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CONTENTS

PART I: PLANT SCIENCES

PART II: HOME SCIENCE

PART III: VETERINARY SCIENCES

PART IV: SOCIAL SCIENCES

PART V: RESEARCH NOTES

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J. Res. ANGRAU 52 (1) 01-08, 2024

ASSOCIATION AND DIVERGENCE STUDIES FOR YIELD AND ITS ATTRIBUTING TRAITS IN UPLAND COTTON

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ABSTRACT

The study was executed during *Kharif,* 2022 to evaluate the character association and genetic variability of yield and its associated traits in cotton. Correlation studies revealed that seed cotton yield exhibited a substantial positive kinship with the number of bolls per plant, the number of sympodial branches per plant, seed index, ginning out turn (GOT), and lint yield.The first three principal components (PCs) out of the 12 PCs displayed eigen values more than one and exhibited maximum share to total variability. The attributes that contributed a maximum portion to the total divergence included the number of sympodia per plant, lint yield, seed cotton yield, ginning outturn percent, and number of bolls per plant. The traits, number of bolls per plant, number of sympodia per plant, seed index, ginning out turn, and lint yield can be utilized for direct selection for further heterosis breeding. Based on D^2 analysis the lines, L 2251 and L 2252 were found divergent and may be used in further heterosis breeding programs.

Keywords: Correlation, D^2 analysis, Principal Component Analysis, Upland Cotton

INTRODUCTION

Cotton (*Gossypium hirsutum* L.) Mexican cotton or upland cotton is a unique commercial and largely planted crop species with approximately 90 percent of global cotton production. India occupies the first position in the cotton area (37% of the world's acreage) and the largest cotton producer (22% of world cotton production) (Cotton Corporation of India, 2023). However, productivity has stagnated nearly at 447 kg ha^{-1} over the past decades. Hence, there is a greater need to enhance productivity by focusing on a selection of high-yielding divergent cultivars based on the genetic relationship (correlation) and divergence studies of yield with its attributing traits under upland conditions. The principal component analysis (PCA) is the apt statistical method to divide total variation that eases the selection of elite parental lines. It also manifests the importance of the main contributors to total variability. The informationon divergence provided by PCA is essential to select genetically and agronomically important genotypes (Isong *et al.,* 2017). The main objective of this study was to understand the character association and the genetic divergence in seed cotton yield and its attributing traits in cotton genotypes.

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MATERIALS AND METHODS

The experiment was executed with the experimental material of 11 elite cotton lines developed from pedigree breeding method along with two commercial checks during *Kharif*, 2022-2023 season in randomized block design and replicated thrice at the Regional Agricultural Research Station (RARS), Lam, Guntur. Each genotype was sown in four rows in each replication with a spacing of 105 cm x 60 cm with a row length of 6 m.The recommended agronomic practices were followed during the crop season. Five random plants / genotype in each replication were selected and data was recorded on 10 yield attributing character namely days to fifty percent flowering (number of days), plant height (cm), number of sympodia per plant, number of monopodia per plant, number of bolls per plant, seed index (g), lint index (g), ginning out turn (%), lint yield (kg ha^{-1}) and seed cotton yield (kg ha^{-1}).The genotypic correlations, Principal Component Analysis (PCA) and D^2 analysis were performed using the statistical package XL Stat and R studio version 1.4.1717 © 2009-21.

RESULTS AND DISCUSSION

Correlation analysis

The results on genotypic correlation (Table 1) exhibited that the traits *viz.,* number of sympodia/ plant*,* (r=0.336), number of bolls per plant (r=0.464), number of seed index (r=0.373), ginning out turn (GOT) (r=0.367) and lint yield (r=0.871) a substantial positive significant correlation with seed cotton yield (kg ha^{-1}) while, plant height manifested significant negative correlation (r=-0.374). Similar findings were stated by Jangid *et al.* (2022), Memon *et al*. (2022**)**, Amein *et al.* (2020), Satish *et al.* (2020). Whereas, the number of bolls per plant exhibited a significant positive association with number of sympodia per plant, number of bolls per plant, GOT (%) and lint index as reported by Kumbhar *et al.* (2020), Jarwar *et al.* (2019), Manonmani *et al.* (2019) and Chaudhari *et al.* (2017).

Not only knowing the relationship between seed cotton yield and its associated characteristics, it is also very essential to understand the kinship among yield attributing traits as well, as these characteristics have a high influence on yield. The number of sympodia per plant showed a significant positive correlation with number of bolls per plant, the seed index, GOT (%), lint yield and seed yield, whereas, number of bolls per plants showed a significant positive correlation with number of sympodial branches per plant, seed index, GOT (%), lint yield and seed cotton yield. Seed index manifested significant positive correlation with number of sympodia per plant, number of bolls per plant, GOT (%), lint yieldand seed cotton yield while the trait lint index exhibited a positive association with GOT (%) (r=0.325) and lint yield (r=0.374). Similar reports of a positive association of number of sympodia/plant with number of bolls/ plant, GOT% with seed index, lint index, staple length and micronaire, seed index with lint index, upper half mean length and micronaire were reported by Kumbhar *et al*. (2020).

Principal Component Analysis (PCA)

To specify the pattern of the existing variability in the germplasm and to the probability of grouping the population into components, the mean data of cotton elite lines were analysed using principal component (PC) analysis.

A total of 12 PCs were observed (Table 2), out of which three PCs exhibited an eigen value of more than one and contributed a cumulative variation of about 86.96%. Hence, the three PCs, therefore, used to explain the total variation and the grouping of the PCs, the remaining nine PCs exhibited eigen values of less than one and were less significant. The

Table1.Genotypic correlation coefficients among yield and its contributing traits in *G. hirsutum* L. genotypes

	DFF	PH	NM	NS	NB	SI	LI	GOT	LY	SCY
DFF	1	0.128	0.365*	0.206	0.305	0.053	0.007	0.194	0.080	-0.048
PH		1	-0.042	0.016	-0.002	-0.006	-0.207	-0.094	-0.299	-0.374*
NM			1	0.151	0.271	0.311	-0.172	0.086	0.086	0.044
NS				1	0.651**	0.595**	0.239	0.545**	0.508**	0.336*
NB					1	0.667**	0.302	0.587**	0.629**	0.464**
SI						1	0.281	0.483**	0.507**	0.373*
LI							1	0.325*	0.374*	0.312
GOT								1	0.774**	0.367*
LY									1	0.871**
SCY										1

** Significant at 0.01 level; * Significant at 0.05 level

DFF: Days to fifty percent flowering; PH – Plant height; NS: number of sympodia/plant; NBP: Number of bolls/plant; SI: Seed Index; GOT: Ginning Out Turn; LI: Lint Index; LY: Lint Yield and SCY: Seed Cotton Yield

contribution of variables under each PC is presented (Table 3). The PC1 manifested the highest variability of 57.73% and was most related to number of sympodia per plant (14.05), lint yield (13.93), seed cotton yield (13.85), ginning outturn percent (12.80) and number of bolls per plant (10.00). The second PC contributed a variability of 19.23% and most of the contributed traits were seed index (27.46), lint index (24.69), number of monopodia per plant (16.41), plant height (15.32) and days to 50 percent flowering (13.99) while the third PC exhibited a variability 9.98% and the traits more associated were number of monopodia per plant (31.05), days to 50 percent flowering (30.68), plant height (13.82), lint index (13.45) and seed index (5.87).

The correlation between variables and PCs is presented (Table 3). PC1 exhibited a positive correlation with all the traits except plant height. In PC2 days to 50 percent flowering, plant height, number of monopodia per plant, and lint yield while in PC3, the traits days to 50 percent flowering, number of monopodia per plant, and seed index manifested a positive correlation. Similar findings of positive correlation of principal components were noticed by Sahar *et al.* (2021); Isong *et al.* (2017).

The PC biplot (Fig.1) demonstrated genotypes and variables are superior and imposed as a vector on the plot. The gap within traits about PC-1 and PC-2 depicted the contribution of these traits in creating variation of genotypes. However, the overall biplot diagram demonstrated that cotton genotypes Rasi magic (check), L 2251, L 2241, L 2252, L 2242, NDLH 2051-1 (Check) and L 2243 identified as divergent as they are distant to the origin and among the morphological traits, number of monopodia/plant, lint index, days to 50% flowering,seed index yield, number of

Table 2. Estimates of Principal Component Analysis (PCA) in *G. hirsutum* L. genotypes

S. No.	Principal component	Eigenvalue	Percentage of variance (%)	Cumulative percentage of variance (%)
1	PC1	6.9	57.7	57.7
2	PC2	2.3	19.2	77.0
3	PC3	1.2	10.0	87.0
4	PC4	0.8	6.5	93.5
5	PC5	0.4	3.2	96.7
6	PC6	0.3	2.4	99.1
7	PC7	0.1	0.6	99.8
8	PC8	0.0	0.1	99.9
9	PC9	0.0	0.1	99.9
10	PC10	0.0	0.0	100.0
11	PC11	0.0	0.0	100.0
12	PC12	0.0	0.0	100.0

Table 3. Contribution of variables and correlation between the variables and PCs

Variables	Contribution of variables on PCs			Correlation between variables and PCs		
	PC1	PC2	PC3	PC1	PC2	PC3
DFF	1.333	13.99	30.686	0.304	0.568	0.606
PH	0.955	15.328	13.827	-0.257	0.595	-0.407
NM	0.951	16.415	31.051	0.257	0.616	0.61
NS	14.045	0.255	0.074	0.986	0.077	-0.03
NBP	10.115	0.151	0.074	0.837	-0.059	-0.03
SI	2.878	27.463	5.875	0.447	-0.796	0.265
GOT	12.803	0.053	2.237	0.942	-0.035	-0.164
LI	2.366	24.693	13.459	0.405	0.755	-0.402
LY	13.931	0.043	0.238	0.982	-0.031	-0.053
SCY	13.854	0.49	1.869	0.98	-0.106	-0.15

DFF: Days to fifty percent flowering; PH – Plant height; NS: number of sympodia/plant; NBP: Number of bolls/plant; SI: Seed Index; GOT: Ginning Out Turn; LI: Lint Index; LY: Lint Yield and SCY: Seed Cotton Yield

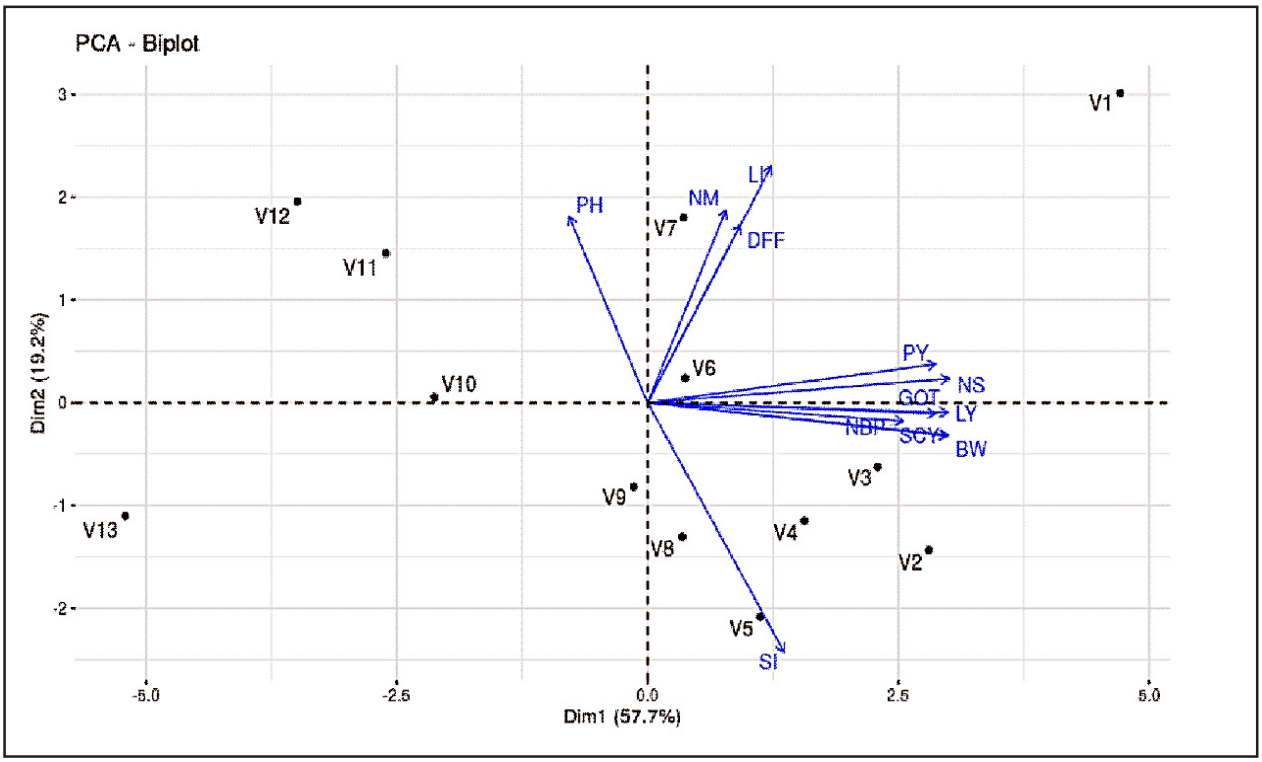

Figure 1. Biplot among PC-1 and PC-2 display the contribution of different traits in the variability of upland cotton

V1- Rasi Magic, V2- L 2251, V3-L 2241, V4 - L 2252, V5- L 2244, V6- L 2242, V7-L 2248, V8- L 2250, V9- L 2245, V10- L 2249, V11- L 2253, V12- NDLH 2051-1 and V13- L 2243; DFF: Days to fifty percent flowering; PH – Plant height; NS: Number of sympodia/plant; NBP: Number of bolls/ plant; SI: Seed Index; GOT: Ginning Out Turn; LI: Lint Index; LY: Lint Yield and SCY: Seed Cotton Yield

Table 4. Grouping of 13 genotypes of cotton in various clusters based on D^2 statistic

S. No.	CLUSTER	Genotype(s)
1	I	Rasi Magic, L 2243, L 2250
2	II	L 2245, NDLH 2051-1, L 2244
3	III	L 2242, L 2249
4	IV	L 2251
5	V	L 2248, L 2253
6	VI	L 2252
7	VII	L 2241

Table 5. Contribution of various traits towards divergence in cotton

S. No.	Character	% contribution towards the divergence
1	DFF	1.28
2	PH	5.13
3	NM	20.51
4	NS	14.10
5	NBP	11.54
6	SI	14.10
7	GOT	10.26
8	LI	10.26
9	SCY	6.41
10	LY	6.41

DFF: Days to fifty percent flowering; PH – Plant height; NS: number of sympodia/plant; NBP: Number of bolls/plant; SI: Seed Index; GOT: Ginning Out Turn; LI: Lint Index; LY: Lint Yield and SCY: Seed Cotton Yield

Table 6. Average intra and inter- cluster incidents among seven clusters in cotton

S.No.	CLUSTER	I	II	III	IV	V	VI	VII
1	I	2.09	8.40	8.40	14.92	19.20	16.98	11.00
2	II		2.28	8.03	6.39	20.79	21.57	26.22
3	III			2.34	6.47	13.99	19.60	10.69
4	IV				0.00	23.90	26.59	23.48
5	V					3.70	7.02	7.66
6	VI						0.00	19.63
7	VII							0.00

bolls/plant contributed significantly towards the diversity in germplasm.

D^2 Analysis

Based on the results obtained by D^2 statistics, the 13 genotypes were grouped into seven clusters. Cluster I and are the largest comprised of three genotypes, followed by clusters III and V (2 genotypes) and IV,VI and VII (1 genotype) (Table 4.). The contribution of various traits towards divergence in *G. hirsutum* L. Cotton presented (Table 5). It was observed that the maximum contribution towards the genetic divergence was observed in number of monopodia/plant (20.51%), number of sympodia/plant (14.10%), seed index (14.10%), number of bolls/plant

(11.54%), ginning out turn (10.26%) and lint index (10.26%). The average intra and inter-cluster incidents among seven clusters in cotton is presented in Table 6. The intra cluster distance was maximum for cluster V (3.70) followed by cluster II (2.28), cluster III (3.34), cluster I (2.09) while for cluster IV,VI and VII it was zero. In cluster IV, the intra-cluster distance was maximum which indicated the presence of wide genetic diversity among the genotypes present within this cluster. The inter-cluster distance was maximum between cluster IV and VI (26.59) followed by cluster II and VII (26.22), cluster IV and V (23.90), cluster IV and VII (23.48), cluster II and VI (21.57), cluster II and V (20.79). These results envisaged that there was genetic diversity between these clusters. Hence, crosses may be attempted to obtain superior heterotic hybrids as these distant clusters are highly heterotic which inturn yield wide range of segregants and there upon direct selection may be practiced. Based on intra-and inter-cluster distances, it is desirable to make crosses between the genotypes of cluster IV (L 2251) and cluster VII (L2252), cluster II (L 2245, NDLH 2051-1, L 2244) and cluster VII (L 2252). These findings are in agreement with the reports of Satish *et al.* (2021).

CONCLUSIONS

The correlation coefficient analysis and Principal Component Analysis (PCA) revealed that direct selection can be practiced for traits such as the number of sympodia per plant, number of bolls per plant, seed index, ginning outturn, and lint yield in cotton crop as these traits exhibited a positive association with seed cotton yield with less environmental influence.The lines L 2251, L 2252, L 2245, NDLH 2051-1, L 2244 may be utilized in the crossing programme to attain superior heterotic hybrids for further development in the varietal improvement programme after analysing their combining ability.

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INFLUENCE OF ENDOPHYTIC BACTERIAL CONSORTIUM ON MAIZE RHIZOSPHERIC SOIL NUTRIENT STATUS UNDER MOISTURE STRESS CONDITIONS

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ABSTRACT

Under conditions of moisture and nutrient limitations growth of the plants is greatly reduced. The investigation was aimed to evaluate the developed endophytic bacterial consortium which is an alternative approach to minimize the use of chemical fertilizers and to mitigate stress conditions in maize under field conditions during *Rabi* of 2020-21. The available nitrogen in soil was more in T_9 (225.29 kg ha^{-1}) and T_8 (209.09 kg ha^{-1}), available phosphorous was higher in T_9 (70.34 kg ha^{-1}) and T_8 (69.92 kg ha^{-1}), available potassium is more in T_9 (390.62 kg ha^{-1}), T_8 (360.59 kg ha^{-1}). Soil enzyme activity has shown significantly higher in microbial inoculated treatments than control. Dehydrogenase activity was found higher in T_9 (58.90 ìg of TPF g^{-1} of soil day^{-1}) at flowering stage and maximum phosphatase activity was found in T_8 (94.97 µpNP g^{-1} of soil h^{-1}). This research advocates the use of endophytic microbial consortium to mitigate stress and to improve soil nutrient status which ultimately enhances the plant health and yield.

Key words: Endophytic bacteria, Moisture stress, Soil enzymes, Soil nutrients

INTRODUCTION

Literature indicates that the world population is expected to hike 8.6 billion by 2030 and 98 billion by 2050 (Union, 2017). Such an increase will involuntarily require decisive production of additional agricultural products. On the other hand Mooney *et al.* (2009) reported that climate change has become main source of creating stress for foliar growth and emerged as an alarming threat to natural ecosystems.The production of maize in India has coverage of cultivated in 9.09 M ha producing over 23.29 M tonnes with 2563 kg ha^{-1} productivity. In global production of maize India stands as fifth largest producer by contributing over 3% of production (Suganya *et al.,* 2020). Among the environmental stresses, moisture stress has concern of major impact on crop growth, productivity and quality throughout the world. By 2050 more than 50% of arable lands are expected to have antgonistic impact on crop growth because of severe drought and climate change (Vinocur and Altman, 2005). Climate change and drought poses a major effect on the stability of agricultural production. The top ten global crops, which include sugarcane,

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palm, soybean, wheat, maize and rice, have seen a significant impact on yields in many places throughout the world (Ray *et al.,* 2019).Drought stress has been reported to cause yield reductions of up to 21 - 22% in wheat and 40 - 42% in maize around the world (Daryanto *et al.,* 2016).

The cultivation of high-yield cultivars and genotypes has increased the requirement for chemical fertilizers (Souza *et al.,* 2015). To maintain proper growth, quality and yield, maize requires an adequate assured supply of Agro-chemical fertilizers. Phosphorus is one of the important and primary macronutrients which is necessary for the maintenance of biochemical and physiological processes in the early and late phenophases of maize crop growth and that's why it is regarded as the key for life (Onasanya *et al.,* 2009)The important functions of P in plant are related to root growth, stalk strength, crop quality, grain production and maturity Mandale *et al.,* 2019).

