipase enzymes on solid media (Fig. 4B and 4C). Many researchers have documented lipase production capacity of fungal isolates. For instance, Takó et al., (2012) screened 35 fungal isolates for lipase production, and all the *fungal isolates* were found positive. Similarly, a strain of *Trichoderma harzianum* IDM14D isolated from soil in Turkey was found positive for extracellular lipase production (Ülker et al. 2011).

The growth of both the fungal isolates was evident on the CMC agar plate. The cellulase enzyme produced by the fungal isolates hydrolyzes CMC, and such hydrolyzed products can be further utilized for fungal growth. The fungal cellulase has an important role in nature as well as in the industry, as it degrades complex insoluble cellulose into simple sugars. For instance, in the process of bioconversion of lignocellulose into simple sugars, *Trichoderma reesei* finds its potential owing to production and secretion of large quantities of cellulase-rich secretome (Berrin *et al*., 2014). Reddy *et al*., (2014) screened 23 fungal isolates for cellulase production; nine fungal isolates displayed positive results for cellulase production.

The screening of extracellular chitinase production showed positive results with both the fungal isolates *Aspergillus ochraceus* and *Trichoderma yunnanens*. The chitinase production was indicated by the formation of clear zones around fungal growth due to hydrolysis of chitin. The production of chitinase has a protective role, as it acts and hydrolyses the cell wall of plant pathogenic fungi (Castillo et al. 2016, Suman *et al*. 2016). Our results are in accordance with previous study, wherein seven fungal isolates isolated from Egyptian soil were positive for chitinase production (Sherief *et al.* 1991).

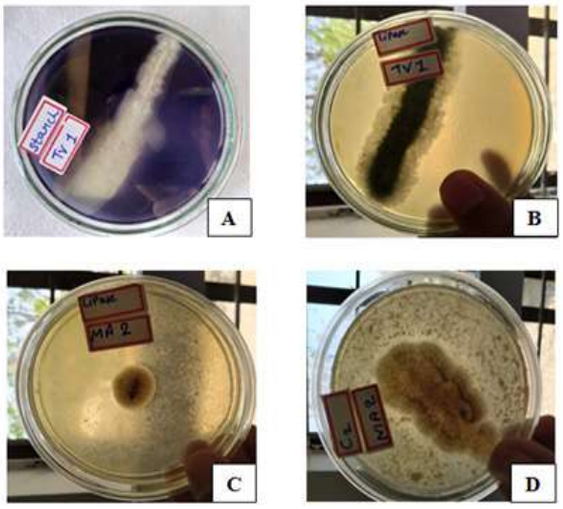


Figure 4: Screening of fungal isolates for extracellular enzyme production: Positive results of amylase (A) and lipase (B) production by TV1 (*Trichoderma yunnanens)*; lipase (C) and cellulase (D) production by MA2 (*Aspergillus ochraceus)*

The extracellular production of protease was indicated by the formation of a clear zone on skimmed milk agar plate. In our study, both the fungal isolates were found to be positive for extracellular protease production. Protease enzyme mediates proteolysis, the breakdown of larger proteins into smaller polypeptides and single amino acid residues. Such amino acids are further utilized for the synthesis of new protein products. Similar to our findings, Sharma et al., (2015) carried out screening of five fungal isolates for protease production on gelatin agar and skimmed milk agar; three isolates among five showed positive results for protease production. In another study, *Panicillium bilaiae* Pb14, *Panicillium bilaiae* C11, *Talaromyces pinophilus* T14, *Aspergillus* sp. D1 and *Beauveria bassiana* T7 displayed production of different extracellular enzymes like amylase, protease and lipase (Brazhnikova et al., 2021).

Numerous industrial as well as biotechnological applications, directly or indirectly are linked with fungal extracellular enzymes. The extracellular enzymes of fungal origin have a potential role in the decomposition and degradation of numerous organic residues in the soil and formation of humus. The degraded metabolites released in the soil serve as source of nutrients for other organisms as well as for plants (Frąc *et al*. 2018; Wösten 2019). Therefore, fungal isolates having capacity to produce large arrays of extracellular enzymes are quite promising candidates for enhancing soil fertility and thereby agricultural crop yield (Imran *et al*., 2021). In our study, soil dwelling fungal isolates *Aspergillus ochraceus* and *Trichoderma yunnanens* possess both PGP as well as extracellular enzyme production capacity, which ultimately help in the improvement of soil fertility and productivity.

4. Conclusion

In the present study, two fungal strains were isolated from agricultural soil and tested for plant growth promoting activities and extracellular hydrolytic enzymes production capacity. The isolates demonstrated positive results for the production of siderophore, ammonia, indole acetic acid (IAA) and gibberellic acid. The fungal isolates also produced extracellular hydrolytic enzymes, mainly amylase, lipase, protease, chitinase and cellulase. Therefore, the fungal isolates *Aspergillus ochraceus* and *Trichoderma yunnanens* hold potential as plant growth promoting fungi as well as source of extracellular enzymes, and can be explored for fungal bioinoculant formation.

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Effect of different carbon and nitrogen sources on growth and pathogenicity of *Rhizoctonia solani* and *Meloidygyne incognita* disease complex in tomato c.v.Pusa Ruby.

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Abstract

Studies were made to study the effect of different carbon and nitrogen sources on the pathogenicity of disease complex formed *Rhizoctonia solani* and root knot nematode *Meloidogyne incognita* on tomato cv Pusa Ruby. For this *R. solani* was cultured on different carbon and nitrogen sources. Selected carbon sources were sucrose, arabinose, glucose, fructose, rhamnose, xylose and starch. Selected nitrogen sources were KNO_3, NH_4NO_3, $(NH_4)_2SO_4$, Asparagine, Glycine, Lysine, Methionine and Urea**.** Seedlings of tomato were inoculated with *R. solani* and RKN separately and in combined inoculations. Plant growth, nematode multiplication and phenol contents of plants were observed for development of disease complex. Different sources show varied degrees of pathogenicity. Reduction in growth of plants was highest with fructose in case of carbon sources both in separate and combined inoculations. For nitrogen sources lysine has shown the most adverse effect on growth of plants in mono-pathogenic inoculations while urea and glycine in bi-pathogenic inoculations. Nematode multiplication was highest in glucose, glycine and KNO_3 for carbon and nitrogen sources respectively. A positive correlation was observed in disease complex development and phenol concentration.