In the future, excessive usage of Agro-chemical fertilizers will leads to negative impact on the environment and ecology and thus affecting human health. The low solubility of phosphate in the soil and excessive use of nitrogenous fertilizers leads to easily runoff into water bodies like lakes, rivers, and oceans causing degradation of water quality, aesthetic value which finally leading to create eutrophication (Kour *et al.,* 2020). Thus the alternative approach to minimize application of chemical fertilizers without affecting ecosystem is the use of microbial inoculants and their intricate biological studies occurring in soils is need of. Microbial inoculants are primarily made up of living cells of selected microbes that use various mechanisms and transformations such as those that convert nutrients to available forms for plant uptake through the root system thereby improving plant fitness that are operative in soils which in turn favor to increase soil fertility to increase crop productivity as well as quality and also play an important role in nutrient cycling and their economic significance (Mond al*et al.,* 2020). Literature have shown that endophytic bacteria promotes plant growth by fixing atmospheric nitrogen, solubilizing inorganic phosphate, solubilizing potassium, synthesizing phytohormones and producing biomolecules like siderophores, exopolys accharides and HCN. In addition to growth promotion, endophytic bacteria protect host plant from both biotic and abiotic stresses (Zhu and She, 2018). In recent studies endophytic *Bacillus, Enterobacter*, and *Klebsiella* were reported to enhance the growth and yield of maize (Mowafy *et al.,* 2021). *Bacilus flexus, Bacillus subtilis* and *Bacillus megaterium* strains isolated from maize roots have showed positive results in laboratory experiment and in vivo experiment and application of *Bacillus* as biofertilizer for agriculture improvement can reduce the use of agrochemicals and support eco-friendly crop production (Lipkov *et al.,* 2021). Therefore, isolation and evaluation of endophytic bacteria for their plant-growth promoting abilities is a significant area of research for the plant health improvement.

Thus investigation was aimed to study the influence of developed endopytic microbial consortium on soil nutrient status of Maize rhizosphere under induced deficit moisture stress in field conditions were created by varying fertility levels and the number of irrigations with different categories.

MATERIALS AND METHODS

Microbial isolates

Endophytic bacteria were isolated from surface sterilized plant tissues of maize samples collected from major maize growing regions of Andhra Pradesh, India. Upon screening for the plant growth promoting traits and stress mitigating ability four efficient bacterial strains were selected for field studies.

The endophytic bacterial isolates were identified by 16S rRNA sequencing and the efficient endophytic bacterial strains used as consortium are *Priestia megaterium* (NL3E3), *Bacillus licheniformis*(VaR3E1), *Klebsiella pneumonia* (LS3E3) and *Methylorubrum populi* (LL3E1) (Moturu *et al.,* 2022). Maize seeds were treated with developed microbial consortium before sowing and foliar application was given twice at vegetative stage at 15 days interval at the rate of 500ml per acre in the form of liquid formulation having an average population of $1X10^{-8}$ of each isolate.

Experiment Site:

Experiment was conducted at Regional Agricultural Research Station (RARS), Anakapalle, Acharya NG Ranga Agricultural University, LAM, India located at 17°38' N Latitude 83°01 E Longitude during Rabi of 2020-21. Experiment was laid out following randomized block design (RBD) and replicated thrice.

Soil characteristics:

The soil of the selected area was previously characterized as dark yellowish brown, weakly structured, well-drained loamy, mildly alkaline to moderately akaline soil.The pre-experimental soil characteristics . Available Nitrogen, Phosphorous, and Potassium in soil, dehydrogenase and phosphatase activity are provided in Table 1.

Fertilizers and irrigation:

The recommended dose of fertilizers (RDF) for maize in Andhra Pradesh, India were applied at the rate of 200, 150 and 100 kg ha^{-1}, using urea, single super phosphate (SSP) and muriate of potash (MOP) as source of NPK, respectively . Three splits of N were applied as basal application before sowing, 2nd dose at (40 DAS) maximum vegetative stage and 3rd dose at (60 DAS) flowering stage, while K was applied in 2 splits as basal dose and before flowering stage and whole P was applied at the time of sowing.

Considering water requirement by maize as 450 - 600 mm for its optimum growth, moisture stress was created by formulating number of irrigations in each treatment providing 60 mm per irrigation. Required number of irrigations to maintain 100 % WHC are 9 which is considered as fully irrigated category. Treatments given with 7 irrigations were taken under assured category, treatments given with 5 irrigations were considered as moderate category and limited category treatments were given with 3 irrigations.

Nutritional status of the rhizospheric soil:

Available N in soil: The alkaline potassium permangante method of Subbiah and Asija (1956) was followed for the estimation of available N content in soil.

Available P in soil : Available P in soil was determined by Olsen's procedure of method described by Olsen *et al*. (1954).

Available K in soil : Available potassium content from soil was extracted by using 1N

Table 1. Chemical properties of initial soil of field before cultivation

1.	Available N in Soil (kg ha^{-1})	83.46
2.	Available P in Soil (kg ha^{-1})	40.21
3.	Available K in Soil (kg ha^{-1})	172.83
4.	Alkaline phosphatase activity (μpNP g^{-1}soil h^{-1})	38.76
5.	Dehydrogenase activity (μ of TPF g^{-1} of soil day^{-1})	23.21

Table 2. Details of the treatments used in the field experiment

1.	Crop	Maize
2.	Variety/Genotype	PIONEER 3396
3.	No. of treatments	10
4.	No. of replications	3
5.	Plot size	4 m × 4.8 m
6.	Spacing	60 cm × 20 cm
7.	Experimental design	Randomized Block Design
8.	Date of sowing	27-12-2020
9.	Date of harvesting	17-04-2021
10.	Treatments	T_1:Limited Irrigations + 50 % RDF + MCT2: Limited Irrigations + 75 % RDF + MCT3:Limited Irrigations + 100 % RDF + MCT_4:Moderate Irrigations + 50 % RDF + MCT5:Moderat Irrigations + 75 % RDF + MCT6:Moderat Irrigations + 100 % RDF + MCT7: Assured Irrigations + 50 % RDF + MCT_8: Assured Irrigations + 75 % RDF + MCT9: Assured Irrigations + 100 % RDF + MCT10: Full Irrigations + 100 % RDF (Control) (RDF: Recommended dose of fertilizer; MC: Microbial consortium 3,5,7 and 9 irrigations were given to limited, moderate, assured and full irrigated treatments, respectively)

neutral normal ammonium acetate as described by Jackson (1973).

Dehydrogenase activity : Method was described by Casida *et al*. (1964). The enzyme activity in 1 gm soil was determined by adding 0.05 g of $CaCO_3$, 2.5 ml of distilled water and by using 1 ml of 3 % TTC where it is reduced to light pink TPF on the incubation for 24 h. Later it was dissolved in 10 ml of methanol and finally made up to 25 ml. The intensity of the red color was measured on a spectrophotometer at 485 nm wave length. Expressed as µg of TPF g^{-1} soil day^{-1}.

Phosphatase activity : The procedure followed was of Eivazi and Tabatabai (1977) for alkaline phosphatases. The principle in the estimation of Phosphatase enzyme activity is that the soil extract from 1g of soil was allowed to react with para nitro phenol, which was estimated using UV Spectrophotometer.

Phosphatase activity was estimated by taking 1g of soil sample and mixed it with 0.2 ml of toluene 4 ml of MUB (Modified Universal Buffer) and 1 ml of disodium para nitrophenol solution. This solution was kept in an incubator at 37 °C for 1 h. Later 1 ml of 0.5 M $CaCl_2$ and 4 ml of 0.5 M NaOH were mixed properly and the intensity of the yellow color of filtrate was read on spectrophotometer at 420 nm. Expressed as µgp NP g^{-1} soil h^{-1}

Statistical analysis

Data on different characters were subjected to analysis of variance as given by Gomez and Gomez (1984) for Randomized

Block Design (RBD) using OP Stat software beta version. Statistical significance was tested by F–value at 0.05 level of probability and critical difference was worked out where ever the effects were significant.

RESULTS AND DISCUSSION:

Available nitrogen in Soil:

Initial status of available soil nitrogen before cultivation (83.46 kg ha^{-1}) has increased predominently at vegetative stage of the crop and further increase of available N was recorded at flowering stage (tasseling and silking) but reduction of available N was observed as the crop proceed to harvesting stage. At all the growth stages available nitrogen in soil is significantly more in treatments given with seven irrigations recording highest 171.43, 225.79 and 179.80 kg ha^{-1} in T_9 at vegetative, flowering and harvesting stage, respectively. While in treatments given with five irrigations (100% RDF) available nitrogen in soil was found on par with control (full irrigations) (Table 3). This implies that even under water stress conditions the developed microbial consortia could able to improve soil available nitrogen. The nitrogen fixing ability of bacterial isolates applied as consortia to the crop might has improved available nitrogen content in soil though provided with 25% lesser RDF.

The results were in agreement with the studies of Ahmad *et al.* (2008) who reported that the increase in available nitrogen and phosphorus in soil was due to biological nitrogen fixation (BNF) and mineralization of organic matter by microbial inoculants. Inoculation of *Azospirillum* spp. in sugarcane contributed 26.7 kg N ha^{-1} or 70% of total required N by plant by process of nitrogen fixation (Boddey *et al.,* 1995). A significant increase in available N, P and K in soils treated with microbial consortium was observed which positively correlated with increase in plant dry weight, plant height, pigment synthesis and seed production (Raklami *et al.,* 2019). The endophytic bacterial isolates *Paenibacillus* sp., *B. subtilis*, *Pseudomonas* sp., *Burkholderia* sp., and *Brevibacillusagri* were foud to have efficient N accumulation in maize and sorghum (Aquino *et al.,* 2019). Supplementation with two strains of *Enterobacter cloacae*, ASD-48 and ASD-21, enhanced the amount of NH^{4+} and NO_3, the total N uptake, and the grain yield of sesame (Thuc *et al.,* 2022)

Available phosphorus in soil (kg ha^{-1})

The initial available phosphorus in soil was found to be 40.21 kg ha^{-1} (Table 1) and it was greately increased at vegetative and further at flowering stage. At harvesting stage reduce available P was observed. The treatments given with water stress (7 and 5 irrigations) and limited nutrients (75% RDF) were found to have significantly higher available P in soil when treated with developed endophytic microbial consortia (Table 3).

Available soil status of the P is highest in treatments with assured irrigations and available P under conditions of winter season *(Rabi)* than other nutrients was more due to its immobile nature and its diffusion is reduced that increases available P status due to conversion of Fe^{3+} phosphate to soluble Fe^{2+} phosphates and hydrolysis of aluminium phosphates by the inoculated microbial consortium which lead to proper utilization and uptake for its growth and development.

Mehta *et al.* (2014) reported that microbes solubilize P by producing metabolites like organic acids that aid in decreasing the pH of the culture media. Application of endophytic bacteria increases acidification of soil due to production of organic acids attributing to convert non-available phosphorus to available P form in soil (Posso *et al.,* 2017).Our results were in accordance with the results of Sood *et al.* (2018) in terms

of soil properties including available N, P, K, soil enzyme activities and phosphate-solubilising bacterial population were significantly increased in the wheat crop inoculated with bacterial inoculants with 80% of recommended dose of N and P in consecutive years over the uninoculated control.

Available potassium in soil (kg ha^{-1})

The initial availability of potassium in soil is 172.83 kg ha^{-1} and it was prominently increased at vegetative and flowering stage while the available K at harvesting stage was observed to get reduced (Table 3). The microbial consortia could able to compensate 25% of potassium in treatments given with 75% RDF even at water stress conditions (7 and 5 irrigations) as applied consortia was developed with efficient K solubilizers along with other PGP characters which converts unavailable forms of K into available forms and further the K ion concentration protects plants under stress conditions by maintaining membrane integrity.

The treatments T8 and T9 were on par due to assured irrigations which create episaturation that reflects to maximum K fixation which would be prone to microbial solubilization and its availability in the rhizosphere by secreting some organic acids which decreases soil pH that dissolve and chelate the silicon ions from the potassium bearing minerals by the inoculated microbial consortium.

By increasing available potassium in soil and enhanced K uptake with the inoculation of *Bacillus mucilaginosus* has promoted the growth of eggplant (Han *et al.,* 2006). Soil essential nutrients such as available N (49.47 kg ha^{-1}), available P (25.41 kg ha^{-1}), and available K (43.01 kg ha^{-1}) observed to increase significantly with inoculation of endophytic microbial consortia in both pot and field soil of chickpea than untreated control soil (Mukherjee *et al.,* 2021).

Dehydrogenase activity (of TPF g^{-1} of soil day^{-1})

Among all enzymes in the soil environment, dehydrogenases are significant and used as an indicator of microbial activity in rhizospheric region. The dehydrogenase activity of the initial field soil was found as 23.21 μg of TPF g^{-1} of soil day^{-1}. The soil enzyme activity was obsereved to get increased in vegetative stage and the same trend followed till flowering stage, however, at harvesting stage soil dehydrogenase activity was found reduced. At all the growth stages significant activity of dehydrogenase enzyme was observed in treatments inoculated with microbial consortia though water stress and nutrient stress was given which implies prominent microbial activity in amended plots.

The activation of soil enzymes are directly linked with the microbial activity and results obtained in the current study clearly showed an increase in soil enzymes in treatments inoculated with microbial consortia. Soil enzyme activity is an important visual indicator of microbial population intensity and their efficiency in plant growth promotion thus application of these endophytes may enhance soil fertility by improved bio-mineralization (solubilization, chelation and dissolution), crop productivity, soil resiliense and its sustainability under status of stress condition. The results were in accordance with previous investigations (Chouhan *et al.,* 2021; Singh *et al.,* 2018).

Alkaline phosphatase (μg pNP g^{-1} of soil h^{-1})

Phosphatase enzymes involves in hydrolyzing recalcitrant form of P into available form of P to the plants. The phosphatase activity in the initial field soil was found as 38.76

Table 3: Influence of endophytic microbial consortia on available nutrients in maize rhizosphere soil under water stress imposed field conditions

S.No	Treatments	Available N in soil (kg ha^{-1})			Available P in soil (kg ha^{-1})			Available K in soil (kg ha^{-1})		
		Vegetative Stage	Flowering Stage	Harvest Stage	Vegetative Stage	Flowering Stage	Harvest Stage	Vegetative Stage	Flowering Stage	Harvest Stage
1.	T_1: Limited Irrigations + 50 % RDF + MC	96.17	129.62	79.44	42.28	48.22	41.28	216.62	267.02	166.48
2.	T_2: Limited Irrigations + 75 % RDF + MC	129.62	150.53	87.81	45.33	47.20	41.29	243.96	281.11	186.64
3.	T_3: Limited Irrigations + 100 % RDF + MC	146.34	188.16	112.90	52.89	53.74	47.40	280.89	326.72	221.00
4.	T_4:Moderate Irrigations+ 50 % RDF + MC	112.90	158.89	121.26	47.27	51.29	39.24	219.77	272.92	187.29
5.	T_5: Moderate Irrigations + 75 % RDF + MC	133.80	175.62	121.26	58.23	62.31	54.71	293.17	373.88	212.45
6.	T_6: Moderate Irrigations+100 % RDF + MC	129.62	200.70	137.98	59.04	68.96	58.47	327.87	376.37	215.42
7.	T_7: Assured Irrigations + 50 % RDF + MC	146.35	175.62	125.44	48.74	53.18	45.24	239.11	280.86	183.88
8.	T_8: Assured Irrigations + 75 % RDF + MC	150.53	209.07	171.44	57.30	69.92	58.81	337.71	360.59	224.96
9.	T_9: Assured Irrigations + 100 % RDF + MC	171.43	225.79	179.80	60.46	70.34	62.24	372.16	390.62	203.01
10.	T_{10}:Full Irrigations + 100 % RDF (Control)	145.65	154.03	129.62	50.08	55.37	45.62	236.04	262.72	189.16
11.	CD @ 5%	29.55	40.00	35.48	7.28	5.80	7.28	37.56	36.78	N/A
12.	S.Em. ±	9.86	13.36	11.85	2.43	1.93	2.43	12.54	12.28	16.22
13.	S.Ed	13.95	18.89	16.76	3.44	2.74	3.43	17.74	17.37	22.94
14.	CV (%)	12.54	13.08	16.20	8.08	5.78	8.52	7.85	6.66	14.11

Table 4: Influence of endophytic microbial consortia on soil enzymes activity of maize rhizosphere under water stress imposed field conditions

S.No	Treatments	Dehydrogenase (µg of TPF g^{-1} of soil day^{-1})			Alkaline phosphatase (µgpNP g^{-1} of soil h^{-1})		
		Vegetative Stage	Flowering Stage	Harvest Stage	Vegetative Stage	Flowering Stage	Harvest Stage
1.	T_1: Limited Irrigations + 50 % RDF + MC	29.44	42.93	20.17	51.57	84.57	39.97
2.	T_2: Limited Irrigations + 75 % RDF + MC	32.42	43.97	21.21	56.87	84.83	40.70
3.	T_3: Limited Irrigations + 100 % RDF + MC	33.47	43.10	20.84	57.10	87.27	37.83
4.	T_4:Moderate Irrigations+ 50 % RDF + MC	37.47	49.90	22.51	56.87	88.53	45.57
5.	T_5: Moderate Irrigations + 75 % RDF + MC	38.81	50.73	23.58	66.83	88.60	40.80
6.	T_6: Moderate Irrigations + 100 % RDF + MC	40.83	52.73	24.10	70.93	89.37	48.63
7.	T_7: Assured Irrigations + 50 % RDF + MC	41.57	50.80	24.78	75.00	88.57	47.00
8.	T_8: Assured Irrigations + 75 % RDF + MC	44.43	58.63	25.40	74.10	94.97	57.20
9.	T_9: Assured Irrigations + 100 % RDF + MC	46.93	58.90	26.62	75.40	92.90	56.27
10.	T_{10}:Full Irrigations + 100 % RDF (Control)	38.13	49.56	23.17	62.50	81.23	42.10
11.	CD @ 5%	2.57	4.82	1.93	5.60	5.30	4.99
12.	S.Em. ±	0.86	1.62	0.65	1.87	3.01	1.67
13.	S.Ed	1.22	2.29	0.92	2.64	4.26	2.36
14.	CV (%)	3.91	5.61	4.85	5.01	5.93	6.34

μgpNP g^{-1} of soil h^{-1}. Upon inoculation of developed endophytic microbial consortium the soil enzyme activity has been significantly improved in all the treatments. The phosphatase activity was found to be higher at flowering stage and decreased activity was observed as the crop proceeds to harvesting stage.

The treatment T_8 (94.97 μg pNP g^{-1} of soil h^{-1}) has encouraged the enzyme activity of alkaline phosphatase at flowering stage under assured irrigation category and it was on par with T_9 (92.90 μg pNP g^{-1} of soil h^{-1}) (Table 4). Even so, it was significantly higher with rest of the treatments and extent of increase in its activity of T8 was about 17% with T_{10} (Control) which was amended with no inoculum. This might be due to activity of alkaline phosphatase is more pronounced at assured irrigations with 75% RDF which shows less mobility and higher microbial load of retention due to lower availability of chemical traces and their residues which enhances activity of inoculated microbial consortium.

The developed endophytic microbial consortia in the present investigation consist of potential phosphate solubilizing isolates and due to their application the inoculated treatments were found to have increased alkaline phosphatase activity in soil which was assumed due to the exudations of microbial consortia bacteria. Phosphorus solubilizing microbes were developed with different mechanisms to release phosphates from prevailing sources in soil which include release of alkaline, acid phosphatases and phytase enzymes that involves conversion of organic phosphates to inorganic phosphates and exudates organic acids to solubilize inorganic phosphates (Rinu *et al.,* 2012; Adhikari and Pandey, 2019). The bacterial endophytes GBPI_TWL and GBPI_TWR which were found to have potential of solubilizing phosphorus in three forms including calcium phosphate, aluminium phosphate, and iron phosphate. Field experimentation and quantitative estimation of mechanism involved was revealed the production of soil enzymes alkaline phosphatase, acid phosphatase and phytases (Adhikari and Pandey, 2020).

CONCLUSIONS

In this study to provide an alternative approach to mitigate moisture stress and minimize chemical fertilizer application a developed endophytic microbial consortia was studied under field conditions and the results indicates that at moderate and assured irrigations significantly higher or on par amounts of available N,P,K in soil was reported even with 75% RDF when compared with control given with full irrigations and 100% RDF, indicating limited application of fertilizers by 25% didn't affect the nitrogen, phosphorous and potassium availability in soil under consortium inoculation.Enhanced soil dehydrogenase activity and alkaline phosphatase activity was found in treatments provided with 100% and 75% RDF even under water deficit conditions and the enzyme activity was found prominent at flowering stage.

It is concluded that enhanced levels of available nitrogen [T_9 (225.29 kg ha^{-1}) and T_8 (209.09 kg ha^{-1})], phosphorous [T_9 (70.34 kg ha^{-1}) and T_8 (69.92 kg ha^{-1})] and potassium [T_9 (390.62 kg ha^{-1}), T_8 (360.59 kg ha^{-1})] in maize rhizosphere was observed in combination of 75% RDF & 100% RDF and developed microbial consortium (*P. megaterium*, *B.licheniformis*, *K. pneumonia* and *M. populi*) under assured (7) irrigations.Hence, the investigation recommends that nearly 120 mm of water (2 irrigations) and 25% of RDF can be minimized for crop production without affecting nutrient availability to the crop growth.

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EFFECTS OF VARIOUS FUNGICIDES AGAINST *CORYNESPORA CASSIICOLA IN VITRO*

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ABSTRACT

In order to determine the efficacy of eleven fungicides against *Corynespora cassiicola* causing Corynespora leaf spot of cotton, an experiment was carried out during 2012-22 employing poisoned food technique. The results of radial growth and inhibition of growth over control revealed that variation existed in the sensitivity of *C.cassiicola* towards different test fungicides. Among the six straight fungicides, carbendazim @ 0.1% and hexaconazole @ 0.2% amended medium promoted zero growth *i.e.,* complete inhibition of *C. cassiicola*. In propiconazole @ 0.1% amended medium, the growth was 0.9 cm equivalent to 89.7% inhibition. Among the five combination fungicides,fluxapyroxad + pyraclostrobin @ 0.06% and metiram + pyraclostrobin @ 0.3% gave 100% inhibition.

Keywords: Cotton, Corynespora leaf spot, fungicides, *in vitro* evaluation

INTRODUCTION

Cotton is one of the most significant commercial crops in Andhra Pradesh, covering an area of 5.24 lakh ha with a productivity of 584 kg lint/ha. In India, cotton occupies an area of 120.69 lakh ha with a yearly production of 362.18 lakh bales of 170 kg and a productivity of 510 kg lint/ha. (ICAR- AICRP on Cotton, 2022). Among the fungal leaf spots caused by *Alternaria macrospora*, *Corynespora cassiicola*, *Myrothecium roridum*, *Helminthosporium gossypii* and *Cercospora gossypii* in cotton, Corynespora leaf spot is a major disease in Andhra Pradesh.Circular to oval or irregular concentric spots with tan to light brown centre develop with yellow halo around the margin. These spots enlarged and concentric zonations were formed resulting in target board symptom. Spots were initially spotted in the lower, interior canopy and extended upwards and also observed on petioles,stems, squares with minute oval to irregular brick red coloured spots. On severe infection, these lesions coalesced causing chlorosis and necrosis followed by complete senescence resulting in premature defoliation (Fig. 1). *Corynespora* leaf spot caused loss of 100-200 lb/ac of lint in cotton (Hagan and Sikora, 2012). According to Fulmer *et al.* (2012), target spot damage on cotton could range up to 75%. In susceptible cotton cultivars, the disease resulted in lint yield losses as high as 224 - 448 kg ha^{-1} equivalent to 5 to 40% (Conner *et al*., 2013; Hagan, 2014; Hagan *et al.*, 2015). Based on the importance of *Corynespora* leaf spot, an *in vitro* experiment was conducted

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to find out the efficacy of fungicides against this pathogen.

Colony growth of *C. cassiicola* on PDA was effuse, grey to light olive green with abundant aerial growth. On lower side, mycelium appeared greyish to black colour (Plate 2). Mycelium was smooth, sub hyaline, light brown in colour, thin-walled, branched and septate with a radial growth of about 9 cm in 8 days. Conidiophores are cylindrical, straight or curved and unbranched, 3-10 septate, smooth and pale brown. Conidia are variable obclavate to cylindrical, either singly or in chains, with truncate base and rounded apex with 5-14 pseudoseptate, subhyaline to pale, brown and smooth.

MATERIAL AND METHODS

The experiment was conducted in the Department of Plant Pathology, Agricultural College Bapatla. Samples of cotton leaves infected with *Corynespora* were collected from Regional Agricultural Research Station, Lam during the 2012-22 *kharif* season. The affected leaf sections were cut into small pieces and surface sterilized with 0.1 N sodium hypochlorite (NaOCl) solution for 30 seconds. They were then thoroughly washed four times with sterile waterand transferred to Petri plates (4 bits per Petri plate) containing Potato Dextrose Agar (PDA).

Eleven fungicides, as detailed in Table 1, were evaluated *in vitro* at recommended doses against *C.cassiicola* by poisoned food technique using PDA as basal medium. To the sterilized medium, the required quantity of test fungicides (Table 1) were added. In the asceptic conditions of the inoculation chamber, three sterilized Petri dishes were equally filled with the poisoned medium. Each plate was inoculated in the centre with a 5 mm diameter of five days old fungal culturedisc cut from the periphery of the actively developing culture under aseptic conditions. The plates were then incubated at 28±1 ˚C in a BOD incubator. Three PDA plates that were inoculated with the fungus but did not contain fungicide served as controls. Radial growth of the fungus was recorded daily in the control plate starting from initiation of the fungal growth in correspondence to treatment plates till the fungal growth was full in control.

The efficacy of different fungicides against *C. cassiicola* was evaluated *in vitro* by poisoned food technique using PDA as basal medium.

$$I = \frac{C - T}{C} \times 100$$

Where, I stands for percent inhibition, C for fungal growth in non-poisoned food medium and T for fungal growth in poisoned food medium.

RESULTS AND DISCUSSION

In fungicide unamended medium, *C. cassiicola* attained a maximum radial growth of 8.7 cm after 14 days of inoculation. When compared to un-amended medium, the growth of *C.cassiicola* in fungicide amended medium was significantly reduced in all the treatments. However, variation existed in the sensitivity of *C.cassiicola* towards different test fungicides (Fig.3).

Among the straight fungicides, in carbendazim @ 0.1% and hexaconazole @ 0.2% amended medium, *C. cassiicola* showed zero growth *i.e.,* complete inhibition (100%) was observed. In propiconazole @ 0.1% amended medium, the growth was 0.9 cm equivalent to 89.7% inhibition.Kresoxim methyl @ 0.1%, propineb @ 0.3% and azoxystrobin @ 0.05% gave 74.7%, 50.6% and 28.7% inhibition, respectively, indicating comparatively lesser sensitivity of *C.cassiicola* to these three test fungicides when tested at field recommended concentrations. With respect to combination fungicides, fluxapyroxad + pyraclostrobin @ 0.06% and metiram + pyraclostrobin @ 0.3% gave 100% inhibition, whereas, trifloxystrobin +

Figure 1. Symptoms of Corynespora leaf spot on different plant parts

Figure 2. Pure culture of *Corynespora cassiicola*

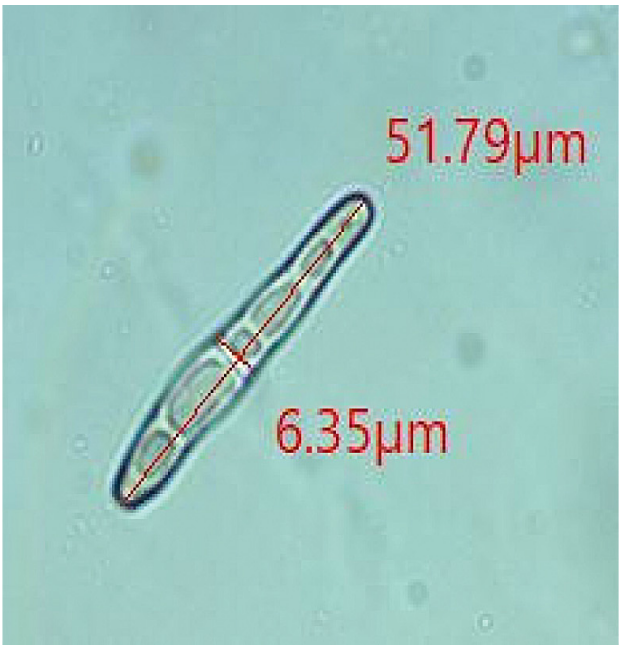

Figure 3. Conidium of *Corynespora cassiicola*

Table 1. List of fungicides used in poisoned food technique *in vitro*

S. No.	Common Name	Trade Name	Formulation
1	Hexaconazole	Trigger	5% EC
2	Carbendazim	Bavistin	50% WP
3	Propiconazole	Tilt	25% EC
4	Azoxystrobin	Amistar	23% SC
5	Kresoxim methyl	Ergon	44.3% SC
6	Propineb	Antracol	70% WP
7	Captan + hexaconazole	Taqat	75% WP
8	Trifloxystrobin + tebuconazole	Nativo	75% WG
9	Zineb + hexaconazole	Avatar	72% WP
10	Fluxapyroxad + pyraclostrobin	Priaxor	50% SC
11	Metiram + pyraclostrobin	Carbrio Top	60% WG
12	Control	-	-

Table 2. Efficacy of fungicides on mycelial growth of *Corynespora cassiicola*

S. No.	Treatments	Conc. (%)	Mycelial growth (cm)*	Per cent Inhibition	Mode of action
1	Hexaconazole (5%)	0.2%	0.0 (1.00)[a]	100.0	Inhibition of Melanin biosynthesis
2	Carbendazim (50%)	0.1%	0.0 (1.00)[a]	100.0	Inhibition of mitosis and cell division by interfering with spindle formation
3	Propiconazole (25%)	0.1%	0.9 (1.37)[b]	89.7	Inhibition of Melanin biosynthesis
4	Azoxystrobin (23%)	0.05%	6.2 (2.69)[a]	28.7	Blocks electron transport at quinol oxidation site
5	Kresoxim methyl (44.3%)	0.1%	2.2 (1.80)[d]	74.7	Inhibition of electron transport in the mitochondria
6	Propineb (70%)	0.3%	4.3 (2.29)[g]	50.6	Denaturation of proteins & enzymes
7	Captan (70%) + hexaconazole(5%)	0.1%	1.5 (1.58)[c]	82.8	Enzyme inactivation through interaction with their $-NH_2$ and –SH group + Inhibition of Melanin biosynthesis
8	Trifloxystrobin (50%) + tebuconazole (25%)	0.05%	2.5 (1.88)[e]	71.3	Blocks electron transport at quinol oxidation site + Inhibition of Melanin biosynthesis
9	Zineb (68%) + hexaconazole (4%)	0.2%	3.2 (2.06)[f]	63.2	Denaturation of proteins & enzymes by reaction with their –SH group + Inhibition of Melanin biosynthesis
10	Fluxapyroxad (17%) + pyraclostrobin (33%)	0.06%	0.0 (1.00)[a]	100.0	Inhibition of succinate dehydrogenase of mitochondrial respiratory chain + Blocks electron transport at quinol oxidation site
11	Metiram (55%) + pyraclostrobin (5%)	0.3%	0.0 (1.00)[a]	100.0	Denaturation of proteins & enzymes by reaction with their –SH group + Blocks electron transport at quinol oxidation site
12	Control	-	8.7 (3.11)[i]	-	
	SEm (±)		0.02		
	CD @ 5%		0.06		
	CV (%)		2.05		

*Mean of three replications; Treatment means with the same alphabet do not differ significantly
Figures in the parenthesis are square root transformed values

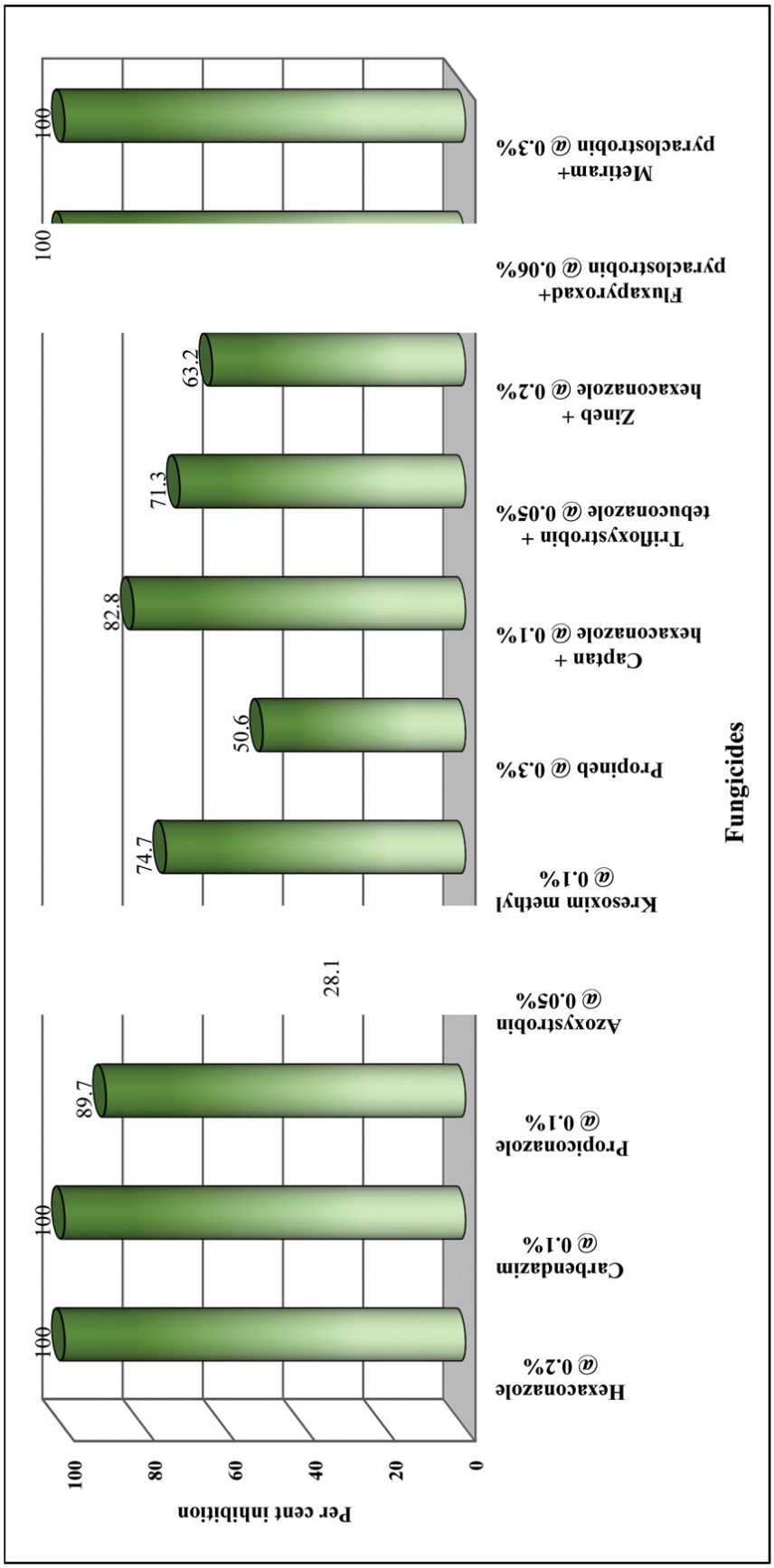

Figure 4. Effect of different fungicides on radial growth of *Corynespora cassiicola*

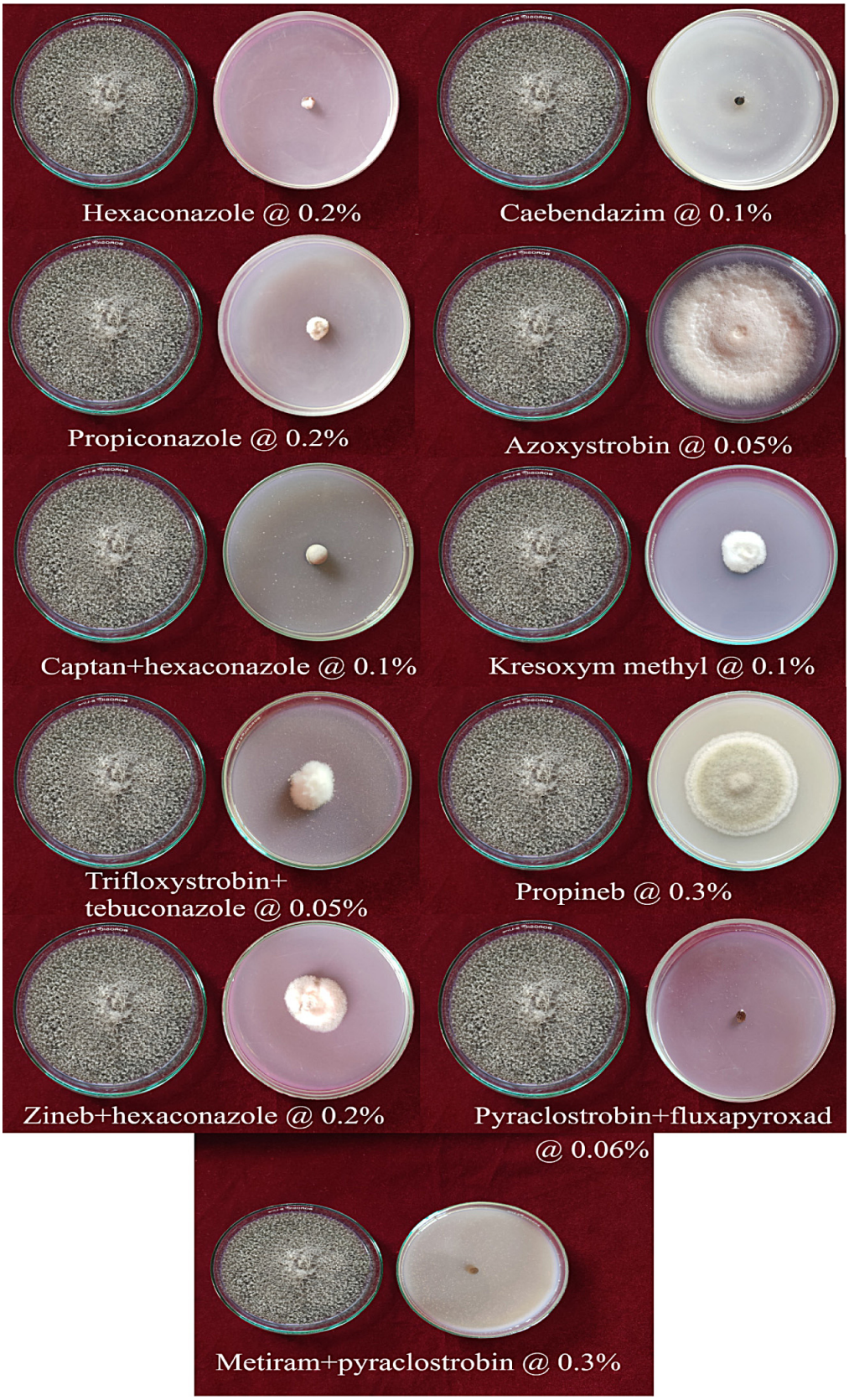

Figure 5. Effect of fungicides on radial growth of *Corynespora cassiicola in vitro*

tebuconazole @ 0.05% gave 71.3% inhibition in the radial growth of *C. cassiicola*. The study also revealed that hexaconazole alone was highly effective against *C. cassiicola* compared to two of its combination products tested, *i.e.,*captan + hexaconazole @0.1% (82.8%) and zineb + hexaconazole @0.2% (63.2%). Such a decreased efficacy of hexaconazole is probably due to its decreased active ingredient in the combination product zineb (68%) + hexaconazole (4%). In case of captan (70%) + hexaconazole (5%) the decreased efficacy could be attributed to incompatible reaction between captan and hexaconazole (Table 2 and Fig. 4).

When compared to control, Arvind *et al*. (2017) observed minimum inhibition of mycelial growth of *C.cassiicola* by mancozeb and pyraclostrobin at 50 ppm and 100 ppm concentrations. Carbendazim @ 0.1% was found to completely inhibit the mycelial growth of the pathogen (Yamuna *et al*., 2020). Ishwari *et al.* (2020) observed that propiconazole inhibited radial growth of *C. cassiicola* with 94.44% inhibition over control under *in vitro* conditions. Metiram + pyraclostrobin @ 0.2% completely inhibited the radial growth of mycelium against *C. cassiicola* over control (Mushrif *et al*., 2020). The results are also in line with the findings of Mahesh *et al.* (2022) who reported complete inhibition of mycelial growth of *C. cassiicola* with hexaconazole @ 0.2% followed by propiconazole @ 0.1% (99.75%), metiram + pyraclostrobin @ 0.3% (98.61%), fluxapyraxad + pyroxystrobin + @ 0.06% (97.50%).

By inhibiting the action of 14-á-sterol demethylase propiconazole reduces fungal development by inhibiting the production of essential fungal cell membrane ergosterols. Shortly after the spores germinate,metiram kills the fungus.By inhibing succinate dehydrogenase in complex II of the mitochondrial respiratory chain, fluxapyroxad inhibits the formation of mycelial structures, germ tubes and spore germination.Pyraclostrobin restricts the fungus energy source by blocking which leads to the death of the target fungus. Thus dual action of pyraclostrobin in combination with metiram or fluxapyroxad was found synergistic with 100% inhibition (Table 2).

CONCLUSIONS

In vitro evaluation of fungicides showed that hexaconazole @0.2%, carbendazim @ 0.1%, fluxapyroxad + pyraclostrobin @ 0.06% and metiram + pyraclostrobin @ 0.3% induced 100% inhibition of *C.cassiicola*, the most important leaf spot pathogen of cotton.