Key Words. Carbon Sources, Nitrogen Sources , Growth, Pathogenicity*, Rhizoctonia solani, Meloidogyne incognita*

INTRODUCTION:

Tomato occupies a unique position among fruits! vegetables with its large number of uses. It is grown in almost all parts of the country occupying approximately 2 lakh hectares. The yield is 25 tonnes per hectare (Horticulture Statistics at a Glance, 2018) The yield is affected by losses to crop by various pests and diseases (Singh, 1987). In India, tomato productivity loss has been estimated to range from 10 to 80% (Sharma and Sharma 2005), Large number of diseases due to fungi have been reported (Kalloo, 1986). *Rhizoctonia solani* Kuhn is a widespread fungal pathogen causing pre and post emergence damping -off, root rot and stem rot in tomato. Root knot nematode, *Meloidogyne incognita* (Kofoid and White,1919), Chitwood,1949 is another pathogen causing great losses in tomato (Reddy, 1985). Both are soil borne pathogens and occupy the same niche forming a disease complex. The losses are compounded many times in combined presence of these than the two pathogens caused in mono-pathogenic conditions. It was reported that, the root rot pathogen along with a nematode could cause 38.64–56.74 percent yield loss in tomato (Kumar, D and Singh, K.P. 2006a)

Pathogenicity of soil borne pathogen is affected by their nutritional status.Carbon and nitrogen sources have been reported to alter the pathogenicity of certain pathogenic fungi (Tripathi and Grover, 1983).Hrushovetz (1957), suggested that pathogenicity of some heterokaryotic fungi is influenced by amino acid present in external medium. Keeping this in view *Rhizoctonia solani* has been grown in medium containing different carbon and nitrogen sources in separate studies and its pathogenicity has been determined in disease

complex with root knot nematode *Meloidogyne incognita.*

Material and Methods: Raising inoculum of *Rhizoctonia solani*. Isolated from the rhizosphere of tomato c.v. Pusa Ruby in a previous study (Arya and Saxena 2009). Pure culture of fungi was raised in 250 ml Erlenmeyer flasks on Richard's solution containing different carbon sources separately (Sucrose, arabinose, fructose, glucose, rhamnose, xylose and starch) keeping the amount of carbon equivalent to 50 g of sucrose. Nitrogen sources selected were (KNO_3, NH_4NO_3, $(NH_4)_2SO_4$, Asparagine, Glycine, Lysine, Methionine, and urea keeping the amount of nitrogen equivalent to 10 g of KNO_3.The flasks were sterilized at 15 lbs pressure for 15 minutes in an autoclave. After cooling these were inoculated with test fungi under aseptic conditions. The flasks were incubated at 28^0 C. After 15 days the mycelial mat was filtered through what man filter paper no. 1 and washed with sterilized distilled water. It was later blended in a blender for obtaining homogeneous suspension in sterilized distilled water in such a way so as to obtain 1g of mycelium/10 ml.

Inoculum of root knot nematode. Roots of 6-12 week old infected tomato roots were cut into 1-2 cm segments and shaken vigorously in 200 ml solution of 0.5% solution of sodium hypochlorite. (Barker, 1985) for 2 minutes. The solution was quickly transferred to 200 mesh sieve placed on 500 mesh sieve to collect freed eggs. The 80 mesh sieve containing eggs was placed under tap water and rinsed to remove residual sodium hypochlorite. The remaining portion of roots were also rinsed to collect the rest of the eggs. The eggs were transferred to 100 mesh sieves of 3 cm diameter placed in 5 cm Petri dishes containing water and incubated at 25^0C. The larvae emerged out each day, were collected and used for inoculation. A suspension of larvae was made in sterile distilled water in such a way so as to contain 1000 larvae/ml. The 1 ml suspension was poured in the four holes around the roots zone with the help of pipettes. The holes were covered with soil

Raising of Seedlings. Seeds of tomato c.v. Pusa Ruby after having been sterilized with 0.1% sodium hypochlorite and rinsed three times with sterilized distilled water. Seeds were sown in 10cm pots. The soil of pots was first sterilized in an autoclave and then infested with 5g /kg soil *of Rhizoctonia solani* and 1000 j2 larvae of *Meloidogyne incognita* separately and in combination. Un- inoculated seeds served as a check. 25 days old seedlings were transplanted in 15 cm pots containing autoclaved sterilized soil. The soil was infested with *R. solani* and root knot nematode separately and in combination.

Recording of observations. After inoculation of plants with test organisms the plants were kept in a greenhouse in a randomized manner. There were ten replicates of each treatment.

After 45 days of inoculation the plants were uprooted, and the root system was thoroughly washed with running water. Length of roots and shoots was measured. The plants were kept between blotting papers to remove excess of water before determining the fresh weight of roots and shoots. After cooling, the dry weight of plants was determined. Roots were examined for the intensity of root-knot development rated as follows.

Nematode population estimation. The soil from each pot was mixed thoroughly and a sub sample of 250 g soil was processed through a set of sieves of different meshes by following Cobb's sifting and gravity method followed by Baermann funnel technique. (Southey, 1970.)

The suspension was collected in a beaker and volume was made up to 100 ml. After stirring, 1 ml of the suspension was drawn by a pipette and transferred to a counting dish. The number of juvenile larvae was counted under stereoscopic microscope. Ten such counts were made , meaning all such counts were taken for the final population of nematodes/kg soil.

Resistance assessment*:* For all assessment methods, tomato plants were uprooted and washed clean of soil 45 days after inoculation on the basis of gall index Gall index 1 was on a scale of 0 to 5 (Taylor and Sasser, 1978).

Category	Number of galls.	Reaction
0	0 galls / root system	Immune
1	01-02 galls/ root system	Resistant
2	03-10 galls/ root system	Moderately resistant
3	11-30 galls/ root system	Moderately susceptible
4	31-100 galls/ root system	Susceptible
5	101 and above galls/ root system	Highly susceptible

Phenols estimation. Total phenols and ortho dihydroxy phenols contents of culture filtrate were also estimated. Phenols were extracted by the methods of Biehn *et al.* (1968) and estimated by using Folin Ciocalteu reagent (1954). Ortho dihydroxy phenols were estimated by the method of Johnson and Shaal(1952) using Arnow's reagent. P Cresol and catechol were used to prepare the standard curves.

Result. **a**. **Growth of plants.** Results shown in table 1 and 2 and 3 show that the growth and phenolic contents of tomato were greatly influenced as a result of inoculation with *R. solani* grown in medium containing different carbon and nitrogen sources.

The growth of plants was most adversely affected when plants were inoculated with the fungus grown on medium containing fructose as carbon source followed by medium containing sucrose, rhamnose, starch, ribose, glucose, arabinose, and xylose as carbon sources. However when plants were inoculated with the nematode together with fungus, the reduction in plant growth was highest when *R. solani* used in inoculum was grown in medium containing fructose and ribose followed by that grown in medium containing starch, sucrose, xylose, rhamnose, arabinose and glucose. Reduction in growth of plants is more when fungus was grown in a medium containing fructose, irrespective of the fact that plants were inoculated with the fungus alone or together with nematode. But the difference lies in the order of reduction in growth of plants in inoculation made with fungus alone or in combination with root knot nematodes.