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NUTRIENT UPTAKE AND YIELD OF RICE AS INFLUENCED BY DIFFERENT SYSTEMS OF RICE CULTIVATION AND ORGANIC NUTRIENT MANAGEMENT PRACTICES

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ABSTRACT

The field experiment was conducted during *late Kharif* season of 2021-22 and 2022-23 in split-plot design and replicated thrice to record the nutrient uptake and grain yield as influenced by different organic nutrient management practices under different systems of rice cultivation. Among the different systems of rice cultivation, significantly higher dry matter production, nutrient uptake and yield were noticed under normal transplanted rice which was at par with wet direct seeded rice. Among the organic nutrient management practices tested, significantly higher nutrient uptake as well as yield were obtained with the application of 100% RDN (Recommended Dose of Nitrogen) through poultry manure which was at par with 50% RDN through VC + 50% RDN through poultry manure but both of them were significantly inferior to 100% RDN (120-60-40 kg N, P_2O_5, K_2O ha^{-1}) through inorganic fertilizers on sandy clay loam soils of Southern Agro-Climatic Zone of Andhra Pradesh. Application of 100% RDN through Poultry Manure in transplanted rice was found effective in obtaining higher nutrient uptake and yield than rest of the systems of rice cultivation and organic nitrogen management practices.

Keywords: Inorganic fertilizers, Organic nitrogen management practices, Poultry Manure, Systems of rice cultivation, Vermi compost

INTRODUCTION

Rice is the primary staple food crop and the major source of daily calorie intake for almost one-third of the global population. In India, the rice crop occupies an area of 46.28 million hectares with 129.47 million tons of production and productivity of 2798 kg ha^{-1}. Continuous use of inorganic fertilizers leads to deterioration in chemical, physical and biological properties of soil and soil health. The negative impacts of chemical fertilizers coupled with escalating prices, have led to growing interest among the farmers to use locally available organic manures. Organic sources such as farmyard manure, vermicompost, poultry manure, green manuring, neem cake and biofertilizers are important components in organic cultivation of crops, to maintain soil fertility and to produce reasonably good crop yields. The use of organic manures benefitted the sequence crops as they leave some residues in the soil. In recent years, there has been a growing interest in organic foods

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among the consumers which warrants the use of organic manures as a source of plant nutrients for producing quality food grains and sustaining soil health for future generations.

The area under traditional method of transplanted rice in world is going to decrease due to the limitation of water and labour, therefore, an alternate method of establishment should be promoted to enhance the crop and water productivity. Wet Direct Seeded Rice (W-DSR) and Dry Direct Seeded Rice (D-DSR) are the best alternate methods of rice sowing. Nitrogen is the major essential plant nutrient and key input for rice production. An increase of 70 % - 80% in the yield of rice could be obtained by the application of nitrogen fertilizers. The application of the organic fertilizers such as the farmyard manure and poultry manure could increase the soil organic carbon content as well as sustain the nutrient availability throughout crop growth. It has several advantages such as conservation and slow release of nutrients, improve soil chemical and physical conditions. Nitrogen is a highly dynamic nutrient which undergoes various changes under different systems of rice cultivation *i.e.,* normal transplanted rice, wet DSR and dry DSR. Keeping these in view, the nutrient dynamics in different systems of rice cultivation was investigated under varied organic sources for increasing the productivity and profitability of organic rice cultivation in Andhra Pradesh state.

MATERIAL AND METHODS

The field experiment was conducted during late *kharif* season of 2021-22 and 2022-23 at the S. V. Agricultural College Farm, Tirupati. The soil of the experimental site was sandy clay loam with a bulk density of 1.43 g cc^{-1} having pH 7.85, EC 0.44 dsm^{-1}, low in organic carbon (0.38 %), available nitrogen (187 kg ha^{-1}), medium in phosphorus (25.5 kg ha^{-1}) and potassium (218 kg ha^{-1}). Rice variety "*NLR-34449*" was taken as the test variety. The experiment was laid out in split-plot design with different systems of rice cultivation as main plots and organic nitrogen management practices as sub-plots and replicated thrice. The main plots comprised of *viz.,*Normal transplanted rice (N-TPR), Wet Direct Seeded Rice (W-DSR) and Dry Direct Seeded Rice (D-DSR). Sub- plots comprised of seven organic nitrogen management treatments *viz.,*100% RDN through Farm Yard Manure (FYM), 100% RDN through Vermicompost (VC), 100% RDN through Poultry Manure (PM), 50% RDN through FYM+ 50% RDN through VC, 50% RDN through FYM + 50% RDN through PM, 50% RDN through FYM + 50% RDN through PM and 100% RDN through inorganic fertilizers. The RDF for rice crop in Southern Agro-ClimaticZone of Andhra Pradesh was 120:60:40 kg N, P_2O_5, K_2O ha^{-1}.

Recommended seed rate for N-TPR (75 kg ha^{-1}), W-DSR (37.5 kg ha^{-1}) and D-DSR (37.5 kg ha^{-1}) was used for sowing directly or nursery raising on the same day. Twenty four days old seedlings were transplanted in well puddled field manually in transplanted rice. In W-DSR, seeds were soaked in water for 24 h, then, wrapped in jute gunny bag and kept in dark for 24 h to induce better sprouting. These sprouted seeds were sown at 20 cm x 10 cm spacing with the help of drum seeder. In D-DSR, recommended seed rate was sown manually at spacing of 20 cm x10 cm. In all the systems of rice cultivation, seeds were sown on 19-08-2021 and 16-08-2022 during first and second years of experimentation. Required quantity of organic sources as per the treatments were applied on N-equivalent basis 15 days before sowing or transplanting. The data on nutrient uptake, yield, net returns and benefit-cost ratio were calculated and presented here under.

Biometric observations

Number of panicles m^{-2}

The number of panicles m^{-2} were counted with the help of quadrant and expressed as number of panicles m^{-2}.

Total number of grains panicle^{-1}

Grains from each randomly selected five panicles were counted, averaged and expressed as number of total number of grains panicle^{-1}.

Number of filled grains panicle^{-1}

Number of filled grains panicle^{-1} from randomly selected panicles were counted. Mean of five panicles was arrived and expressed as number of filled grains panicle^{-1}.

Test weight

Thousand grains were drawn randomly from the composite sample of grain yield obtained from each of the net plot area, weighed and expressed as test weight in g.

Grain yield and straw yield

The grains obtained from each net plot area were sun dried, cleaned, weighed and expressed in kg ha^{-1}. Straw from each net plot was thoroughly sun dried to a constant weight, weighed and expressed in kg ha^{-1}.

Post-harvest soil nutrient status

Five soil samples at 0-15 cm depth were collected at randomly in the experimental field before ploughing the experimental area and the composite soil sample was prepared by quartering method. Similarly, post-harvest soil samples were also drawn treatment wise and air dried under shade and then passed through 2 mm sieve which were used for analysis of available N, P_2O_5 and K_2O. The methods used for analyzing the nutrients are presented in Table 1.

Nutrient uptake by rice crop

The plant samples of the rice crop were collected from the respective treatments at 30 DAS, 60 DAS, 90 DAS/DAT and at harvest and were oven dried, finely grounded and used for estimation of total N, P, K, Fe and Zn content by following the standard procedures. Nutrient uptake was calculated by using the formula given below.

Nutrient uptake (kg ha^{-1}) =

$$\frac{\text{Nutrient content (\%) x Dry matter production kg ha}^{-1}}{100}$$

Nitrogen Uptake

The nitrogen content in dry matter of crop was estimated by kelplus method as suggested by Subbiah and Asija (1956). The nitrogen uptake

Table 1. Methods employed for soil analysis

S.No.	Nutrient	Method	Instrument used (Model)	Authority
1	Available N(kg ha^{-1})	Alkaline $KMnO_4$ method	Kelplus supra-LX	Subbiah and Asija (1956)
2	Available P_2O_5 (kg ha^{-1})	Olsen's extractant method	Double beam UV visible spectrophotometer (ECIL- UV570455)	Olsen's *et al*. (1954)
3	Available K_2O(kg ha^{-1})	Neutral normal ammonium acetate method	Flame photometer (Elico-CI-361)	Jackson (1973)
4	Iron and Zinc (mg kg^{-1})	DTPA- soil extract method	Atomic Absorption Spectrophotometer	Lindsay and Norvell (1978)

was calculated by multiplying the nitrogen content (%) with respective dry matter production and expressed in kg ha^{-1}.

Phosphorus Uptake

The di-acid digested plant samples were analyzed for phosphorous content by vanadomolybdo phosphoric yellow colour method (Jackson, 1973). The intensity of yellow colour developed was measured at 420 nm using spectrophotometer (Pipper, 1966). The phosphorus uptake was calculated by multiplying the phosphorus content (%) with respective dry matter production and expressed in kg ha^{-1}.

Potassium Uptake

The di-acid digested plant samples were analyzed for potassium content by using flame photometer (Jackson,1973) and potassium uptake was calculated by multiplying the potassium content (%) with respective dry matter production and expressed in kg ha^{-1}.

Iron and Zinc Uptake

The iron and zinc content in dry matter of crop were estimated by Atomic Absorption Spectrophotometer method as per the method outlined by Lindasy and Norvell (1978). The iron and zinc uptake were calculated by multiplying the iron and zinc content (%) with respective dry matter production and expressed in g ha^{-1}.

Economics

The total cost of cultivation was calculated for individual treatments on the basis of inputs used for different systems of rice cultivation and organic nutrient management practices.

Gross Returns

Gross returns were computed by multiplying economic yield with prevailing market price (Rs.19.6 and Rs. 20.6 during 2021-22 and 2022-23, respectively) and expressed in ha^{-1}.

Net Returns

Net returns were arrived by subtracting the cost of cultivation from gross returns of corresponding treatment and expressed as ha^{-1}.

Net returns (ha^{-1}) = Gross returns (ha^{-1}) – Cost of cultivation (ha^{-1})

Benefit-Cost Ratio

Benefit-cost ratio was calculated for each treatment in rice and mungbean by dividing gross returns with corresponding cost of cultivation. Benefit-cost ratio was calculated by using the following formula.

$$\text{Benefit-Cost Ratio} = \frac{\text{Gross returns (Rs. ha}^{-1})}{\text{Cost of cultivation Rs. ha}^{-1})}$$

RESULTS AND DISCUSSION

Among different systems of rice cultivation, Normal transplanted rice recorded significantly higher dry matter production and nutrient uptake(nitrogen, phosphorous, potassium, iron and zinc) which was comparable with W-DSR,whereas, significantly lower nutrient uptake and dry matter production were obtained with D-DSR. The superiority of transplanted rice over dry direct seeded rice might be due to favourable environment for increased uptake of nutrients and thereby increase the dry matter production. The increased nutrient availability under puddled condition due to formation of reduced zone for enhanced nutrient availability and reduced weed infestation compared to D-DSR (Deo *et al.,* 2019;Shahane *et al.,* 2019).

Yield attributes *viz.,* number of panicles m^{-2}, total number of grains $panicle^{-1}$, number of filled grains $panicle^{-1}$, test weight and yield (grain and straw) of rice were significantly higher with N-TPR, which was on par with W-DSR as this might be due to better environmental and eco-

physiological conditions prevailed because of less crop weed competition resulted in increased grain yield of rice. The decrease in yield of rice in D-DSR was 53.5% compared to N-TPR.

Among different nitrogen sources tested, significantly higher nutrient uptake by crop was recorded with the application of 100% RDN (120-60-60 kg N, P_2O_5, K_2O ha^{-1}) through inorganic fertilizers followed by the application of 100% RDN through poultry manure which was comparable with 50% RDN through vermicompost + 50% RDN through poultry manure. Poultry manure might have helped in improved root biomass which in turn increased the uptake of major and micro nutrients because of higher availability of major and minor nutrients. Application of organic manures enhances the microbial activity that releases different organic acids which helps in solubilization of native soil nutrients and makes available for the uptake of plants(Rao *et al.,* 2013).

Among the nitrogen management practices, yield attributes and yield were significantly higher with the treatment 100% RDN through inorganic fertilizers. Among organic sources, 100% RDN through poultry manure recorded significantly higher yield attributes and yield which was comparable with 50% RDN through vermicompost + 50% RDN through poultry manure and both of them were significantly superior over other organic sources due to higher nitrogen content in poultry manure which is readily available throughout the crop. The poultry manure is acidic in nature which might have helped in increasing the availability of nutrients due to faster mineralization process of soil. Concentration and steady nutrient release of essential nutrients for plants in the poultry manure were significantly higher compared to other organic manures. Similar results were reported by Sankaramoorthy and Rangasamy (2019) in rice crop. Whereas, significantly lower grain and straw yields were noticed with the application of 100% RDN through FYM which might be due to less availability of nutrients at critical stages of crop growth period. Similar results were found by Kyi *et al.* (2019) in rice.

The highest post-harvest soil available nutrients (nitrogen, phosphorous, potassium, iron and zinc) were estimated with N-TPR (M_1) which was statistically at par with W-DSR (M_2) and both of them were significantly superior to D-DSR (M_3) during both the years of study and in the pooled mean. Puddling results in the formation of an impervious layer that reduces the deep percolation losses of water as well as the loss of nutrients too. Puddling leads to the incorporation of weed stuff which on decomposition adds nutrients to the soil. The lowest soil available nitrogen was estimated with D-DSR as a result of various losses of nutrients. These results are in accordance with Hou (2019).Similarly, application of 100% RDN through PM (S_3) resulted in higher soil available nitrogen which was comparable with 50% RDN through VC + 50% RDN through PM (S_6) and 50 % RDN through FYM + 50% RDN through PM (S_5) which were statically at par with each other during both years of study as well as in pooled mean. This might be due to higher N content, continuous and slow release of nutrients from poultry manure.Similar findings were also reported by Prasanthrajan *et al.* (2008).

Significantly higher net returns and benefit-cost ratio was registered with treatment W-DSR compared to N-TPR and D-DSR due to decreased cost of cultivation by saving labour (Rana *et al.,* 2014). Similarly, application of 100% RDN through inorganic fertilizers recorded significantly higher net returns and benefit-cost ratio followed by application of 100% RDN through PM which might due to higher grain and through PM which might due to higher grain and straw yield.

Table 1. Nutrient uptake of rice at harvest as influenced by different systems of rice cultivation and organic nutrient management practices (Average of two years)

Treatments	Dry matter production (kg ha^{-1})	Nitrogen (kg ha^{-1})	Phosphorous (kg ha^{-1})	Potassium (kg ha^{-1})	Iron (g ha^{-1})	Zinc (g ha^{-1})
Mainplot: Systems of rice cultivation (3)						
M_1: Normal Transplanted Rice	11086	94.2	19.8	108.2	2680	297
M_2: Wet Direct Seeded Rice	10358	90.7	18.9	105.5	2666	292
M_3: Dry Direct Seeded Rice	5614	75.5	12.1	68.1	2340	198
SEm±	**189**	**1.97**	**0.41**	**2.12**	**50.5**	**3.9**
CD @ 5%	**742**	**7.7**	**1.6**	**8.3**	**198**	**15**
Subplot: Organic nitrogen management practices (7)						
S_1: 100% N-FYM	7110	69.1	14.0	75.2	2460	246
S_2: 100% N-VC	8060	79.4	15.6	84.0	2500	253
S_3: 100% N-PM	10046	96.0	18.4	103.7	2576	265
S_4: 50% N-FYM + 50% N-VC	7902	76.0	15.5	82.5	2476	250
S_5: 50% N-FYM + 50% N-PM	9214	88.6	17.2	96.6	2513	257
S_6: 50% N-VC + 50% N-PM	9608	92.7	17.8	100.6	2532	261
S_7: 100% N-RDF	11196	105.6	20.1	114.9	2876	304
SEm±	**217**	**1.49**	**9.22**	**1.37**	**58.0**	**6.5**
CD @ 5%	**622**	**4.3**	**0.66**	**4.1**	**167**	**19**
Interaction						
M at S						
SEm±	**396**	**3.09**	**0.54**	**3.06**	**105.9**	**11.2**
CD @ 5%	**1232**	**10.2**	**1.87**	**10.3**	**329**	**33**
S at M						
SEm±	**376**	**2.58**	**0.37**	**2.38**	**100.5**	**10.8**
CD @ 5%	**1078**	**7.4**	**1.1**	**6.8**	**289**	**31**

Table 2. Yield attributes and yield of rice as influenced by different systems of rice cultivation and organic nutrient management practices (Average of two years)

Treatments	Number of panicles m^{-2}	Total number of grains $panicle^{-1}$	Number of filled grains $panicle^{-1}$	Test weight (g)	Grain yield ($kgha^{-1}$)	Straw yield ($kg\ ha^{-1}$)	Net Returns (ha^{-1})	B:C ratio
Mainplot: Systems of rice cultivation (3)								
M_1 : N-TPR	285	143	128	13.78	4117	4461	51120	1.72
M_2: Wet Direct Seeded Rice	274	138	125	13.31	3922	4262	53019	1.90
M_3: Dry Direct Seeded Rice	211	109	97	10.17	1914	2842	3703	0.95
SEm±	**5.1**	**2.8**	**2.4**	**0.28**	**57.1**	**69.3**	**588**	**0.031**
CD @ 5%	**20**	**11**	**10**	**1.1**	**234**	**271**	**2303**	**0.12**
Subplot: Organic nitrogen management practices (7)								
S_1: 100% N-FYM	217	105	95	10.74	2161	2554	17151	1.16
S_2: 100% N-VC	237	119	107	10.83	2670	3219	8794	0.92
S_3: 100% N-PM	278	144	128	13.17	3849	4498	55261	1.78
S_4: 50% N-FYM + 50% N-VC	234	115	105	10.80	2486	2999	14856	1.05
S_5: 50% N-FYM + 50% N-PM	260	132	119	13.00	3490	4070	48887	1.73
S_6: 50% N-VC + 50% N-PM	268	138	124	13.06	3689	4289	43193	1.46
S_7: 100% N-RDF	301	158	139	15.34	4878	5357	63488	2.58
SEm±	**4.7**	**3.2**	**2.8**	**0.34**	**78.2**	**76.7**	**702**	**0.035**
CD @ 5%	**14**	**9**	**8**	**1.0**	**225**	**220**	**2014**	**0.10**
Interaction								
M at S								
SEm±	**9.1**	**5.9**	**5.1**	**0.61**	**137.9**	**141.1**	**1269**	**0.063**
CD @ 5%	**29**	**18**	**16**	**NS**	**420**	**441**	**3929**	**0.20**
S at M								
SEm±	**8.2**	**5.6**	**4.8**	**0.58**	**135.5**	**132.8**	**1215**	**0.060**
CD @ 5%	**23**	**16**	**14**	**NS**	**389**	**381**	**3489**	**0.17**

Table 3. Post-harvest soil available nutrient status (kg ha^{-1}) of rice as influenced by different systems of cultivation and organic nutrient management practices (Average of two years)

Treatments	Nitrogen	Phosphorous	Potassium	Iron	Zinc
M_1 : N-TPR	229	39.9	289	2.84	2.84
M_2: Wet Direct Seeded Rice	225	39.1	282	2.81	2.81
M_3: Dry Direct Seeded Rice	197	32.6	248	1.43	1.43
SEm±	**4.5**	**0.77**	**5.1**	**0.046**	**0.046**
CD @ 5%	**18**	**3.0**	**20**	**0.18**	**0.18**
S_1: 100% N-FYM	194	33.7	245	2.22	2.22
S_2: 100% N-VC	202	35.4	253	2.29	2.29
S_3: 100% N-PM	226	39.9	285	2.39	2.39
S_4: 50% N-FYM + 50% N- VC	197	34.2	250	2.26	2.26
S_5: 50% N-FYM + 50% N-PM	218	38.3	271	2.31	2.31
S_6: 50% N-VC + 50% N-PM	224	38.7	278	2.35	2.35
S_7: 100% N-RDF	191	32.8	242	2.16	2.19
SEm±	**3.3**	**0.95**	**5.0**	**0.060**	**0.059**
CD @ 5%	**10**	**2.7**	**15**	**0.18**	**0.18**
M at S					
SEm±	**6.8**	**1.41**	**7.7**	**0.096**	**0.096**
CD @ 5%	**NS**	**NS**	**NS**	**NS**	**NS**
S at M					
SEm±	**5.4**	**1.28**	**6.3**	**0.091**	**0.091**
CD @ 5%	**NS**	**NS**	**NS**	**NS**	**NS**

CONCLUSIONS

The highest nutrient uptake, yield and benefit-cost ratio were obtained in normal transplanted rice which was at par with wet direct seeded rice, whereas, net returns and benefit-cost ratio were significantly higher in W-DSR. Among the nitrogen management practices, significantly higher nutrient uptake, yield and benefit-cost ratio were recorded with 100% RDN (120-60-60 kg N, P_2O_5, K_2O ha^{-1}) through inorganic fertilizers. Among the organic sources tested, application of 100% RDN through PM in N-TPR resulted in higher grain yield and nutrient uptake in sandy clay loam soils of Southern Agro-Climatic Zone of Andhra Pradesh.

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BIOCHEMICAL ANALYSIS OF RESISTANT AND SUSCEPTIBLE COTTON GENOTYPES AGAINST ALTERNARIA AND CORYNESPORA

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ABSTRACT

Cotton leaf samples of 10 genotypes LAHB-1, L799, L 389, L770 and LPS 141 (resistant); L 1060, L 2551, L 2503, L 397 and L 2559 (susceptible) along with one control (Jaadoo BG II hybrid) against *Alternaria* and *Corynespora* were analyzed at flowering and boll development stages for the estimation of total phenols, total proteins, total sugars, reducing sugars, non-reducing sugars, chlorophyll and peroxidises. Results showed that all the biochemical parameters,except peroxidase enzyme, showed decreasing trend from flowering to boll development stage. There was significant reduction in phenols at flowering stage (16.23% to 53.30%) and at boll development stage (22.71% to 58.37%), total proteins at flowering stage (16.91% to 58.19%) and at boll development stage (9.51% to 51.43%), total sugars at flowering stage (15.71% to 51.32%) and at boll development stage (28.10% to 65.05%), reducing sugars at flowering stage (9.59% to 53.00%) and at boll development stage (35.56% to 69.93%), non-reducing sugars at flowering stage (18.38% to 49.53%) and at boll development stage (10.53% to 92.84%) and total chlorophyll at flowering stage (24.80% to 48.46%) and at boll development stage (9.19% to 32.86%). However, there was increase in peroxidase content at flowering stage (59.94% to 225.35%) and at boll development stage (38.79% to 138.79%) as the disease severity increased.

Key Words: *Alternaria, Corynespora,* Cotton, Peroxidase, Phenols

INTRODUCTION

Cotton is one of the most important commercial crops of the world, referred to as “King of Fibres” and also known as “White Gold”. India produced 590 lakh bales of 170 kg lint in 2021-2022 from an area of 126.50 lakh ha with a productivity of 466 kg ha^{-1}. Andhra Pradesh stood 8th in area (5.78 lakh ha), 6th in production (20.26 lakh bales) and 4th in productivity (596 kg/ha) during 2021 – 2022 (ICAR-AICRP, 2022). Globally cotton crop is affected by fungal, bacterial and viral diseases. In India, foliar diseases were estimated to cause yield losses ranging from 20% to 30% (Bhattiprolu and Monga, 2018). Percent seed cotton yield loss of 16.14 in Jaadoo BG II, 20.34 in RCH 2 BG II and 26.28 in L 1060 due to foliar diseases was recorded (Roshan *et al*., 2022). In India, corynespora leaf spot was first reported from Junagadh

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district of Gujarat in cotton Hybrid-4 and Hybrid-6 during 1984-1985 (Parakhia *et al.,* 1989) and yield loss of 100 to 200 lb per acre of lint was reported (Hagan and Sikora, 2012). Since 2019 Corynespora has been developing as major leaf spot of cotton in Andhra Pradesh. In view of their economic importance, influence of Alternaria and Corynespora leaf spot pathogens on different biochemical components present in resistant and susceptible genotypes was investigated to understand their role in relation to disease grade and their significance in disease resistance.

MATERIAL AND METHODS

Leaf samples from ten entries *viz*., LAHB-1, L799, L 389, L770 and LPS 141 (resistant); L 1060, L 2551, L 2503, L 397 and L 2559 (susceptible) infected with *Alternaria* and *Corynespora* along with one control (Jaadoo BG II hybrid) were collected at flowering and boll development stages from cotton field trials at RARS, Lam, Guntur during *kharif* 2020-21. Biochemical parameters including total phenols,total proteins, total sugars, reducing sugars, non-reducing sugars, chlorophyll and peroxidises were estimated (Sadasivam and Manickam, 1996). Each treatment was replicated thrice.

Estimation of total Phenols: Total phenols were estimated by Folin-Ciocalteau Reagent method (Malick and Singh, 1980).One g of cotton leaf sample was homogenized in 10 ml of 80% ethanol and centrifuged. The supernatant was reextracted and then evaporated to dryness. The residue was dissolved in distilled water and Folin Ciocalteau reagent followed by 20% Na_2CO_3 was added. The contents were mixed thoroughly, the tubes were placed in boiling water for exactly one min, cooled and absorbance at 650 nm was measured against a reagent blank. A standard curve was prepared using different concentrations of catechol. Using the standard curve the concentration of phenols in the test sample wasmeasured andexpressed as mg phenols/100 g plant sample on fresh weight basis.

Protein estimation: Protein estimation was carried out as per Lowry *et al*. (1951). 500 mg of leaf tissue ground in 5-10 ml of buffer, centrifuged and supernatant was used for protein estimation. BSA was used for preparation of standard graph and from it amount of protein in the test sample was calculated and expressed as mg protein/100 mg of sample.

Estimation of total sugars: Estimation of total sugars was carried by following anthrone method (Gerhardt *et al*., 1994). Sugars were extracted twice with hot 80% ethanol, supernatant was evaporated in a water bath at 80 °C and sugars were dissolved in water. The amount of sugars present in the extract was calculated using a standard curve prepared from glucose.

Estimation of reducing sugars: A series of standards were run using glucose (0 to 500 µg) and plot a graph.The amount of reducing sugars in the leaf sample was calculated by using standard graph (Miller, 1959).

Estimation of Non-reducing Sugars: The amount of non-reducing sugar was calculated by deducting the reducing sugar content from that of the total soluble sugars.

Chlorophyll Estimation: Total chlorophyll was estimated by following protocol of Arnon (1949). The leaves of healthy and diseased samples from upper and lower leaves were collected, washed with distilled water and blot dried. One gram of leaf sample was weighed exactly into clean mortar and was ground to a fine pulp by adding 20 ml of 80% acetone. After centrifugation at 5000 rpm for

5 min, the supernatant was transferred to 100 ml volumetric flask.The residue was further ground with 20 ml of 80% acetone, centrifuged and supernatant was transferred to the same volumetric flask.The procedure was repeated until the residue was colourless. Finally the mortar and pestle were washed thoroughly with 80% acetone and clear washings were collected into the volumetric flask.Volume was made up to 100 ml with 80% acetone and absorbance at 645, 663 and 652 nm against the solvent (80% acetone) blank were measured.The amount of chlorophyll a, chlorophyll b and total chlorophyll was estimated by using the following formulae:

Chlorophyll a (mg g^{-1}) = [12.7 (A663) - 2.69 (A645)]×V/1000 × W

Chlorophyll b (mg g^{-1}) = [22.9 (A645) - 4.68 (A663)]×V/1000× W

Total Chlorophyll (mg g^{-1}) = [20.2 (A645) +8.02 (A663)]× V/1000 × W

Where,

A = Optical density at respective wavelength (nm)

V = Final volume of chlorophyll extract in 80% acetone

W = Fresh weight of the tissue extracted

Assay of Peroxidase activity: Peroxidase activity was determined according to Hammerschimidt *et al*. (1982).The enzyme extract from leaves of cotton plants was prepared by homogenizing 1g of sample in 2 ml of 0.1 M sodium phosphate buffer (pH-6.5) at 4 ˚C. The homogenate was centrifuged at 10000 rpm at 4 ˚C for 30 min in a cooling centrifuge. The supernatant was used as enzyme source. In a sample cuvette, 1.5 ml of 0.05 M pyrogallol and 100 µl of enzyme extract were taken. In the reference cuvette, 100 µl of inactivated enzyme extract (by boiling) was taken along with 1.5 ml of 0.05 M pyrogallol. The reading for absorbance was adjusted to zero at 420 nm in a spectrophotometer with the reference sample. To initiate the reaction, 100 µl of 1% hydrogen peroxide (v/v) was added to the sample cuvette and the absorbance values were read at 420 nm. The enzyme activity was expressed as changes (Δ) in absorbance min^{-1} g^{-1} fresh weight.

RESULTS AND DISCUSSION

Variation in all biochemical components was observed in cotton leaves that contained mixed infection of *A. alternata* and *C. cassiicola* when compared with healthy leaves as control. Mixed infection of cotton leaves of resistant entries varied significantly in susceptible entriesat flowering and boll development stages. Mukewar and Mayee (2001) reported that different reactions of cotton cultivars to diseases may be related to anatomical, morphological and histochemical factors of each cultivars that cotton cultivars may show higher or lower disease severity due to those factors.

Total Phenols (mg g^{-1})

The amount of phenol varied from 3.22 to 2.46 mg g^{-1} and 2.22 to 1.79 mg g^{-1} at flowering; 3.43 to 2.57 mg g^{-1} and 2.30 to 1.85 mg g^{-1} at boll development stage in resistant and susceptible entries, respectively (Table 1). Among the resistant entries, LAHB-1 recorded the highest total phenol content at flowering (3.22 mg g^{-1}) and boll development stages (3.43 mg g^{-1}), whereas, the lowest total phenol content was observed in the susceptible entry, L 397 at both flowering (1.79 mg g^{-1}) and boll development stages (1.85 mg g^{-1}) and was on par with L 2551 having 1.85 and 1.92 mg g^{-1} total phenol at both stages, respectively (Table 1). Jaadoo BG II recorded total phenol of 3.84 mg g^{-1} (flowering) and 4.84 mg g^{-1}(boll development). Decrease in total phenol content ranged from 53.30% to 16.23% at flowering and 58.37% to 22.71% in boll development stage.

Total Phenol content increased from flowering to boll development stage in both infected and healthy leaves (Fig. 1a and b).

Mixed infection of *A. alternata* and *C. cassiicola* led to drastic reduction of total phenol content at flowering and boll development stages in resistant and susceptible entries. During infection, phenols were used as substrates for toxin production of pathogen. When infection increases slowly depletion of phenols occurred. Phenolic substances are known to be antimicrobial and act as precursor for the synthesis and accumulation of lignin, regulate the signal molecules that are directly associated with defence related genes that provides barrier against plant pathogens.

This study results are in accordance with Sumith *et al.* (2021) who reported high production of total phenol content suppressed early blight of potato caused by *A. solani*. Lalesh (2020) observed that Periwinkle leaves infected with *A. alternata* contained higher amount of phenols than healthy leaves. Satish *et al.* (2020) reported that moderately resistant genotypes had higher amount of total phenols than susceptible genotypes infected with Alternaria blight. The amount of phenol content was high in resistant genotypes than susceptible genotypes infected with karnal bunt (Pandey *et al.,* 2019). The amount of total phenol was higher in healthy leaves compared to infected leaves irrespective of the genotype reaction to *Alternaria* (Venkatesh *et al*., 2016). Hosagoudar *et al*. (2008) observed the highest total phenol content in healthy leaves compared to Alternaria infected leaves of cotton. Bashan (1986) found resistant plants infected with *A. macrospora* contained relatively higher phenol content than susceptible plants in leaves, whereas, Ramasami and Shanmugam (1976) reported that old seedlings of resistant cotton contained more quantity of total and orthodihydroxy phenols than susceptible young seedlings.

Total Protein (mg g^{-1})

The amount of total protein ranged from 5.66 to 4.75 mg g^{-1} and 4.45 to 2.85 mg g^{-1} atflowering; 8.99 to 8.08 mg g^{-1} and 7.07 to 4.83 mg g^{-1} at boll development in resistant and susceptible entries, respectively (Table 1). LAHB-1 recorded the highest total protein at flowering stage (5.66 mg g^{-1}) which was on par with L 389 (5.61 mg g^{-1}) and L 770 (5.52 mg g^{-1}) and the lowest protein content was observed in L 2259 (2.85 mg g^{-1}). However, at boll development stage L 799 expressed the highest total protein (8.99 mg g^{-1}) and the lowest total protein was noticed in L 2551 (4.83 mg g^{-1}).Total protein content variedfrom 58.19% to 16.91% at flowering stage and 51.43% to 9.51% at boll development stage, whereas, healthy leaves recorded 6.81 mg g^{-1} at flowering and 9.94 mg g^{-1} at boll development stages, respectively. It was observed that total protein content increased from flowering stage to boll development stage in both infected and healthy leaves (Fig. 1a and Fig. 1b).

Total proteins, amino acids and PR-proteins are responsible for plant resistance against diseases. When both *A. alternata* and *C.cassiicola* infected the resistant and susceptible entries there was disturbance in protein metabolism which led to decrease in protein content due to degradation of existing proteins or blockage of protein synthesis. Suhail *et al.* (2020) reported significant decrease in total soluble protein in the susceptible genotype as compared to moderately resistant and resistant genotype infected with *mungbean yellow mosaic India virus* (MYMIV). Satish *et al.* (2020) observed that moderately resistant genotypes contained maximum and significantly higher protein content in comparison to susceptible genotypes that are infected with Alternaria blight.The

amount of protein was higher in healthy plants than diseased plants among all genotypes (Mandhania *et al.,* 2018). Venkatesh *et al.* (2016) reported that irrespective of the genotype, the amount of total protein was significantly highest in resistant followed by moderately resistant, moderately susceptible and highly susceptible genotypes. Total protein content was reduced due to break down by the proteolytic enzymes and subsequent utilization of total soluble proteins by the plant pathogens at a faster rate (Sambrsoki *et al*., 1958 and Agarwal *et al*. (1982).

Total Sugars (mg g^{-1})

Among the entries, LAHB-1 recorded the highest total sugar content at flowering stage (7.44 mg g^{-1}) which was on par with L 799 (7.37 mg g^{-1}) and LPS 141 (7.17 mg g^{-1}) and the lowest sugar content was found in L 2559 (4.30 mg g-1) (Table 1). At boll development stage, LPS 141 was significantly superior over all other entries (3.95 mg g^{-1}); L 397 (1.92 mg g^{-1}), L 2551 (1.98 mg g^{-1}) and L 2559 (2.04 mg g^{-1}) were on par with each other with low total sugars. Decrease in total sugars content ranged from 51.32% to 15.71% in flowering and 65.05% to 28.10% in boll development stage.The amount of total sugars varied from 7.44 to 6.61 mg g^{-1} and 5.57 to 4.30 mg g^{-1} at flowering; 3.95 to 3.20 mg g^{-1} to 2.39 to 1.92 mg g^{-1} at boll development in resistant and susceptible entries, respectively. Comparatively healthy leaves registered 8.83 mg g^{-1} at flowering and 5.49 mg g^{-1} at boll development stage. Data showed that total sugar content decreased from flowering to boll development stage in both resistant and susceptible entries (Fig. 1a and b). Reduction of sugars, reducing sugars and non-reducing sugars were due to complete utilization of carbohydrates by the *A. alternata* and *C. cassiicola,* because sugars are the primary choice for nutrition of pathogen and breakdown of sugar contents by the pathogens as the infection starts.

These results agreed with Lalesh (2020) who reported that periwinkle leaves infected with *A. alternata* showed significant reduction in total soluble sugar content than healthy leaves. Pandey *et al.* (2019) observed that total sugar content was higher in resistant genotypes than susceptible genotypes. Venkatesh (2016) found that total sugars were significantly higher in resistant genotype followed by moderately resistant, moderately susceptible and susceptible genotypes. Nath *et al*. (2015) reported that total sugars play an important role in disease resistance as they are the precursors for the synthesis of phytoalexins and phenolic compounds which suppress the cellulolytic enzymes and pectolytic enzymes that are essential for the disease development.

Reducing Sugars (mg g^{-1})

The amount of reducing sugars ranged from 4.11 to 3.95 mg g^{-1} and 3.04 to 2.14 mg g^{-1} at flowering; 2.25 to 1.96 mg g^{-1} and1.78 to 1.05 mg g^{-1} at boll development in resistant and susceptible entries, respectively (Table 1) of all the entries, LAHB-1 exhibited the highest reducing sugar content at flowering (3.95 mg g^{-1}) which was on par with L 799 (3.99 mg g^{-1}) and the lowest reducing sugar content was recorded in L 2559 (2.14 mg g-1). At boll development stage, LAHB-1 expressed the highest total reducing sugars, on par with the resistant entries except L 799 (1.96 mg g^{-1}). The lowest total reducing sugars were observed with L 2551 (1.05 mg g^{-1}); L 2559 (1.12 mg g^{-1}) and L 1060 (1.18 mg g-1) were on par with it (Fig 1a and b).

These results were in accordance with Satish *et al*. (2020) who reported that leaves infected with Alternaria blight had less content of reducing sugars in susceptible genotypes than resistant varieties. Pandey *et al.* (2019) reported that reducing sugar content was

higher in resistant cultivars than susceptible. The magnitude of reduction in reducing sugar was 12.7% in tolerant varieties than susceptible genotypes (Srivastava, 2018).

Non-Reducing Sugars (mg g^{-1})

The amount of non-reducing sugars varied from 3.06 to 3.49 mg g^{-1} and 2.83 to 2.16 mg g^{-1} at flowering; 1.70 to 1.24 mg g^{-1} and1.21 to 0.14 mg g^{-1} at boll development in resistant and susceptible entries, respectively (Table 1). Comparatively healthy leaves showed 4.28 mg g^{-1} at flowering stage and 1.90 mg g^{-1} at boll development stage. Except LPS 141, all other resistant entrieswere found on par with each other with high non reducing sugars at flowering stage.The lowest non reducing sugar was observed in L 2559 (2.16 mg g^{-1}) and was on par with L 2503 (2.30 mg g^{-1}). The entry, LPS 141 expressed the highest non reducing sugar content at boll development stage while L 397 exhibited the lowest non reducing sugar content at boll development stage (0.14 mg g^{-1}) (Fig. 1a and 1b).

Pandey *et al.* (2019) reported that non-reducing sugar content was higher in resistant cultivars than susceptible genotypes. Oke **(**1988) reported that *C.cassiicola* infected tobacco leaves expressed decrease in total sugars and reducing sugars both in young and mature leaves when compared to healthy leaves. The *Bt* cotton genotypes infected with *A. macrospora* were reported with decreased reducing and non-reducing sugars (Hosagoudar *et al.*, 2008). Suryawanshi *et al.* (2017) reported decrease in reducing sugars in resistant, moderately resistant and susceptible cotton genotypes (5.7%, 18.1% and 24.6% respectively) and non-reducing sugars (13.6%, 18.3% and 34.9%, respectively), 10 days after inoculation with *A. macrospora*. It was reported that the amount of decrease was high in susceptible genotypes than resistant genotypes.

During successful infection process pathogens bring about changes in the synthesis of biochemical molecules for their establishment. They utilize carbon from the sugars for their biomass development which lead to reduction in sugar content in diseased plants. Thus, for a successful host-pathogen interaction, there will be requirement of sugars for increased respiration or utilization of sugars by the fungi which in turn depends on the capability of fungi to secrete carbohydrate degrading enzyme (Prasad *et al*., 1960).

Chlorophyll a (mg g^{-1})

There was significant reduction of Chlorophyll 'a' content in mixed infection of *Alternaria* and *Corynespora* in resistant and susceptible entries than healthy leaves at flowering and boll development stages (Table 2). At flowering stage, it ranged from 1.30 to 1.44 mg g^{-1} in resistant entries and 1.11 to 1.26 mg g^{-1} in susceptible entrieswhen compared to healthy leaves (1.73 mg g^{-1}); in boll development stage it varied from 1.63 to 1.70 mg g^{-1} in resistant entries and 1.37 to 1.53 mg g^{-1} in susceptible entries when compared to healthy leaves (1.85 mg g^{-1}) (Fig. 1a and 1b).

Chlorophyll b (mg g^{-1})

At flowering stage, chlorophyll 'b' ranged from 0.32 to 0.52 mg g^{-1} in resistant entriesand 0.16 to 0.32 mg g^{-1} in susceptible entries when compared to healthy leaves (0.75 mg g^{-1}). Entries, L 770 (0.52 mg g^{-1}) and LAHB-1 (0.50 mg g^{-1}) recorded high chlorophyll 'b' and found to be on par with each other, whereas, L 2551 (0.16 mg g^{-1}) and L 2559 (0.20 mg g^{-1}) were on par. At boll development stage it varied from 0.64 to 0.78 mg g^{-1} in resistant entries,significantly superior in L 389 with maximum chlorophyll b (0.78 mg g^{-1}) and minimum chlorophyll 'b' was obtained in L 2503 (0.46 mg g^{-1}) which was on par with L 2559 (0.51 mg g^{-1}) and 0.46 to 0.57 mg g^{-1} in

susceptible entries when compared to healthy leaves (0.88 mg g^{-1}) (Fig 1a and b).

Total Chlorophyll (mg g^{-1})

Total chlorophyll content decreased at both flowering and boll development stages when leaves contained mixed infection of *A. alternata* and *C. cassiicola* (Table 2). It ranged from 1.67 to 1.86 mg g^{-1} and 1.31 to 1.55 mg g^{-1} in resistant and susceptible entries at flowering stage compared to healthy leaves (2.47 mg g^{-1});at boll development stage, from 2.29 to 2.47 mg g^{-1} and 1.83 to 2.08 mg g^{-1} in resistant and susceptible entriescompared to healthy leaves (2.72 mg g^{-1}), respectively. Reduction in the total chlorophyll content varied from 48.46% to 24.80% in flowering stage and 32.86% to 9.19% in boll development stage (Fig 1a and Fig 1b).