Nitrogen sources used in the medium to grow fungus, influenced the pathogenicity of *R.solani.* The growth of plants was most adversely affected when the fungus was grown in the medium containing lysine and least affected when the fungus was grown with methionine as a nitrogen source. Among the plants inoculated with nematodes together with the fungus, the most adversely affected plants were those inoculated with fungus grown on media containing urea/ glycine and least where the nitrogen source was methionine.

Nematode multiplication. Nematode population was lesser in the plants treated with fungus and nematode together than nematode alone.

The nematode number was least affected when the fungus component was grown in the medium containing glucose and most affected when fungus was grown with arabinose. On the other hand gall formation was less in concurrent inoculation (nematode and *R. tsolani)* where the fungus was grown in the medium containing sucrose and highest number of galls was observed with glucose as carbon source.

In nitrogen sources the least number of nematodes was observed when methionine was used as nitrogen source to grow *R. solani*, while highest number with glycine. Highest number of galls was observed in plants treated with fungus grown with KNO_3 and lowest with methionine.

Phenolic contents of plants. There has been increase in phenols and ortho dihydroxy phenols contents of plants as a result of inoculation with the two pathogens separately and together. The increase in phenols was more when two pathogens present together. However, the carbon and nitrogen sources in which the fungus was grown influenced the phenolic contents of plants. For carbon sources, the phenolic contents was highest when fungus was grown in xylose and least for fructose for separate inoculations. For combined inoculations highest phenols were observed when glucose was used as carbon source and least in ribose and fructose.

For nitrogen sources highest phenolic content was observed in the plants inoculated with the

fungus containing methionine and lowest in lysine used as nitrogen sources. When plants were inoculated with two pathogens together, the phenolic content was highest with the fungus grown in methionine and least in glycine.

Discussion: The disease development due to *R.solani*either alone or with root knot nematode, has been highest when the fungus has been grown in a medium containing fructose. It is interesting to note that fructose as carbon source not only support better growth.

The pathogenicity, besides other factors , also depends on the metabolites of fungus which in turn is influenced by content of culture media (Toussoun et al , 1960).. Therefore in the present studies the changed pathogenicity could be in part due to change in metabolites of fungus brought about by different carbon and nitrogen sources. This is in conformity with Hrushovetz (1957) wherein it has been reported that amino acid in the external medium influenced the pathogenicity. The mycelium of R. solani is somewhat heterokaryotic (Ogoshi, 1987) , which could explain its behavior. These results are also in agreement with those of Weinhold et al (1969), who reported that nutritional status of the inoculum of *R. solani* plays an important role in the pathogenicity. Gangopadhyay and Wylie (1979) also reported the damage to the plants by *Macrophomina phaseoli* (Maubl) Ashby, besides inoculum level , is determined by the carbon nitrogen ratio of the medium in which it was grown. It appears that some mechanism of preference on the part of nuclei as suggested by Hrushovetz (1957) might be operating in the present case.

There appears to be a positive correlation between the damage of plants as a result of infection with root knot nematode and *R.solani* (separate or combined) and the phenol contents of plants. Phenols seem to play some role. They are already known to interfere with the respiratory mec.

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Table 1 - Effect of inoculation of seedlings of tomato cv Pusa Ruby with Rhizoctonia solani (grown in medium containing different Carbon and Nitrogen Sources separately) alone and together with root knot nematode on phenolic content of plants.

	Carbon Treatment/ Carbon Source in which fungus grown	Phenols (mg/ml)	Ortho dihydroxy phenol (mg/ml)		Nitrogen Source	Phenols (mg/ml	Ortho dihydroxy phenol (mg/ml)
Uninoculated		0.393	0.161	Uninoculated		0.357	0.123
Inoculated with nematode		0.675	0.168	Inoculated with nematode		0.413	0.131
R	Sucrose	0.431	o.171	R	KNO_3	0.524	0.172
R+N		0.712	0.211	R+N		0.673	0.231
R	Arabinose	0.607	0.189	R	NH_4NO_3	0.493	0.161
R+N		0.769	0.225	R+N		0.618	0.198
R	Fructose	0.399	0.169	R	$(NH4)_2SO_4$	0.435	0.152
R+N		0.693	0.195	R+N		0.495	0.168
R	Glucose	0.524	0.178	R	Asparagine	0.522	0.168
R+N		0.778	0.257	R+N		0.595	0.188
R	Rhamnose	0.482	0.172	R	Glycine	0.396	0.418
R+N		0.751	0.219	R+N		0.471	0.159
R	Ribose	0.524	0.178	R	Lysine	0.371	0.146
R+N		0.693	0.198	R+N		0.671	0.228
R	Xylose	0.694	0.199	R	Methionine	0.573	0.197
R+N		0.741	0.213	R+N		0.756	0.261
R	Starch	0.482	0.172	R	Urea	0.431	0.152
R+N		0.694	0.214	R+N		0.488	0.168
C.D.at 5%		0.033	0.016	C.D.at 5%		0.0365	0.0116

Each figure is mean of 10 replicates in each treatment,

R= Rhizoctonia solani,

N= Nematode (Meloidogyne incognita)

R+N= R.solani + Nematode

Table 2. Effect of inoculation of seedlings of tomato cv ,"Pusa Ruby" with Rhizoctonia solani (grown in the medium containing different carbon and nitrogen sources)

Treatment /Carbon sources in which R. solani grown		Length (cm)	Fresh wt. (g)	Dry wt. (g)	No of galls /root sys.	RKI	No of larvae/ root sys	No of ♀/ root sys	No. of larvae / kg soil	Total nematode population
Uninoculated		69.88	26.63	4.65						
Inoculated with nematode		65.13	20.95	3.83	73	4		69	1365.0	3524
R	Sucrose	51.25	24.48	3.13			2090.0	33		
R+N		44.00	14.19	2.54	39	4			880.0	2253.0
R	Arabinose	68.38	24.35	4.11			1340.0	43		
R+N		66.13	21.75	3.17	48	4			880.0	2108.5
R	Fructose	39.38	12.05	1.96			1134.5	51		
R+N		41.75	11.64	1.91	53	4			931.0	2811.0
R	Glucose	59.93	22.50	3.88			1730.0	56		
R+N		44.33	21.50	3.71	60	4			1345.0	3221.0
R	R h a m - nose	66.63	24.38	3.39			1820.0	42		
R+N		44.75	17.88	2.99	48	4			794.0	2148.0
R	Ribose	65.75	25.00	3.80			1312.0	48		
R+N		38.75	14.18	1.91	54	4			970.0	2629.5
R	Xylose	66.63	24.75	4.19			1611.5	42		
R+N		51.00	17.75	2.97	45	4			805.0	2147.5
R	Starch	51.80	23.28	3.44			1300.5	53		
R+N		47.00	15.91	1.94	56	4	1730.0		970.0	2753.0
C.D. at 5%		02.091	01.063	0.220	3.442		20.82	03.65	30.77	

S= Standard (100 percent) Each figure is a mean of ten replicates.