At flowering and boll development stages there was overall reduction of chlorophyll content in all entries due to breakdown of photosynthetic pigments and damage to photo systems that was affected by the metabolites of toxic production. Results obtained are in line with the report of Lalesh Kumari (2020) who reported there was a significant reduction of total chlorophyll, chlorophyll 'a' and chlorophyll 'b' of peri winkle leaves infected with *A. alternata* at all levels of disease intensities due to breakdown of photosynthetic apparatus. Satish *et al.* (2020) found that moderately resistant genotypes had higher chlorophyll content than the susceptible genotypes in cluster bean leaves due to infection of Alternaria blight. Suryawanshi *et al.* (2017) reported that chlorophyll a content was decreased when inoculated with *A. macrospora* in resistant (12.9%), moderately resistant (14.4%) and susceptible (18.2%) cotton genotypes similarly, with decreased chlorophyll b and total chlorophyll. Reduction in chlorophyll content causes decrease in photosynthesis rate which led to decline in total sugar level under Alternaria blight stress condition in mustard genotypes (Mallick *et al.*, 2015). Reduction in chlorophyll was due to production of toxic metabolites by the pathogen or inhibited the synthesis of chlorophyll rather than the degradation of pre-existing pigments.

Peroxidases

Significant variation was observed in peroxidases when leaves were infected with *Alternaria* and *Corynespora* in resistant and susceptible entries at flowering and boll development stages (Table 2). At flowering stage, it ranged from 0.43 to 0.56 Δ abs^{-1} min^{-1} g^{-1} and 0.63 to 0.87 Δ abs^{-1} $min^{-1}g^{-1}$ in resistant and susceptible entriescompared to healthy leaves (0.27 Δ abs^{-1} min^{-1} g^{-1});at boll development stage, it varied from 0.54 to 0.73 Δ abs^{-1} $min^{-1}g^{-1}$ and 0.75 to 0.93 Δ $abs^{-1}min^{-1}g^{-1}$ in resistant and susceptible entriescompared to healthy leaves (0.39 Δ $abs^{-1}min^{-1}g^{-1}$), respectively. Increase in peroxidases varied from 59.94% to 225.35% in flowering stage and 38.79% to 138.79% in boll development stage (Fig 1a and b).

Mixed infection of *A. alternata* and *C. cassiicola* in flowering and boll development stages caused damage to the cell walls which are gateway to other pathogens for their establishment so that as infection rises peroxidases are required.Peroxidases produce reactive oxygen species that are lethal to the pathogen and also involved in the wound healing, suberization and lignification of plant cell walls. Suhail *et al*. (2020) reported that after inoculation of *Mungbean yellow mosaic India virus* (MYMIV), the activity of peroxidases increased in all resistant and susceptible genotypes. Sidra Hameed *et al.* (2017) observed increased POD activity in leaves infected by phytoplasma over the healthy plants in Mungbean. Peroxidase activity was higher in Alternaria infected leaves than in healthy leaves of all genotypes (Venkatesh *et al.,* 2016).

Table 1. Total phenols, proteins, sugars, reducing and non-reducing sugars in Alternaria and Corynespora infected leaves of Cotton

Treatment	Total phenols		Total proteins		Total sugars		Total reducing sugars		Non reducing sugars	
	Flowering stage (mg g^{-1})	Boll development stage (mg g^{-1})*	Flowering stage (mg g^{-1})	Boll development stage (mg g^{-1})*	Flowering stage (mg g^{-1})	Boll development stage (mg g^{-1})*	Flowering stage (mg g^{-1})	Boll development stage (mg g^{-1})*	Flowering stage (mg g^{-1})	Boll development stage (mg g^{-1})*
Resistant Entries										
LAHB-1	3.22	3.43	5.66	8.35	7.44	3.71	3.95	2.26	3.49	1.45
L799	2.74	2.76	4.75	8.99	7.37	3.20	3.94	1.96	3.43	1.24
L389	3.02	3.06	5.61	8.22	7.01	3.45	3.72	2.20	3.30	1.25
L770	2.89	2.99	5.52	8.08	6.61	3.60	3.34	2.16	3.27	1.44
LPS 141	2.46	2.57	5.29	8.52	7.17	3.95	4.11	2.25	3.06	1.70
Susceptible Entries										
L 1060	2.21	2.29	3.56	5.56	5.77	2.39	3.04	1.18	2.73	1.21
L 2551	1.85	1.92	4.45	4.83	5.46	1.98	2.66	1.05	2.80	0.93
L 2503	2.20	2.22	3.88	6.83	4.80	2.39	2.50	1.48	2.30	0.91
L 397	1.79	1.85	4.22	7.07	5.56	1.92	2.73	1.78	2.83	0.14
L 2559	2.28	2.30	2.85	5.88	4.30	2.04	2.14	1.12	2.16	0.91
Control (Jaadoo BG II)	3.84	4.44	6.81	9.94	8.83	5.49	4.55	3.50	4.28	1.90
SEm±	0.03	0.07	0.08	0.06	0.09	0.07	0.08	0.06	0.12	0.05
CD ($P \leq 0.05$)	0.10	0.21	0.25	0.18	0.27	0.22	0.22	0.17	0.36	0.15
CV (%)	2.23	4.56	3.02	1.44	2.45	4.12	3.93	5.38	6.92	7.25

*Mean of three replication

Table 2. Chlorophyll a, b, Total chlorophyll and Peroxidases in Alternaria and Corynespora infected leaves of Cotton

Treatment	Chlorophyll a		Chlorophyll b		Total chlorophyll		Peroxidases	
	Flowering stage (mg g^{-1})	Boll development stage (mg g^{-1})*	Flowering stage (mg g^{-1})	Boll development stage (mg g^{-1})*	Flowering stage (mg g^{-1})	Boll development stage (mg g^{-1})*	Flowering stage (Δ $abs^{-1}min^{-1}g^{-1}$)	Boll development stage (Δ $abs^{-1}min^{-1}g^{-1}$)*
Resistant Entries								
LAHB-1	1.37	1.68	0.50	0.72	1.86	2.39	0.56	0.63
L799	1.38	1.63	0.39	0.64	1.77	2.27	0.51	0.68
L389	1.30	1.70	0.38	0.78	1.67	2.47	0.44	0.54
L770	1.30	1.58	0.52	0.71	1.82	2.29	0.54	0.65
LPS 141	1.44	1.68	0.32	0.70	1.75	2.37	0.43	0.73
Susceptible Entries								
L 1060	1.26	1.51	0.25	0.57	1.51	2.08	0.79	0.83
L 2551	1.16	1.48	0.16	0.54	1.32	2.02	0.63	0.83
L 2503	1.23	1.37	0.32	0.46	1.55	1.83	0.72	0.75
L 397	1.19	1.47	0.23	0.53	1.42	2.00	0.74	0.93
L 2559	1.11	1.53	0.20	0.51	1.31	2.04	0.87	0.90
Control (Jaadoo BG II)	1.73	1.85	0.75	0.88	2.47	2.72	0.27	0.39
SEm±	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.02
CD @ 5%	0.03	0.03	0.06	0.05	0.05	0.06	0.05	0.05
CV (%)	1.47	1.11	9.68	4.92	1.78	1.57	4.95	3.93

* Mean of three replications

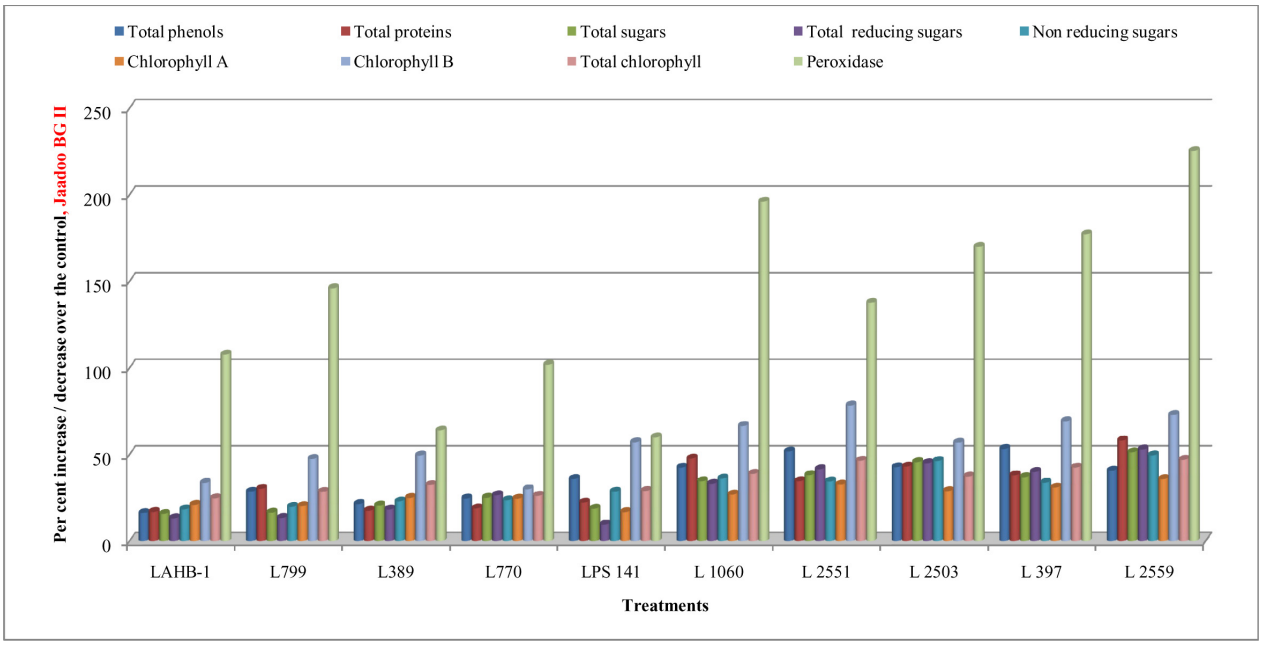

Figure 1a. Biochemical differences in Alternaria and Corynespora infected leaves of Cotton entries at flowering stage

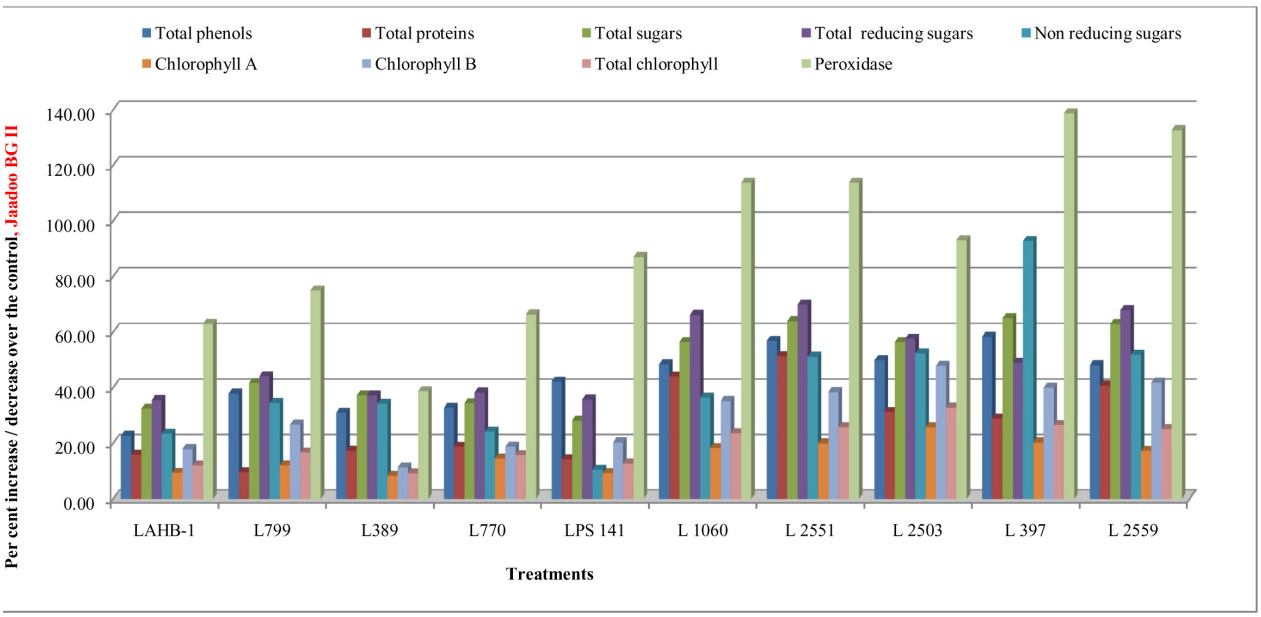

Figure1b. Biochemical differences in Alternaria and Corynespora infected leaves of Cotton entries at boll development stage

CONCLUSIONS

Cotton genotypes infected with *Alternaria* and *Corynespora* showed significant reduction in phenols, total proteins, total sugars, reducing sugars, non-reducing sugars, total chlorophyll but increase in peroxidase content at flowering and boll development stages as the disease severity increased while defending the mixed infection.

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EFFECT OF LEAD NITRATE ON CARBOHYDRATES AND LIPIDS CONTENT OF CHICKPEA DURING GERMINATION

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ABSTRACT

This study has been carried out to examine effects of lead nitrate on the carbohydrates and fats content of *Cicer arietinum* L. during germination. This study reported the carbohydrate content in seedling during germination decreased over time. At lead nitrate concentrations of 0 %, 0.025 %, 0.05 %, 0.1 % the carbohydrate concentration increased by 21.6 %, 19.5 %, 15.8 % and 19.5 %, respectively. It was found that the increase in lead nitrate concentration affects the fat content of cotyledon with increasing time period but decreased with the simultaneous rise in lead nitrate concentration. Fat content was 5.3% after 24 h treatment of 0.1% lead nitrate which reduced to 3.5% after 144 h of lead nitrate treatment at the same concentration. Lead nitrate was responsible for the depletion in the synthesis of carbohydrates and fats in seedlings.

Keywords: Carbohydrate content, *Cicer arietinum* L., Fat content, Germination, Lead nitrate

INTRODUCTION

Cicer arietinum L. (Chickpea) of the family Leguminosae is an important nutritive pulse extensively utilized as a protein adjunct to starchy diets in India. It is consumed as a high fibre and protein containing food (Sharma *et al*., 2021). A whole gram contains protein- 17.1 g; fat- 5.3 g; minerals matters- 2.7 g; fibre- 3.9 g; carbohydrate- 16.2 g; calcium- 0.19 g; phosphorus- 0.24 g for 100 g. Tender shoots of the plant are consumed as vegetable. It is also applied as medicine for treating diarrhea, flatulence, constipation, sunstroke (Singh and Gahlot, 2018).

The gram seed contains large amount of various polysaccharides as reserve food material and the polysaccharides like lipid, carbohydrate are utilized during the germination of seeds. The quantitative changes in various reserve materials like proteins, amino acids, starch and activities of various types of enzymes are related with the germination of chickpea. During germination, starch and polyfructosans were found to be depleted to a level of 20% and 47% of the gram seeds dry weight respectively (Madurapperumage *et al*., 2021).

Heavy metal toxicity is a modern day problem which severely impacts the production and food quality of plants. Though plants need heavy metals in a small amount but any excess of this metal may reduce the nutrient absorption and translocation and also uptake of water which may ultimately cause the plant death. The heavy metal toxicity were found to cause chlorosis, inhibition of photosynthesis, growth etc (Ali and Gill, 2022). Studies showed that heavy metals like Cd, Pb, Hg were highly toxic

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than the other heavy metals like Cu, Zn, Cr, Ni; As because Cd, Pb, Hg can cause toxicity even in low concentration (Pande *et al*., 2022). The heavy metal like lead is considered non essential nutrients of plant and it can cause toxicity in minute quantities. Industrial activities like mining, sewage sludge, paints containing excessive lead causes lead contamination of soil (Osman and Fadhlallah, 2023). Lead usually does not show any significant effect upon short time exposure but it may be highly toxic for any long time exposure. The heavy metals alter physiological, genetical and biochemical profile of the seeds at the time of germination and growth. It is also found that seedling growth is more prone to heavy metals compared to seed germination. Therefore, in this study it was aimed to examine the effect of lead nitrate on physiological and biochemical changes in the course of seed germination of chickpea.

MATERIAL AND METHODS

Collection of Plant Materials

Certified healthy seeds of *Cicer arietinum* L. (chickpea) were collected from the Guwahati branch of Seed Corporation of Assam for experimentation. The collected seeds were cleaned by surface sterilization and washed with distilled water thoroughly.

Seed germination

The required chemicals for experimentation were supplied by the Department of Biotechnology, Gauhati University, Guwahati. Preliminary work on effect of lead nitrate on germination of chickpea seeds showed tangible results while concentrations were used in percentage. So, test solutions were made in percentage. Clear healthy seeds of uniform size were selected and surface sterilized and the seeds were soaked with 0.025%, 0.05% and 0.1% concentration of lead nitrate solution along with a control (0%) for 24 h. The gram seeds were kept soaked for different intervals of time by spraying distilled water and respective test solution of $PbNo_3$ of different concentrations and they were allowed to germinate at room temperature and where they were exposed to 12 h light and 12 h dark period in a 24 h cycle. The germination process was observed at different time interval as 24 h, 48 h, 72 h, 96 h and 144 h.

Determination of Carbohydrates

Total carbohydrates of the sample were determined by Anthron method (Sadasivan and Manikam, 1992). Weighed 100 mg of the collected sample and poured into the boiling tube and they were kept in a water bath for 3 hours where 5 ml of 2.5 N HCl was also added and cooled at room temperature. Then, solid sodium carbonate was used to neutralize the mixture until effervescence ceased; the volume was made to 100 ml and then it is centrifuged. The supernatant was collected and 0.5 and 1 ml of aliquots were taken for analysis; then prepared the standard by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of working standard where zero serves as blank. The volume of the tubes was made up to 1ml by adding distilled water, and then added 4 ml of Anthrone reagent; then it was heated in boiling water bath for eight minutes, and cooled rapidly and read the green to dark green color of 630 nm. By plotting concentration of the standard on X-axis versus absorbance on Y-axis, a standard graph was drawn. From the graph the amount of carbohydrate was calculated which was present in the sample tubes.

The amount of carbohydrate was calculated as– Amount of carbohydrates present in 100 mg of the sample

Estimation of Fat

$$= \frac{\text{Mg of glucose}}{\text{Volume of test sample}} X\ 100$$

The seeds of *Cicer arietinum* L. were allowed to germinate in three replications at 0%, 0.25%, 0.05% and 0.1% concentrations of lead nitrate. After germination the cotyledons and seedlings were separated. The cotyledons of respective replicates peel inside hot air oven at 180 °C for about 2 h. Then the dry weight of the cotyledons was recorded after 24 h, 48, 72, 96 and 144 h of germination for each concentration. Then the seed was ground and estimation of fat was done by cold extraction method with petroleum ether. The extraction was made by adding 5 ml of petroleum ether 8-10 times into extraction tubes which was collected in conical flask followed by evaporation. The weight of the conical flasks was recorded earlier. After evaporation, weight was calculated by deducing the initial weight of the conical flask from the conical flask with fat for each sample.

Analysis of Data

The data obtained from the experiments were statistically analyzed to study the effects of various factors and their interaction by variance ratio.

Critical difference: C.D. was evaluated as follows:

$CD = \frac{\text{EMSS} \times 2}{\text{n}} \times \text{t}$ Value at 5% or 1% level for error difference

in which, n= total number used for calculating the means.

The calculated value of C.D. was utilized for testing the difference between the two mean values as significant or not.

Standard Error: In the case of estimation of carbohydrate, fat content and also the growth, fresh weight and dry weight for each treatment, the mean was calculated from the three replications. From the mean value the standard error was calculated using the following formula

$$SE = \sqrt{[\frac{\sum d^2}{N}]}$$

Where, d=deviation from mean, N=number of replications.

RESULTS AND DISCUSSION

Carbohydrate content in cotyledon during germination

The estimation of carbohydrate content was performed at of 24 h, 48 h, 72 h, 96 h and 144 h hours following Anthrone method. For the experiment, one day old germinated seeds were taken. The mean percentage of carbohydrates (Table 1) was recorded. The carbohydrate content in cotyledon was increased with time but as the concentration of lead nitrate increased, carbohydrate content decreased (Figure 1).The analysis of variance shows the effect of lead nitrate and time period on carbohydrate content to be highly significant establishing carbohydrate content varied considerably during germination period, however the interaction of lead nitrate and time period is not significant (Table 2).

Carbohydrate content in seedling during germination

The procedure for the germination experiment is same as in the previous experiment. The estimation of carbohydrate in seedling was conducted using Anthrone method. The mean percentages of carbohydrates content in seedling is presented (Table 3). It is observed from Table 7 and Figure 2 that carbohydrate content in seedling was decreased with germination time period during germination. Furthermore, with the increase of concentration of lead nitrate, the carbohydrate percentage was increased in seedling. The ANOVA revealed that the effect of lead nitrate was not significant in carbohydrate content of the seedling, but the

$$\% \text{ of Fat} = \frac{\text{Increase in weight of the conical flask after evaporation}}{\text{Initial weight of the conical flask}} \times 100$$

effect of time period is found to be significant. Moreover, the change of carbohydrate content in different time period of the seedling growth is not caused by lead nitrate, therefore lead nitrate do not affect carbohydrate content of seedling (Table 4).