S/10= 010 percent Each Petri-plate containing ten egg masses for hatching

S/100= 001 Percent Each Petri-plate containing 100 larvae for mortality

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Impact of Covid-19 on Stress and Dietary Pattern Among Students, Assessed using an Online Questionnaire

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Abstract

Stress is the way that one reacts physically, mentally, and emotionally to various conditions, changes, and demands in life. High levels of stress can affect physical and mental well-being and performance. The study was community-based, carried out by urban students. The demographic profile, stress factors, and dietary habits were studied. The parameters for stress, diet, and demographic profile were assessed by using Percentage Method. The areas where the students were residing were selected by Convenient Random Sampling and Uncontrolled Instrument Distribution Random Sampling. More than half of the students were facing stress and an erratic diet pattern. The male respondents (45.00%) were able to relax more than female respondents (37.70%) during COVID-19. Female respondents (65.58%) were more tense than male respondents (56%) about their future. 40.09% of respondents consumed fried foods at home. 62% of respondents consumed green leafy vegetables. 81.96% of females were adding cumin and 77% of the males were consuming cumin. Out of the total sample, 73.87% of respondents were consuming turmeric every day. Out of the total sample, 59% were consuming milk and milk products in their diet, 62.29% females and 53% males were consuming citrus or other fruits in their diet. 45.08% females and 42% males were feeling difficulty connecting with online classes. The findings of the present study revealed the widespread prevalence of stressful symptoms and erratic changes in dietary patterns which also highlighted a need for improving the health of the community.

Keywords: Mental Wellbeing. Performance. Stressful symptoms

INTRODUCTION:

Stress is the way that one reacts physically, mentally, and emotionally to various conditions, changes, and demands in life. High levels of stress can affect physical and mental well-being and performance (Askew K,2020). With many universities suspending the classes and closing the campuses for safety reasons, students had to suddenly leave the campus, face unexpected changes in their academic and social life with greater feelings of uncertainty and dissatisfaction due to classes being held online for extended period of time and diminished opportunities for experiential learning whether within the campus or outside the campus in the form of internships. The COVID-19 lockdown seems to have prompted some to adopt a healthier diet-but conversely, there are those for whom the opposite is true. While 10% of respondents in a Dutch study said they are making healthier dietary decisions only slightly fewer i.e. 7% reported that they are actually eating less healthily in response to the crisis. Reasons for this include more temptations at home and boredom. A fifth of the participants said they had started snacking and snacking more (Bettermann E, 2020).

When the global focus has mostly been on testing, finding a cure, and preventing transmission; people are going through a myriad of psychological problems in adjusting to the current lifestyles and fear of the disease. Since there is a severe dearth of research on these issues, the researcher decided to conduct

an online survey to evaluate its psychological impact. COVID-19 pandemic has posed many challenges for all age groups and especially for the students. COVID-19 has not only affected the students mentally but also changed a lot in their lifestyle and dietary practices. With this background the present study was conducted with the following objectives:

1. To assess the demographic profile of students using an online tool.

2. To assess the level of stress among college students during COVID-19 using an online tool.

3. To assess dietary pattern and consumption of immunity boosting foods in college going students during COVID-19 using the online tool.

MATERIAL & METHODS

Sample

Selection of samples was done by using a random sampling technique (Uncontrolled Instrument Distribution Technique) which was performed by using an online survey questionnaire (tool). Uncontrolled instrument distribution is the posting of a survey on the Web/mail/What's App group for anyone to fill out.

•The sample consisted of college students in the age group of 17-40 years.

•The sample belonged to urban communities of Aligarh, Agra (lFirozabad, Etah, Debai, Mathura, Jalesar, Hathras, Motihari, Naharkatia, Kasganj, Gangiri, Tundla, Sadabad, Etah, Badaun, Barauli Rao), New Delhi, Kanpur, Indore, Vadodra.

Methodology

In the present study, the general information of the respondents like age, gender, eating habits, zone, type of family structure, COVID-19 treated as the independent variables. Whereas dietary habits and stress were considered as dependent variables. One self-constructed online survey tool entitled **"Stress and Diet Pattern Assessment Tool for students during COVID-19"** was developed using Google forms. Keeping in view the conditions and circumstances prevalent due to COVID-19 only online mode could be used for data collection of the college students. Questionnaire was prepared in three sections as Section A, Section B, and Section C. In the Section "A", questions were designed to assess the demographic profile which covered all the necessary information of respondents like age, class, gender, city, college, family type, income group, respondent's area, eating habits. In Section "B", questions were designed to assess stress during COVID-19 pandemic which covered the symptoms of stress. Section "C" included the dietary habits related questions of students during COVID-19. The three point rating scale was used in the tool and the options were Yes, No, Sometimes.

The pilot study was conducted on 10 students. The pilot study was done to assess the adequacy of the items, language difficulties, and repetition of items in the tool. Phase validity and Content validity was established for the tool.

Questionnaires were filled by the college students through an online tool. Approximately three weeks' time was devoted for the online questionnaire to be filled by the respondents.

Statistical Analysis

Each respondent was assigned a numeric code to maintain confidentiality; data was coded and tabulated. Percentage method was used to study the distribution of both dependent and independent variables. No other test method was further used.

RESULTS & DISCUSSION

A total number of the 222 college going students between 17- 40 years were the subjects of the study. Table 1 shows socio-demographic details of the sample.

Table 1: Sociodemographic Data (N=222)

Age (years)	17-20 years	63.06 %
	20-25 years	19.83%
	25-30 years	9.01%
	30-35 years	4.5%
	35-40 years	3.60%

Gender	Male	45.05%
	Female	54.95%
Family Structure	Joint	52.70%
	Nuclear	47.30%
Eating Habits	Vegetarian	80.63%
	Non-Vegetarian	12.16%
	Eggitarian	6.31%
	Vegan	0.90%
Income Group	LIG	31.98%
	MIG	62.16%
	HIG	5.86%

Stress was assessed in the study group using the online self-structured questionnaire. The following findings related to level of stress among college students during COVID-19 were observed and are tabulated in Table no.2

Table 2 - Distribution of respondents on the basis of stress among college going students during COVID-19

S. No.	Parameters	Categories	Respondents					
			Male		Female		Total	
			N	%	N	%	N	%
1.	Feel difficulty in relaxing mind	Yes	22	22.00	34	27.87	56	25.23
		No	45	45.00	46	37.70	91	40.99
		Sometimes	33	33.00	42	34.43	75	33.78
2.	Eat too much or very little	Yes	35	35.00	25	20.50	60	27.03
		No	44	44.00	50	40.98	94	42.34
		Sometimes	21	21.00	47	38.52	68	30.63
3.	Feel boredom most of the time	Yes	24	24.00	34	27.87	58	26.13
		No	52	52.00	50	40.98	102	45.95
		Sometimes	24	24.00	38	31.15	62	27.92
4.	Experience intense exhaustion	Yes	17	17.00	30	24.59	47	21.17
		No	57	57.00	54	44.26	111	50.00
		Sometimes	26	26.00	38	31.15	64	28.83
5.	Face difficulty meeting dead-lines	Yes	19	19.00	30	24.59	49	22.07
		No	63	63.00	59	48.36	122	54.96
		Sometimes	18	18.00	33	27.05	51	22.97
6.	Feel changes in sleep cycle	Yes	47	47.00	57	46.72	104	46.85
		No	38	38.00	43	35.25	81	36.49
		Sometimes	15	15.00	22	18.03	37	16.66
7.	Feel difficulty in connecting with online classes	Yes	42	42.00	55	45.08	97	43.69
		No	31	31.00	43	35.25	74	33.33
		Sometimes	27	27.00	24	19.67	51	22.98

8.	Availability of stationary material	**Yes**	45	45.00	44	36.07	89	40.09
		No	35	35.00	63	51.64	98	44.14
		Sometimes	20	20.00	15	12.29	35	15.77
9.	Feel tensed about future career	**Yes**	56	56.00	80	65.58	136	61.26
		No	29	29.00	16	13.11	45	20.27
		Sometimes	15	15.00	26	21.31	41	18.47
10.	Face day-to-day conflicts with family	**Yes**	35	35.00	39	31.97	74	33.33
		No	46	46.00	47	38.52	93	41.89
		Sometimes	19	19.00	36	29.51	55	24.78

Dietary pattern and consumption of immunity boosting foods in college going students during COVID-19 was also assessed by using the online tool. The following observations were made after statistical analysis and are tabulated in Table no.3

Table 3 - To assess dietary pattern and consumption of immunity boosting foods in college going students during COVID-19 by using the online tool

S. No.	Parameter	Categories	Respondents					
			Male		Female		Total	
			N	%	N	%	N	%
1.	Craving for junk food	**Yes**	36	36.00	27	22.13	63	28.37
		No	46	46.00	55	45.08	101	45.49
		Sometimes	18	18.00	40	32.78	58	26.12
2.	Try to cook junk food at home to satisfy craving	**Yes**	45	45.00	53	43.44	98	44.14
		No	32	32.00	29	23.77	61	27.47
		Sometimes	23	23.00	40	32.78	63	28.37
3..	Consumption of fried foods increased in home during lockdown	**Yes**	7	47.00	8	31.14	85	38.29
		No	38	38.00	51	41.80	89	40.09
		Sometimes	15	15.00	33	27.04	48	21.62
4.	Intake of balanced diet everyday	**Yes**	65	65.00	83	68.03	148	66.66
		No	24	24.00	13	10.65	37	16.66
		Sometimes	11	11.00	26	21.31	37	16.66
5.	Consume turmeric in diet everyday	**Yes**	72	72.00	92	75.40	164	73.87
		No	16	16.00	17	13.93	33	14.86
		Sometimes	13	13.00	13	10.65	26	11.71
6.	Add ginger/ginger paste in diet everyday	**Yes**	51	51.00	68	55.73	119	53.60
		No	31	31.00	28	22.95	59	26.57
		Sometimes	18	18.00	26	21.31	44	19.81
7.	Intake of any supplements like (Giloy, Vitamin –C Capsules during lockdown	**Yes**	29	29.00	29	23.77	58	26.12
		No	61	61.00	81	66.39	142	63.96
		Sometimes	10	10.00	12	9.83	22	9.90

DISCUSSION

In the present study twenty five percent respondents faced difficulty in relaxing their mind during COVID-19. COVID-19 has caused serious emotional challenges for young people; pandemic anxiety is almost three times more worrying to this age group than any other stress creating experiences pre COVID-19 (Brooks SK e tal.,2020). Thirty percent of respondents reported that they began to eat too much or very little sometimes. Stress is also considered as an important factor which tends to influence snacking and eating patterns among young individuals (Koetsier, 2020). Twenty six percent

respondents were found to have a feeling of boredom most of the time during COVID-19. Similar findings were reported that common stressors during COVID-19 included longer quarantine duration, infection fears, frustration, boredom, inadequate supplies, inadequate information, financial loss and stigma (Kolodner M, 2020). Forty four percent respondents reported that they had no availability of stationary material. In another study it was reported that low-income college students already faced many barriers to graduating. The coronavirus pandemic has multiplied them (Oaklander M, 2020)

Forty four percent respondents felt changes in their eating pattern during COVID-19. In a similar study it was reported that during the COVID-19 lockdown, the sense of hunger and satiety changed for more than half of the population: Seventeen percent of responders had less appetite, while thirty four percent of respondents had increased appetite (Renzo L.D et al.,2020). Twenty eight percent of respondents had a craving for junk food during COVID-19. Studies from across the globe have shown high rates of snacking among young people. Forty four percent respondents tried cooking junk food at home during COVID-19. Thirty eight percent respondents consumed fried food at home during COVID-19 (Bettermann E. 2020). It has also been reported that Cooking at home puts one in control of the ingredients that end up in the meal, unhealthy foods are in wide circulation yet; foods like flour, sugar, canned soups are on sale during the Pandemic in the United States (Selye, Hans.,1956). Thirty eight percent respondents consumed fried food at home during COVID-19.

In the present study fifty three percent respondents consumed ginger/ginger paste in their diet during COVID-19. Twenty five percent respondents added cinnamon in their diet during COVID-19. Thirty five percent respondents added cloves in their food during COVID-19. Twenty five percent respondents added curry leaves in food during COVID-19. Seventy nine percent respondents added cumin in their diet during COVID-19. Fifty nine percent respondents consumed milk, curd, paneer in their diet during COVID-19. In a similar study it was reported that immunity boosting foods could be good for pandemics. Apart from supporting calcium absorption and bone development, vitamin D also enhances immunity by regulating the body's antimicrobial response. Common food sources of vitamin D include mushrooms, eggs, raw milk, salmon, tuna, mackerel, sardines and whole-grain oats, paneer, curd (Sominsky and Spencer S. J. 2014).