Fat content in cotyledon during germination

Seeds of chickpea were allowed to germinate at 0%, 0.025%, 0.05% and 0.1% concentration of lead nitrate. The estimation of fat was conducted at 24 h, 48 h, 72 h, 96 h and 144 h of germination by cold extraction method with petroleum ether. The mean percentage of fat is presented in Table 5. From the mean percentage of fat, the fat content in cotyledon was observed to be decreasing gradually during germination period. With increasing lead nitrate concentration the fat content (%) increased (Figure 3).The analysis of variance (Table 6) revealed the lead nitrate effect on fat content of cotyledon was significant at 5% level. The effect of time period was observed to be highly significant i.e. fat content decreases during different time intervals. The interaction effect was found not significant.

Fat content in seedling during germination of chickpea

Seeds of chickpea were allowed to germinate at 0%, 0.025%, 0.05% and 0.1% concentration of lead nitrate. The estimation of fat content was conducted after 24 h, 48 h, 72 h, 96 h and 144 h of germination using cold extraction method with the help of petroleum ether. The mean percentage of fat is presented in Table 7 and Figure 4. The analysis of variance revealed that the effect of lead nitrate and time period on fat content in seedling was found to be highly significant, indicating, inhibitory effect of lead nitrate with time period (Table 8).

After observing seed germination and development of seedling under laboratory condition, carbohydrates content were found increasing with time in cotyledons. The carbohydrate content was observed more significant after 144 h germination. At lead nitrate concentrations of 0%, 0.025%, 0.05%, 0.1% the carbohydrate concentration increased by 21.6%, 19.5%, 15.8% and 19.5% respectively after 144 h of germination the carbohydrate content in seedling at 0.1% lead nitrate was 72.6% as against 58.3% at 0% lead nitrate. Hence, carbohydrate content in seedling during germination was seen to decrease with progress of time. This decrease in the amount carbohydrate in the seedling indicates that the carbohydrates were being utilized by the seedling. The effect of lead nitrate concentration on fat content of cotyledon was more significant with increasing time period but it also decreased due to the simultaneous rise in concentrations. Lead nitrate may be cause for the reduction in synthesis of fat in seedlings. This kinds of similar observations were reported by Naz *et al*. (2015), Ullah *et al*. (2019), Medda and Mondal (2017) on heavy metal studies on *Cicer arietinum* L. Naz *et al*. (2015) observed that lead concentration reduced the nodule number per plant of *Cicer arietinum* L. at 52.85, 105.70 and 211.40 mg kg/ soil concentration after 60, 90 and 135 days when compared with the control. Ullah *et al*. (2019) studied Cadmium toxicity in *Cicer arietinum* L. and observed that Cadmium reduced the plant height; fresh and dry biomass of three cultivars of chickpea namely (NC234 (NC2)), ICCV89310 (IC8)) and ICCV 89323-B(IC8-B). Medda and Mondal (2017) studied Cr toxicity in chickpea where they observed that Cr affected the growth and development of the plant by reducing elongation of coleoptiles. They also observed the ultra structural deformation of shoot and root. Islam *et al*. (2022) also studied the effect of Chromium on germination and growth of chickpea which also showed the detrimental effect of heavy metal on growth and germination of chickpea.

Table 1. Carbohydrate content (%) in cotyledon during germination treated with lead nitrate

S.No.	Germination period(Hour)	0% $PbN0_3$ (Control)	0.025% $PbN0_3$	0.05% $PbN0_3$	0.1% $PbN0_3$
1	24	58.3 ± 0.07	58.2 ± 0.67	63.8 ± 0.39	58.4 ± 0.47
2	48	66 ± 1.22	66.2 ± 0.69	65.9 ± 0.81	64.3 ± 2.04
3	72	69.8 ± 0.75	65.2 ± 0.98	69.4 ± 0.64	68.2 ± 0.15
4	96	71.2 ± 1.4	69.5 ± 0.56	70.8± 0.85	67.9 ± 0.71
5	144	74.4 ± 0.98	72.5 ± 0.37	75.7 ± 0.37	72.6 ± 0.48

C.D. for Lead nitrate at 1% level (n=12) = 2.13
C.D. for Lead nitrate at 5% level (n=12) = 1.62
C.D. for Lead nitrate at 1% level (n=15) = 12.21
C.D. for Lead nitrate at 5% level (n=15) = 1.45

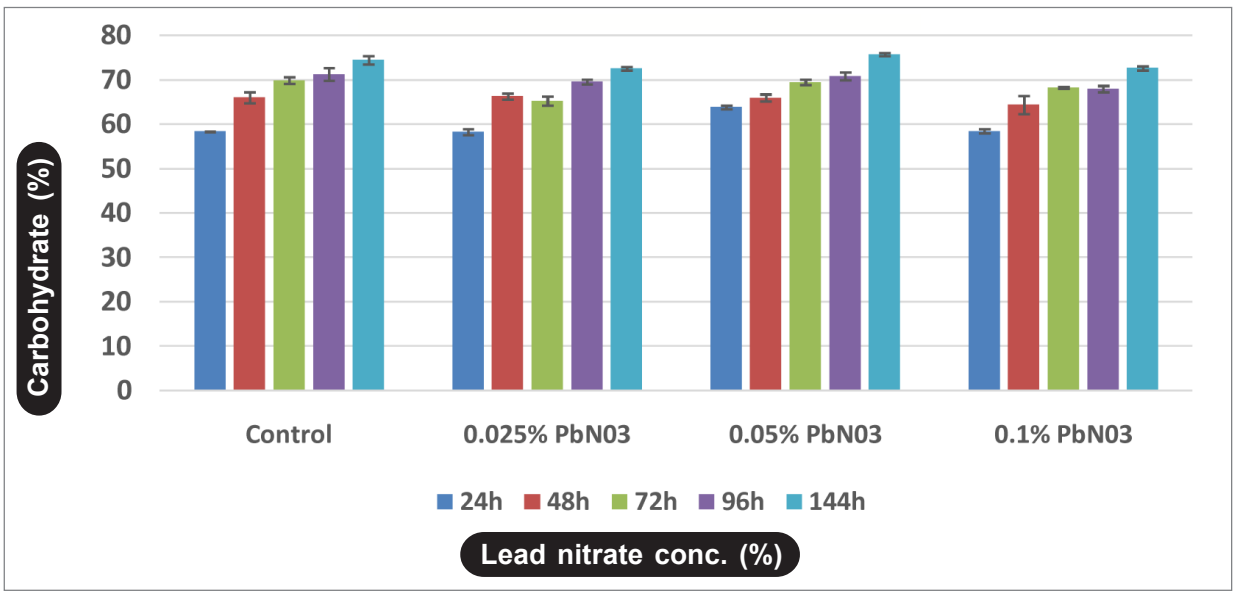

Figure 1. Carbohydrate content (%) in cotyledon during germination when treated with lead nitrate

Table 2. Analysis of variance of carbohydrate content (%) in cotyledon during germination treated with lead nitrate

S.No.	Sources of Variation	Degrees of Freedom(df)	Sum of Squares(ss)	Mean Sum of Squares (Mss)	Variance Ratio
1	PbN03(conc.)	3	91.31	30.44	7.39*
2	TimePeriod	4	1301.46	325.37	78.97 **
3	Interaction	12	76.6	6.34	1.54
4	Error	40	164.82	4.12	
	Total	59			

**** Significant at 1% level**

Table 3. Carbohydrate content (%) in seedling during germination treated with lead nitrate

S.No.	Germination period(Hour)	0% $PbN0_3$ (Control)	0.025% $PbN0_3$	0.05% $PbN0_3$	0.1% $PbN0_3$
1	24	70.8 ± 4.3	70.9 ± 2.8	71.4 ± 0.6	73.2 ± 0.91
2	48	67.4 ± 0.29	68.2 ± 0.46	68.8 ± 0.12	71.2 ± 0.60
3	72	62.3 ± 1.2	60.9 ± 1.7	61.5 ± 0.50	64.2 ± 0.57
4	96	56.1 ± 1.2	56.6 ± 0.17	55 ± 0.89	60.2 ± 1.10
5	144	52.7 ± 0.3	53 ± 1.04	55 ± 0.04	57 ± 0.8

C.D. for Lead nitrate at 1% level (n=12) = 4

C.D. for Lead nitrate at 5% level (n=12) = 3.04

C.D. for Lead nitrate at 1% level (n=15) = 3.57

C.D. for Lead nitrate at 5% level (n=15) = 2.72

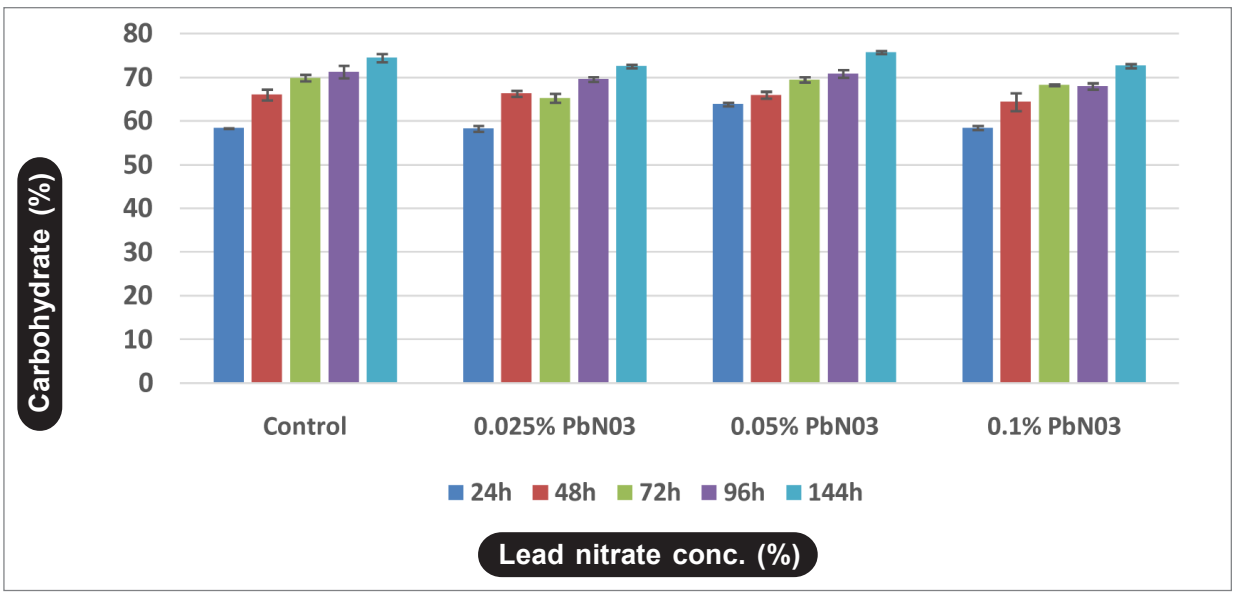

Figure 2. Carbohydrate content (%) in seedling during germination treated with lead nitrate

Table 4. Analysis of variance of carbohydrate content (%) in seedling during germination treated with lead nitrate

S.No.	Sources of Variation	Degrees of Freedom (df)	Sum of Squares(ss)	Mean Sum of Squares (Mss)	Variance Ratio
1	$PbN0_3$ (conc.)	3	112.68	37.56	2.6
2	Time Period	4	2617.76	654.44	45.23 **
3	Interaction	12	28.98	2.415	0.17
4	Error	40	578.85	14.47	
	Total	59			

** Significant at 1% level

Table 5: Fat content (%) in cotyledon during germination treated with lead nitrate

S.No.	Germination period(h)	0% $PbN0_3$ (Control)	0.025% $PbN0_3$	0.05% $PbN0_3$	0.1% $PbN0_3$
1	24	5 ± 0.05	4.9 ± 0.14	5.1 ± 0.08	5.3 ± 0.4
2	48	4.5 ± 0.12	4.6 ± 0.11	4.7 ± 0.08	4.8 ± 0.17
3	72	4 ± 0.16	3.9 ± 0.08	3.5 ± 0.29	4.3 ± 0.09
4	96	3.4 ± 0.22	3.5 ± 0.24	3.7± 0.22	4.1 ± 0.38
5	144	2.5 ± 0.02	2.7 ± 0.12	2.9 ± 0.42	3.5 ± 0.33

C.D. for Lead nitrate at 1% level (n=12) = 0.36
C.D. for Lead nitrate at 5% level (n=12) = 0.48
C.D. for Lead nitrate at 1% level (n=15) = 0.32
C.D. for Lead nitrate at 5% level (n=15) = 0.43

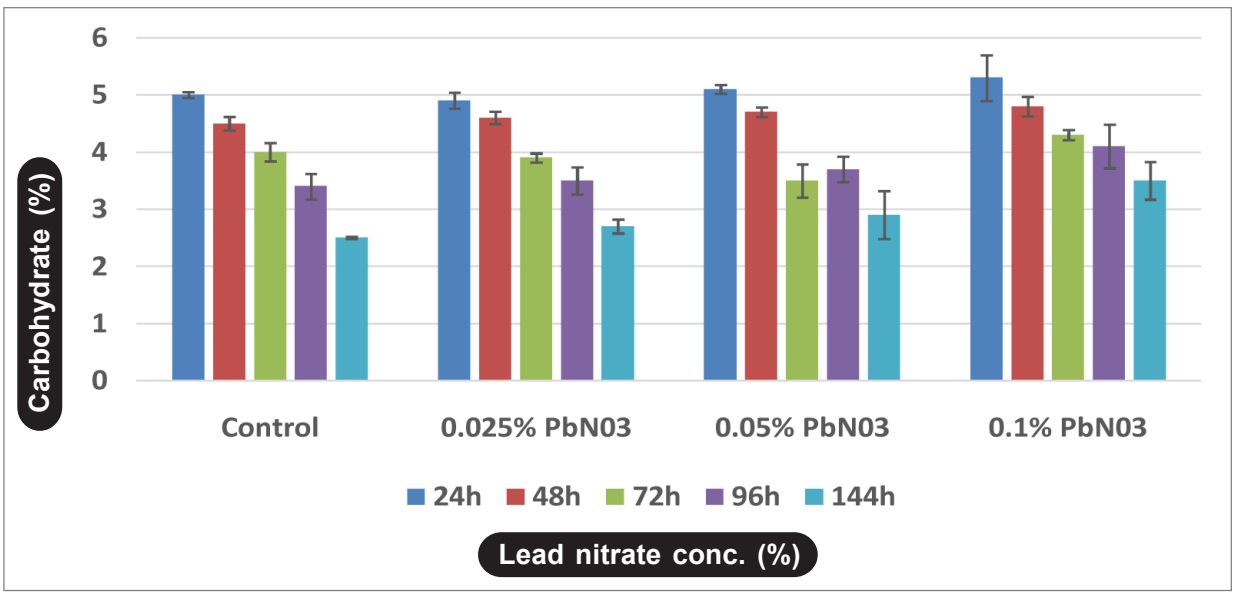

Fig. 3. Fat content (%) in cotyledon during germination treated with lead nitrate

Table 6. Analysis of variance of fat content (%) in cotyledon during germination treated with lead nitrate

S.No.	Sources of Variation	Degrees of Freedom (df)	Sum of Squares(ss)	Mean Sum of Squares (Mss)	Variance Ratio
1	$PbN0_3$(conc.)	3	2.42	0.81	3.86 *
2	Time Period	4	34.65	8.66	41.24 **
3	Interaction	12	0.75	0.063	0.3
4	Error	40	8.55	0.21	
	Total	59			

** Significant at 1% level

Table 7: Fat content (%) in seedling during germination treated with lead nitrate

S.No.	Germination period(Hour)	0% $PbN0_3$ (Control)	0.025% $PbN0_3$	0.05% $PbN0_3$	0.1% $PbN0_3$
1	24	3.2 ± 0.26	3.1 ± 0.43	3.0 ± 0.24	2.6 ± 0.34
2	48	4.2 ± 0.45	4.0 ± 0.04	3.7 ± 0.21	3.0 ± 0.5
3	72	6.2 ± 0.48	6.0 ± 0.04	5.8 ± 0.48	4.9 ± 0.19
4	96	6.8 ± 0.15	6.5 ± 0.34	6.1± 0.09	5.2 ± 0.33
5	144	7.0 ± 0.33	7.0 ± 0.21	6.6 ± 0.04	5.8 ± 0.33

C.D. for Lead nitrate at 1% level (n=12) = 0.62
C.D. for Lead nitrate at 5% level (n=12) = 0.47
C.D. for Lead nitrate at 1% level (n=15) = 0.55
C.D. for Lead nitrate at 5% level (n=15) = 0.42

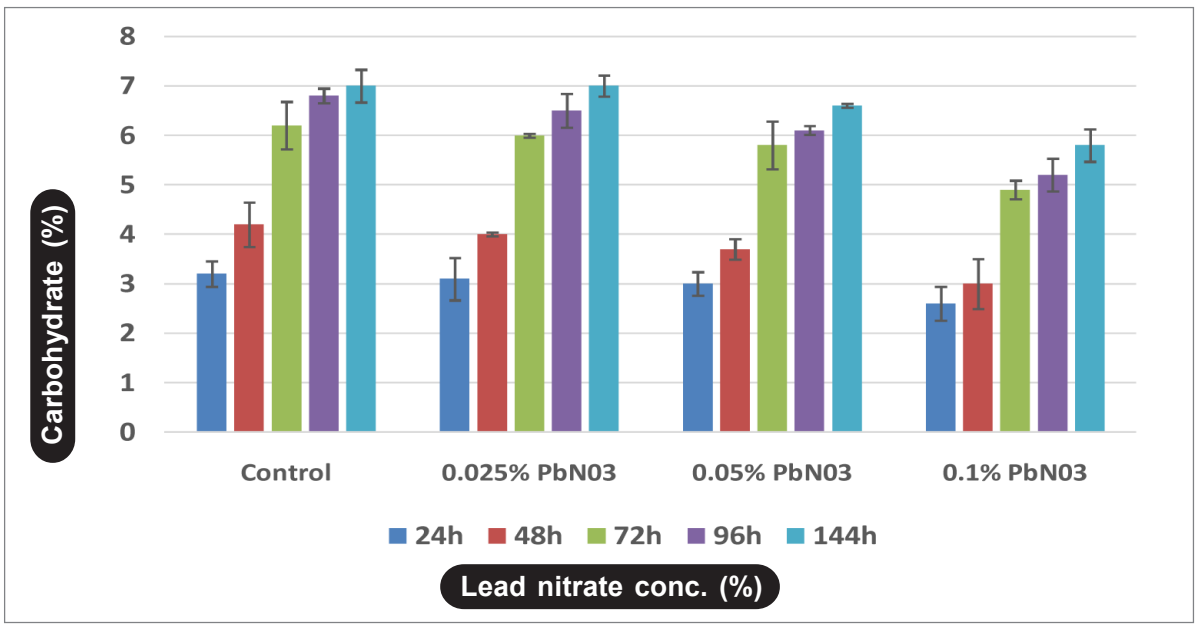

Fig. 4. Fat content (%) in seedling during germination treated with lead nitrate

Table 8. Analysis of variance of fat content (%) in seedling during germination treated with lead nitrate

S.No.	Sources of Variation	Degrees of Freedom (df)	Sum of Squares(ss)	Mean Sum of Squares (Mss)	Variance Ratio
1	$PbN0_3$(conc.)	3	10.23	3.41	9.74 *
2	TimePeriod	4	113.08	28.27	80.77 **
3	Interaction	12	1.9	0.158	0.451
4	Error	40	13.99	0.350	
	Total	59			

** Significant at 1% level

CONCLUSIONS

Lead nitrate affects the carbohydrate and fat content of chickpea during germination. With increasing concentration of lead nitrate, carbohydrate and fat content decreased gradually. The effect was more pronounced at 0.1% concentration. The carbohydrate and fat contents in the seedling was found to decrease with increasing time period. Similarly, with increasing time period carbohydrate and fat content in the cotyledons was found to be decreased. Thus, lead nitrate inhibited the synthesis of carbohydrate and fat during germination of *Cicer arietinum* L. Since lead nitrate is an atmospheric environmental pollutant, it effects adversely the growth of *Cicer arietinum* L. and effect the growth of other crops reducing their nutrient value.

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THE ERGOGENIC POTENTIAL OF AN OAT-BASED ENERGY BAR: A COMPREHENSIVE NUTRITIONAL EVALUATION

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ABSTRACT

The study was conducted during the year 2022 and the objective was to develop a minimally processed energy bar using peanuts, oats, whey, dates, and pumpkin seeds. The ideal composition of the energy bar was determined as a combination of 60% oats and 40% peanuts, based on the sensory analysis. The energy bar exhibited a moisture content of approximately 10%, ash content of 1.55%, crude fat content of 16.42%, crude fibre content of 3.73%, and crude protein content of 20.83%. The energy value was calculated as 213.13 kcal per serving. The hardness value of the energy bar was found to be 150 ± 0.870 N. The nutritional analysis of this minimally processed energy bar suggested it is a convenient and functional ergogenic aid for health-conscious people, particularly athletes, and active individuals.