Fifty eight respondents consumed citrus fruits /other fruits during COVID-19. Only twenty percent respondents consumed non-veg or egg in their diet during COVID-19. Foods such as beef, chicken, oysters, beans, cashews, broccoli, kale and whole-grain oats contain high amounts of iron which is needed for formation of haemoglobin (Sominsky and Spencer S. J. 2014).

There are certain limitations of this study, firstly this study was limited to only college going students. The sample size of this study was limited to only 222 college going students. The data was gathered using an online survey method. Therefore the data was collected only from those students who had access to the internet.

CONCLUSION

COVID-19 Pandemic has affected the stress and dietary patterns of t students as it is evident from the opinions of respondents. During the COVID-19, respondents were feeling changes in their sleep cycle and became more tensed about their future. Students were also facing difficulty in coping with changes in living patterns especially in connecting with online classes. During this stage of emotional and mental crises, the respondents were observed to have some changes in their diet pattern also from the results respondents felt a change in their eating patterns. The respondents also faced craving for junk foods during the ongoing period of stress and boredom which can be seen as a base for such cravings for unhealthy junk foods. A major number of college students started to consume immunity boosting foods like turmeric, cumin, cloves, curry leaves during COVID-19; they were also seen to consume the milk products and pulses in their diet.

Implications

The implications of the study will be best suited for

students, mental health professionals, parents, and dieticians to design stress management strategies and dietary management protocols in the event of online classes and pandemic situations.

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Performance Evaluation of Battery-Operated Power Weeder

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Abstract

The mechanical weed management systems hold promise toward reducing the drudging task of hand weeding. A battery-operated power weeder was evaluated in soybean crop for V-type and straight blade. The average weeding efficiency, field capacity, field efficiency, percentage plant damage, and performance index of V-type blade were found 91.42 %, %, 0.051 ha/h, 90.73 %, 2.37 % and 2496.30, whereas straight blade 86.78 %, 0.048 ha/h, 88.50 %, 3.55 %, and 1497.04 respectively at the operating speed of 2-3 km/h. The average power consumption of V-type blade and straight blade was found to be 185.6 and 262.5 W. The average draft force 269 and 391 were observed with V-type and straight blades. The performance of the V-blade was found better over the straight blade. The overall performance of the developed machine was found satisfactory for small farmers.

Keywords: Battery, power weeder, performance, evaluation, soybean

INTRODUCTION:

Manual weeding is the most efficient method of weed management, but its use has reduced due to a labour shortage and increasing labour costs. Mechanical weeding is preferred over the chemical method because weedicides are expensive and harmful to the environment (Biswas, 1984). Weeding removes weeds that consuming key-resources such as soil, water, minerals, and sunlight and affect crop yield (Yadav, and Pund, 2007). The negligence and delay in weeding activity reduce yield by 40-60 percent (Singh, G. 1988). Mechanized weeding allows farmers to reduce weeding costs, aerate the soil, and protect the environment by minimizing the use of herbicides. The shortage of farm labours during peak season reduces the crop yield (Singh, M.U., 2017). The high levels of vibration exposure in existing power weeders add to the operators' drudgery and can cause dynamic health disorders (Heidary, *et al.,* 2014).

The crop grown by small farmers and the area of this category of farms is very less which does not allow the use of large machines due to size and shape of the field (Mehta, *et al.* 2019). Traditional tools and equipment are in practice due to a lack of improved tools, which may have higher working efficiency. The mechanized weeding in small farms is not practiced well, options available for the weeding operation to increase productivity are only small tools and equipment (Alam A., 2007). The eco-friendly technology and alternate power source are the identified mechanization gap for the small farms. The power requirement can be reduced if the major load is shared with a power source which will enhance the machine performance and operator's productivity (Singh, *et al.,* 2019). The application of electric battery vehicles for light agricultural work can convert about 75% of potential chemical energy to mechanical energy (Mousazadeh, *et al.,* 2009). This technology can be used particularly for such field operations which are considered as light draft requirements such as sowing, weeding at low depth and spraying. Thus a battery-operated weeder was developed for small farms.

MATERIALS AND METHODOLOGY

The field performance evaluation of the weeder was carried out at Instructional Farm of College of Technology and Engineering, MPUAT, Udaipur during 2019-20. The battery, DC motor with speed controller, drive wheel, weeding mechanism, main frame and handle are the principal components of the developed machine. The developed weeder was operated in walk-behind mode, this enabled the operator to ease in operation during weeding operation

(Fig. 1). The battery-operated weeding machine's detailed specifications are described in Table 1.



Fig. 1. Figure showing battery- operated weeder (1) DC motor (2) motor controller (3) battery (4) drive wheel (5) weeding drum (6) weeding tools, V-type and straight blade (7) key switch (8)TPS (9) energy meter (10) battery charge-level indicator (11) brake (12) transport wheels

Power source: DC motors are suitable for agricultural operations because the motor can deliver more torque. In DC motors, rpm, current, voltage, and torque are linearly related. (Hazari *et al.,* 2014 and Singh *et al, 2019*). A brush type DC motor of 350 W and 24 V was used. The speed of the motor is controlled by using a throttle positioning sensor which supplies corresponding throttle position voltage to the motor controller. Two 12-V, 24-Ah lead-acid batteries were in series connection to get the required output voltage. A safety switch with a key lock was provided to on-off the machine during weeding operation. The current, voltage, and power consumption were measured by using an energy-meter during field operation.

Power transmission system: The chain sprocket drive was selected for transmission of power from the motor to the drive wheel shaft. The driving and driven sprockets were apart by 350 mm. To get a speed ratio of 5.61, a small sprocket with 12 teeth for the motor shaft and 48 teeth for the drive wheel shaft was provided based on the operational speed of the drive wheel system. The size of the wheel was kept larger 300 × 50 mm with pegs for getting better traction during weeding operation.

Weeding mechanism: The weedding mechanism is a combination of a drum and a tool. The weeding drum is expected to perform two functions simultaneously shear-off the soil into the small segment as well as partial weeding. The drum blade has a conical shape that shears off the soil and provides traction power for forward motion. Moreover, the weeding drum provides stability as well as maintains the uniform depth of operation. Behind the drum, a tool (V-blade/straight blade) penetrates the soil and cuts the weeds. The width of the weeding tool was less than the crop row spacing to avoid any damage to the crops while operating. The weeding tools are shown in Fig. 2.

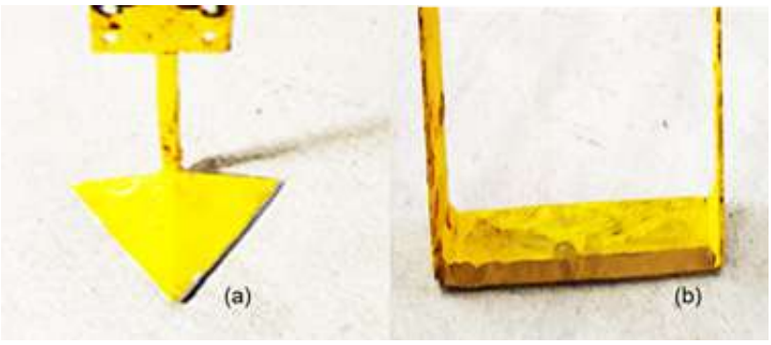


Fig. 2 Weeding blades (a) V type blade and (b) straight blade

Main frame: The main frame provides support for the DC motor, batteries, transmission system, drive wheels, weeding drum and tool, and handle. Two MS flats, each 700 × 50 × 8 mm were used to fabricate the mainframe for the machine. A T-type handle was used for the development of the weeder (Singh *et al.* 2017). The length of the handle was based on the average standing elbow height of male and female operators. So to accommodate 5 to 95% of the operator's two standard MS rectangular conduit pipes 600 × 20 mm were used to fabricate the handle. The handle's operating height can be adjusted from 95 to 105 cm from the ground. A 35 mm outer diameter, 600 mm long plastic handgrip was provided for easy grip and handling of the weeder. Handle height and length were made adjustable to the operator's comfort.

Table. 1. Specifications of battery-operated power weeder

Particulars	Details
Overall dimensions, (l × w × h), mm	765 × 250 × 950
Weight, kg	45
Size of the drive wheel (diameter × width), mm	300 × 50
Handle dimension (l × w), mm	1180 × 200
Size of T- type grip of the handle, (l × dia), mm	20 × 600
Size of weeding drum (total dia × l), mm	245 × 200
No. of blades on the drum	10
Number of weeding tool	1
Cutting width, mm	180
Maximum weeding depth, mm	35
DC motor and controller	350 W, 24 V,
Batteries	2, 12-V, 24 Ah
Energy meter	6.5-100 V, 0-2 kW
Throttle position sensor	0- 4 A, 0-24 V
Transport wheel	2

Field evaluation of machine

The field testing was carried out in soybean crops when the crop was 21-25 days old and the field was infested with the weeds. The main field was divided into six sub-plots each of size 30 m × 10 m. The experiments were laid-out in complete randomized design and three replications of each type of weeding tool. The soil and crop parameters such as soil type, soil moisture content, cone index, bulk density, row spacing, plant height, plant canopy width, weed height were measured at various locations before the experiments. The developed prototype was operated at the forward speed of 2-3

km/h. The different parameters such as power consumption, field capacity, field efficiency, weeding efficiency, percentage plant damage, and performance index were determined.

Weeding efficiency: The number of weeds before and after the weeding operations was counted and the average values were used to determine the weeding efficiency (Yadav and Pund, 2007).

$WE = (N_1 - N_2)/N_2 \times 100$

Where, WE= weeding efficiency of the weeder (%), N_1= number of weeds before weeding operation, and N_2 = the number of weeds after weeding operation.

Percentage plant damage: The percentage of damaged plants, as a quality of work done, is determined (Singh, M.U. 2017).

$D_P = [1- (Q_D/Q_P)] \times 100$

Where, D_P = percentage of plant damage, Q_D = number of plants in 10-meter row length after weeding, and Q_P = number of plants in 10 m row length before weeding.

Field capacity and efficiency: Field capacity (FC) and field efficiency (FE) were determined (Hunt, 1995 and Singh S. 2007).

$$FC = A/ (Tp+Tn)$$

$Ef = (EFC/TFC) \times 100$

Where, FC = field capacity (ha/h), TFC = theoretical field capacity, FE= field efficiency of weeder (%), Tp and Tn productive time, and non-productive time. Total working time includes effective time, time lost for turning, and minor adjustment, rest time.

Power and draft requirement: The draft force of the weeder calculated by measured power and forward speed as follows (Yadav and Pund 2007)

P (kW) = Draft force N/1000 × Speed (m/s)

Performance index: It indicates the overall performance of weeder. Field performance of weeding tools was assessed calculating the performance index as suggested by (Gupta 1981)

$PI = FC [100 – D_P] WE /P$

Where, FC= field capacity (ha/h), D_P=percentage plant damage (%), WE= weeding efficiency (%) and P = power input, W

RESULTS AND DISCUSSION

The experiment was carried out in sandy loam soil. The average soil moisture content, bulk density, and cone index were found as 15.8 % (db), 1.56 g/cc, and 439 KPa respectively. The average plant height, canopy width of plant, plant population, weed count, and weed height were recorded 156 mm, 126 mm, 69, 150, and 95 mm respectively (Table. 2). The developed prototype was operated in crop rows and maintained a safety-zone (distance between the tool edge of the weeder to the crop stem) of about 6 cm to avoid plant damage.

Table 2. Soil and crop parameters of experimental field

Parameters	Value
Crop	Soybean
Type of soil	Sandy loam
Moisture content of soil, % d.b	15.80
Bulk density, g/cc (d.b)	1.56
Cone Index, (KPa)	439
Row spacing, mm	300
No. of plants/m^2	69
Height of plants, mm	156
Plant canopy width, mm	126
Weed count/m^2	150
Weed height, mm	95

Field performance of the weeder

Based on the data, It was observed that the DC motor can take a minimum current of 1.66 A at no load, whereas the consumption of current goes up to 17.08 A during load. The output current characteristics corresponding to the power consumption showed the linear characteristics in load and no-load conditions. In no-load condition, the current drawn and power consumption recorded 1.66-3.89 A and 41.5 to 100.7 W with an average of 2.57 A and 65.29 W. The current drawn and power consumption during field operation of weeder with V-type blade varied from 4.24 to 12.42 A and 105.8 to 307.6 W, whereas with the straight blade it varied from 5.06 - 17.08 and 123.4 - 406.8 W. The average power consumption of V-type blade and straight blade was recorded at 185.62 and 262.53 W respectively. The current and power characteristics of the DC motor are presented in Fig. 3.

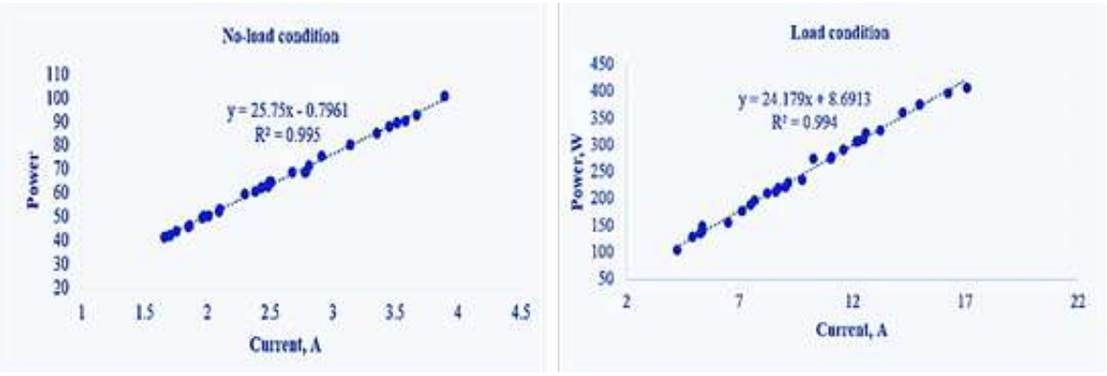


Fig. 3 Current and Power characteristics of the DC motor (No-load and load condition)

The average speed of operation was about 2-3 km/h. The variation in operating speeds were due to field and crop conditions. Weeding efficiency of weeder with V-type blade and straight blade varied from 89.39–93.95 % and 83.78- 90.46 % respectively. The variations in weeding efficiency were due to the undulating surface and the type of tool. The maximum draft force with straight and V-type blade was obtained 391.70 N and 269.50 N respectively. For V-type blades the average operating speed, depth of operation, field capacity, and field efficiency were observed as 2.81 km/h, 30 mm, and 0.051 ha/h, 90.73% respectively. The average weeding efficiency, percentage of plant damage, power consumption, and performance index was observed of 91.42 %, 2.37 %, 262.53 W, and 2496.30 respectively.

In the case of straight blades the average operating speed, depth of operation, field capacity, and field efficiency were observed as 2.66 km/h, 25 mm, 0.048 ha/h, and 88.50 respectively. The average weeding efficiency, percentage of plant damage, power consumption, and performance index was observed 86.78 %, 3.55 %, 185.6 W, and 1497.04 respectively. The weeder with V-type blade and straight blade required 19.60 and 20.83 man-hours per hectare respectively. The weeder field performance presented in Table 3.

Table 3 Field performance of weeding tools

Parameters	V- blade	Straight blade
Speed of operation, km/h	2-3	2-3
Depth of operation, mm	25-35	25-30
Actual field capacity, ha/h	0.051	0.048
Field efficiency,%	90.73	88.50
Labour requirement, man-h/ha	19.60	20.84
Weeding efficiency, %	91.42	86.78
Plant damage,%	2.37	3.55

Draft force, N	269.50	391.70
Power consumption, W	185.60	262.53
Current consumption, A	7.54	10.92
Performance Index	2496.30	1497.04
Labour requirement, man-h/ha	19.60	20.84

The weeder with V- type blade required minimum man-h as compared to the straight blade. During operation, the straight blade weeder tends to entangle with weeds which reduces its efficiency and increases the power consumption. The variations in field capacity were also reflected in-field efficiency which was due to operator, field condition, and moisture content of the soil. Due to use of a combination weeding mechanism (weeding drum and tool) , the possibility of weeds escaping in between the row is reduced and the weeding efficiency increases. The weeder with a V-type blade required minimum power input than a straight blade. However, the maximum performance index was observed for the V-type blade. The weeding efficiency and field capacity of this weeder was found better than the existing wheel hoe 0.022 ha/h, and 79.72% (Singh M.U., 2017 and Singh *et al.* 2016). The higher capacity of the machine due to the higher forward speed and less workload on the operator. The weeder operation in soybean field shown in Fig. 4



Fig 4 : Weeding operation by developed weeder in soybean crop

Battery run time: The battery run time decreased with an increase of current consumption 4-17 A at this load, the power requirement varied from 105 to 406 W. The developed weeding machine consumed 1.6 - 3.7 A during no-load condition while in weeding operation the average current required was 7-10 A. This will allow the developed prototype to run for about 2.5-3 h during weeding operation.

CONCLUSIONS

The developed battery-operated power weeder performed weeding activities with less drudgery and higher efficiency. The performance index was observed higher for V-type blades as compared to straight blades. The V-type blade gave better performance over straight blade with the weeding efficiency of 91.42% and field capacity of 0.051 ha/h at the speed of 2.88 km/h respectively. The average power and current requirement of weeder was obtained 185.60 W and 7.54 A. The man-h requirement of developed weeder is three times less as compared to existing wheel hoe. Weeding efficiency increases as the machine's forward speed increases. The tool type of weeder has a significant effect on the weeding efficiency, field capacity and power consumption. The higher forward speed of the machine is the key explanation for its better performance, as it reduces the operator's workload and minimises turning losses during the weeding operation. For the small size farms, the developed weeding machine found an appropriate technology.

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First report of root-knot nematode (*Meloidogyne incognita*) infecting Tuberose (*Polianthes tuberose L.*) in Gujarat

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SHORT COMMUNICATION

Plant parasitic nematodes are important biotic restrictions in the horticultural systems. Root-knot nematodes are a major economic pest in various crops. Tuberose (*Polianthes tuberose L.*) is an important ornamental flowring crop in India and it has great economic value as a cut flower and in the perfume industry. It is grown in the states of Karnataka, Tamil Nadu, West Bengal, Maharashtra, Odisha and Uttar Pradesh. Recently, during daily routine field visit it was observed that growth of some tuberose plants was reduced, having yellowing of leaves with reduced length of spikes. When such plants were pulled out along with root system, galls were noticed. Such galls were dissected, active juveniles and different stages of female of root-knot nematode were observed. The specific identity of the nematode was determined by cutting perineal patterns of the females. On the basis of perineal pattern and other characters such as stylet length, head shape and larval length (Eisenback, 1985), the nematode was identified as *Meloidogyne incognita*. This is a first report of *Meloidogyne incognita* infecting *P. tuberose* in Gujarat.

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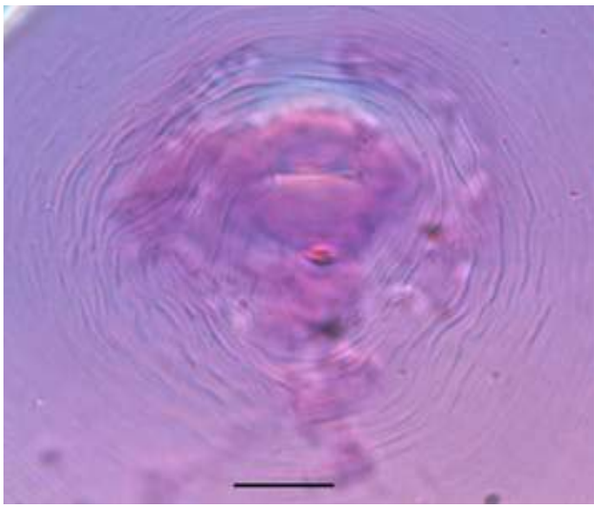
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