Keywords: Energy bar, Ergogenic aid, Oats, Sensory analysis, Whey protein

INTRODUCTION

Amidst the current post-pandemic scenario, people are prioritizing their health and well-being more than ever before and this has led to a raised awareness of dietary choices, Consumers now seek functional food options that promote a healthy lifestyle along with offering convenience in handling, storage, and consumption. Energy bars, also known as snack bars, have gained immense popularity as they cater to these health needs. These bar-shaped snacks are designed as ergogenic aids, providing essential nutrients and serving as energy boosters. They are widelyenjoyed by people of all ages but are especially favored by physically active individuals and athletes (Aljaloud *et al.,* 2020; Barakat and Alfheeaid, 2023).

Energy bar preparation involves a diverse mix of ingredients in the right quantity. These are made with ingredients such as oats, wheat, barley, and millets to increase their fiber content and to provide sweetness, alternative sugar sources like dates, jaggery, or sugar substitutes can be incorporated (Safvi *et al.,* 2023). In energy bars designed for athletes, high-protein ingredients like whey protein isolate, peanuts, and cheddar cheese are often included (Jabeen *et al.,* 2020).

Whey, a valuable by-product of the cheese industry, is often disposed offas waste despite containing essential nutrients (Leon-Lopez *et al.,* 2020). However, recognizing its nutrient value and wastage, food industries are exploring ways to incorporate whey in high-

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protein bars to improve product texture and structure (Maecki *et al*., 2020; Ahmed *et al*., 2023). Also, the energy bar can be topped with nutritious ingredients like pumpkin seeds which are packed with essential nutrients, bioactive compounds, and valuable minerals, promoting a healthy lifestyle (Dotto and Chacha, 2020.)

In recent times, functional foods have become increasingly accessible to consumers through extensive processing. This study aimed to create a minimally processed energy bar using readily available technologies, incorporating ingredients such as peanuts, oats, whey, dates, and pumpkin seeds, and subsequently evaluating its physicochemical properties and sensory attributes.

MATERIAL AND METHODS

The study was conducted in the Experiment Laboratory, at the College of Dairy Science and Technology, Kerala Veterinary and Animal Sciences University, Wayanad, Kerala, during the year 2022.

Raw materials

The raw materials, peanuts, oats, pumpkin seeds, and dates (Mazafati variety), were procured from the local market, Wayanad, Kerala. Milk for the preparation of whey was obtained from Livestock farm, Kerala Veterinary and Animal Sciences University, Wayanad.

Optimization of ingredients

The ingredients used in the preparation of the energy bar underwent some pre-treatments. The dry ingredients (oats, peanuts, and pumpkin seeds) were first roasted for 2 minutes. The dates were then pitted and crushed into a fine paste. One-third of the peanuts were ground to a smooth paste, while the rest were crushed into a powder along with half the amount of oats and pumpkin seeds.The wet ingredient, whey was prepared by heating milk to 90 °C, followed by cooling to 70 °C and adding citric acid at a rate of 1.5% of the milk. After coagulation, the curd was pressed and

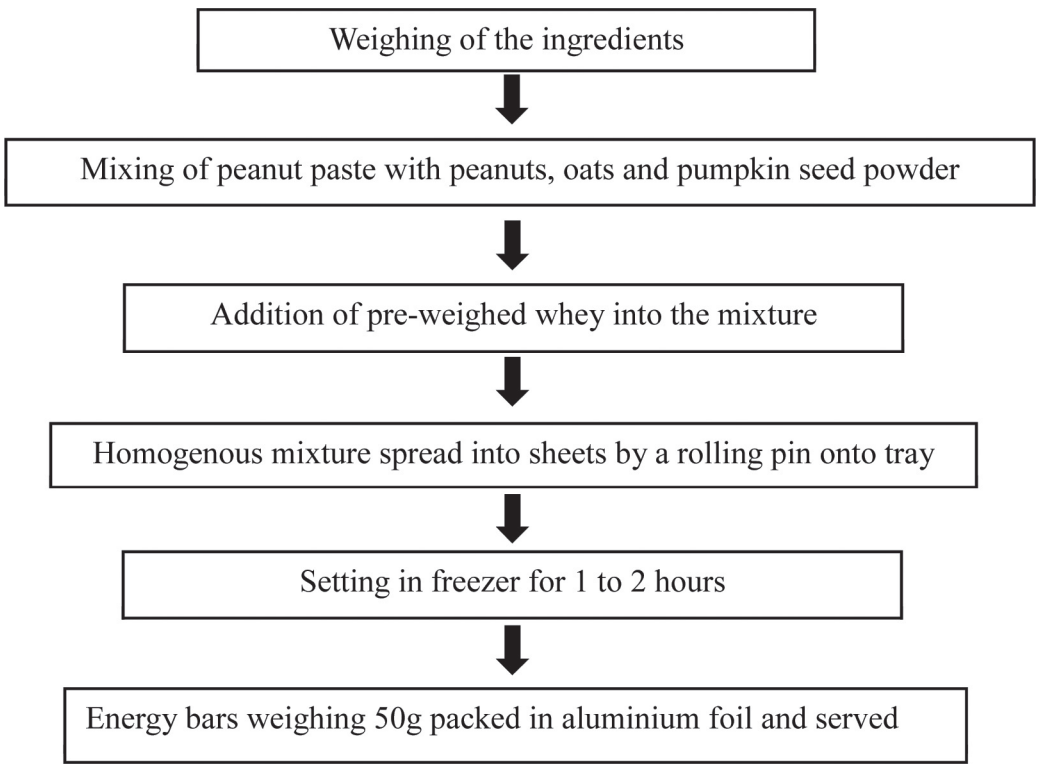

Figure 1. Procedure for the preparation of energy bar

whey was separated as per the method suggested byShanaziya *et al*. (2018) and was concentrated to 60º brix.The rate of addition of pumpkin seed and dates was fixed at levels 18% and 7%, respectively. The quantity of oats: peanuts was adjusted in the ratio 30:70, 40:60, 50:50, and 60:40 and the treatments were labeledas T1, T2, T3, and T4, respectively.

The different treatments were subjected to sensory analysis by a panel consisting of 5 semi-trained judges. The parameters assessed were flavour, color and appearance, sweetness, body and texture, mouth feel and overall acceptance of the samples. The sensory analysis was performed using a 9-point hedonic scale, where a score of 1 indicated 'dislike extremely' and a score of 9 indicated 'like extremely'. The results obtained were analysedusing the 'Kruskal Wallis test' using IBM SPSS STATISTICS (version 26.0).

Procedure for preparation of energy bar

The procedure for preparation of oats-based energy bar is given in Figure 1 and the sliced energy bars are shown in Figure 2.

Figure 2. Sliced energy bar

Proximate analysis

The energy bar was subjected to analysis based on the standard procedures outlined by AOAC International (2007) to determine its moisture, crude ash, crude fat, crudefiber, and crude protein content.The total amount of carbohydrates was estimated by calculating the difference between the sum of the values of other analyses conducted.

Energy value

The total calorie content per serving size of the energy bar was determined by multiplying the protein, carbohydrates, and fat content by the respective factors: 4.0 for protein, 9.0 for fat, and 4.0 kcal/g for carbohydrates.

Texture Profile analysis

The major texture attribute, the hardness of the energy bar was assessed following the method suggested by Kumar *et al*. (2018). A Universal Testing Machine (TRAPEZIUM EZ-SX, Shimadzu, Japan) was used for the analysis. The samples were tempered at room temperature prior to analysis. Five sample replications (2.5 cm * 2.5 cm* 2 cm), were analysed for hardness which was compressed with a 3-point bending rig. The cross-head moved at a constant speed of 1 mm/s.

RESULTS AND DISCUSSION

Optimization of ingredients

The sensory scores obtained for different treatments are given in Table 1. The statistical analysis revealed that while there were no significant differences ($p < 0.1$) in the scores for color and appearance and sweetness among the different treatments, there were significant differences ($p < 0.1$) in the scores for flavor, mouthfeel, body and texture, and overall acceptability.The scores indicated that the energy bar formulated with a ratio of 60% oats to 40% peanuts (T2) achieved the highest scores compared to the other samples. This provides strong evidence that the specific composition used in sample T2 significantly contributes to the superior sensory quality of

Table 1. Sensory scores obtained for different treatments of energy bar

S.No.	Parameters	T1 (70:30)	T2 (60:40)	T3 (50:50)	T4 (40:60)	Kruskal-Wallis H
1.	Flavor	7 ±0.169	8 ± 0.372	7 ± 0.268	7 ± 0.340	12.66**
2.	Color and appearance	8±0.111	8 ±0.372	8 ± 0.250	8 ± 0.360	0.452^{ns}
3.	Sweetness	8±0.158	8.5±0.169	8 ± 0.250	8 ± 0.223	2.99^{ns}
4.	Bodyand texture	8± 0.158	8.5± 0.250	8 ± 0.224	8 ± 0.108	6.97**
5.	Mouth feel	7 ±0.119	8 ± 0.224	7.5± 0.180	7.5 ± 0.119	12.6**
6.	Overall acceptability	7 ± 0.119	8.5± 0.169	8 ± 0.158	7 ± 0.119	14.24**

Figures are mean ± standard error of three replications, **-Significant at one per cent level (p<0.01), ns – Not significant at one per centlevel (p<0.01)

Proximate analysis

The results obtained for proximate analysis is shown in Table 2.

Table 2. The proximate composition of oat-based energy bar

S.No.	Parameters	Sample
1.	Moisture	10±0.02%
2.	Crude ash	1.55±0.21%
3.	Crude fat	16.42±0.75%
4.	Crude protein	20.83±0.75%
5.	Crude fibre	3.73±1.13%
6.	Carbohydrates	48.8g/100g

the product. The sensory quality of bars based on overall acceptability is in the order T2>T3>T1>T4.

Moisture content

The moisture content of energy bars can exhibit significant variability, primarily influenced by the variety of ingredients utilized in their formulation. In this study, the energy bar revealed a moisture content of approximately 10%, demonstrating an optimal balance between dryness and stickiness, ultimately contributing to a desirable mouthfeel for consumers. The carefully selected binding agents, including dates, whey, and peanuts, played a crucial role in achieving this balance. This finding is in line with previous research work by Bhavani *et al*. (2018) on energy bars prepared with popped amaranth, where slightly higher moisture content was reported, potentially attributed to the specific properties of the amaranth and other ingredients used.

Ash content

The ash value of the energy bar has been determined to be 1.55%. The observed ash value indicated the presence of essential minerals in the product, such as calcium, magnesium, potassium, and phosphorus, which are crucial for supporting various physiological functions and overall well-being (Bilge *et al*., 2016). Ahmad *et al*. (2017)

reported an ash value of 1.51% in their study on a high-energy granola bar, which closely aligns with the ash value obtained for the energy bar. This similarity in ash content suggests that both bars may share comparable mineral compositions, potentially offering similar nutritional benefits.

Crude fat

The energy bar had a significant crude fat content of 16.42%. This high-fat content may be attributed tothe presence of healthy fats, which contribute to the bar's higher calorie density, as mentioned by Gill and Singh (2020). Nuts and seeds, as essential ingredients in an energy bar, are rich sources of fats and have been proven to exert a protective effect against cardiovascular disease development (Batool *et al*., 2022). Peanuts in the bar provide a good source of monounsaturated fatty acids, while pumpkin seeds offer both polyunsaturated and monounsaturated fatty acids, as given by Bardaa *et al*. (2016).

Crude fibre

The energy bar contains a crude fiber content of 3.73%. This fairly good amount of crude fiber may be obtained from various ingredients, with oats contributing significantly to the fiber content at 6.16% (Chauhan *et al*., 2018). Additionally, peanuts add to the fiber content with 3.07% (Devhare *et al*., 2021). Including dates and pumpkin seeds in the formulation also contributes to the overall fiber content of the energy bar (Aljaloud *et al*., 2020; Habib *et al*., 2015). Crude fibre contains a considerable portion of dietary fibre which not only enhances the nutritional profile of the product but also provides potential health benefits(Madhu *et al*., 2017).Sports or ergogenic diets are encouraged to include dietary fiber in it, as this promotes optimal bowel function and enhances gut microbial diversity, making their incorporation into energy bar formulations a valuable way to support their performance and overall health (Jang *et al*., 2019).

Crude protein

The energy bar was found to contain 20.83% crude protein, which is noteworthy. This high protein content can be attributed to the inclusion of key ingredients such as peanuts, pumpkin seeds and whey, which are rich sources of protein. Peanuts are a rich source of protein and other macronutrients, providing all the essential amino acids required for promoting body growth and metabolism. (Akhtar *et al*., 2014). Whey is rich in bioactive peptides and amino acids, which are easily digestible and readily absorbed, making it ideal for post-exercise recovery and muscle synthesis (Patel, 2015). Pumpkin seeds are a valuable plant-based protein source, providing a wide range of essential amino acids that support muscle repair and recovery, making them particularly beneficial for athletes and individuals with high-performance requirements (Dotto and Chacha, 2020).

Carbohydrate content

The energy bar, with a total carbohydrate content of 48.8g, is uniquely formulated with a combination of nutritious ingredients such as peanuts, oats, whey, pumpkin seeds, and dates.

Carbohydrates serve as the primary energy source, providing quick energy to the body, superior to any other macronutrient, and act as the primary fuel for exercise and metabolism. (Mul *et al*., 2015). Significantly, in the energy bar, dates act as a natural sweetening agent, eliminating the need for added sugars, which makes it a safer choice for individuals with diabetes (Arshad *et al*., 2022).

Energy content

The energy value of the energy bar was calculated as 213.13 kcal per serving. This high energy value is contributed by the ingredients, such as peanuts, oats, whey, pumpkin seeds, and dates and this value states that the bar can be considered as an ideal ergogenic aid for individuals with high energy requirements.

For highly active individuals and athletes, energy is important as it serves as the fuel that powers their intense physical activities and performance and prevent premature fatigue (Benardot, 2020). The inclusion of healthy fats, proteins, and complex carbohydrates from these ingredients ensures a steady and efficient energy supply, making it a practical choice for athletes and active individuals seeking optimal nutrition (Arenas-Jal *et al*., 2020).

Textural analysis

The hardness of energy bars can vary significantly depending on their ingredient composition. In this study, the energy bar showed a hardness of 150± 0.870N, which is slightly higher than that obtained for a whey protein isolate-based energy bar (100N) developed by McMahon *et al*. (2009), but lower than that of the energy bar made with sea algae proteins and coated with chocolate (276.43N) as studied by Ma³ecki*et al*. (2020), highlighting how different ingredients can influence the texture and firmness of energy bars.

The texture is considered an important factor influencing consumers' perception, which decides their repeated buying. Hardness refers to the amount of force needed to compress food between the tongue and palate to achieve a specific deformation or penetration (Park *et al*., 2020). A study comparing a high-protein bar to commercially available bars found that the high-protein bar was less firm. However, consumer sensory analysis indicated that this slight difference in hardness did not affect the acceptability of the product. Therefore, it can be concluded that the texture of the protein bar may not be a barrier to its acceptance among consumers (Jovanov *et al*., 2021)

CONCLUSIONS

The study focused on formulating a minimally processed energy bar using oats, whey, peanuts, dates, and pumpkin seeds, and the composition was optimized as a ratio of 60% oats to 40% peanuts demonstrating superior sensory quality and nutritional value. The energy bar exhibited a desirable balance of moisture, ash, fat, fiber, protein, and carbohydrates, contributing to its overall appeal as a healthy and efficient ergogenic aid. As proteins play a crucial role in muscle building and repair and by incorporating protein-rich ingredients such as peanuts, pumpkin seeds, and whey into the energy bar formulation, individuals, especially athletes, can conveniently access a nutrient-dense snack that may meet their protein needs for optimal growth, development, and performance. Also, minimal processing techniques used in this energy bar formulation offer the advantage of retaining the natural nutritional properties of ingredients, providing a healthier and more wholesome product compared to energy bars produced using sophisticated processing methods that may involve excessive heat, additives, and preservatives.

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DEVELOPMENT AND EVALUATION OF NOVEL DIABETES NUTRITION EDUCATION TOOLKIT TAILORED FOR MIZO ADULTS

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ABSTRACT

The study aimed to develop and evaluate a Diabetes Nutrition Education Toolkit tailored for Mizo adults to be used interactively between patients and dietitians. The Diabetes Nutrition Education Toolkit was developed between September 2022 and February 2023. It consisted of seven interactive nutrition education modules that provided practical tools to improve diet adherence. The appropriateness of the toolkit was evaluated by the "Suitability of Assessment of Materials (SAM)" by two independent raters. The readability grade level was assessed by the average readability score of seven online tools, with each module rated "superior. The SAM score for each module was rated "superior" (93.5–100%) having a mean score of 97.46%. Cohen's kappa statistically tested inter-rater reliability, and the strength of agreement varied between "substantial" and "almost perfect." The Diabetes Nutrition Education Toolkit is suitable for the intended population and can be used as digital or printed material to supplement diabetes nutrition therapy.

Keywords: Culture, Diabetes nutrition education, Health literacy, Interactive tool kit

INTRODUCTION

Diabetes worldwide has risen over the past decades (Sun *et al.*, 2022). Early diagnosis is the key to managing diabetes well. Individuals with diabetes can benefit from access to appropriate care to prevent or delay complications. Diabetes is a complicated condition that requires daily self-management decisions. People struggling with diabetes can become skilled in their day-to-day self-care with confidence and improved results with diabetes self-management education (Davies *et al*., 2018).

A notable segment of the ADA management of lifestyle for diabetes therapeutic education is nutrition-focused, exhibits actual understanding, and demonstrates skills in simplifying so people can understand.Despite this,nutritional education is not used as a fundamental factor in the treatment of diabetes. There are still information gaps in nutrition and eating habits in people with diabetes irrespective of their types, as well as in adapting Medical Nutrition Therapy to diverse socio-economic and racial/ethnic groups according to the available data (Evert *et al.*, 2019). To complement diabetes nutrition therapy, the healthcare team must provide reliable support and tools that empower people with diabetes to meet their requirements, help them choose healthy food,and improve their overall health (Alison *et al.*, 2019)

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Table 1. Diabetic nutritional education modules and their description

S. No.	Diabetic Nutrition Education Module	Description
1.	Introduction to diabetes nutrition	Overview of function of foods in diabetes.
2.	What to eat?	Overview of different kinds of food groups with their sources. Instruction to select healthy option from each food groups.
3.	When to eat?	Importance of meal timing, Interactive tool for planning personalise meal time.
4.	How much to eat?	Overview of different food groups and importance of their portion size. Illustration of cultural appropriate estimate of portion sizes/ utensils using commonly used glasses, bowls, and spoons of different sizes.
5.	Understanding high carbohydrate serving size	List of commonly consumed high carbohydrate foods with serving size in grams. Illustration of commonly consumed high carbohydrate foods with serving size.
6.	Using a plate to control portion	Step by step instruction to fill up diabetic plate. Interactive tool for planning personalise serving size .
7.	Snack and diabetes	Overview of snacks in diabetes. Interactive tool for planning personalise snacks.

Need for tailored education material

Mizoram is a small state in northeast India with unique dietary habits. The unique way of processing, storing, preparing, and consuming food may affect diabetes in various ways (Lalrohlui *et al.*, 2021). Addressing individual nutrition needs, social and cultural preferences, health literacy demands,and readiness and adeptness to make behavioural changes is crucial. It is recommended to provide practical tools to individuals with diabetes to develop healthy eating habits instead of focusing on single foods or specific macro and micronutrients (Alonso *et al.*, 2018; Liu *et al.,* 2020).

While healthcare settings are limited to enhancing health literacy skills, such as the capacity of an individual to translate knowledge and information to maintain and improve health, instead, more focus has to be on reaching and involving the population demand affected by low health literacy (Nutbeam and Lloyd, 2021).

MATERIAL AND METHODS

Development of the module

The nutritional education toolkit was designed to utilized in diabetic nutrition therapy for the Mizo population. It stresses the AADE nutrition therapy goals for diabetes Adults. The food value and portion were calculated according to the Indian Food Composition Table (IFCT). It was an interactive tool designed to be used between a dietitian and a patient. Each module includes factors particularly intended to enhance active patient and health-provider interaction.

The toolkit was sent to a psychologist, a linguistic expert, and 10 dietitians who were experts in the field of diabetes nutrition therapy. Developing the diabetic nutrition education toolkit was an iterative method for six months between 2022 and February 2023. It soughted responses from the experts until the module was rated as "adequate" and concluded with seven modules in the Mizo language.

Test of readability assessment

To assess how easy it is to read the material, grade level readability was analysed using the Average Readability Score from seven online tools: "Flesch Reading Ease Score, Gunning Fog, Flesch-Kincaid Grade Level, The Coleman- Liau Index, The SMOG Index, The Linsear Write Formula, and the Automated Readability Index". Single words, Tables, and graphics were excluded from the assessment due to online tool's nature. It is deemed "superior" if the score is grade 5th or lower, "adequate" grade 6th- grade 8th and grade 9th and above is considered "not suitable."

Material Suitability assessment

The Diabetic Nutrition Education toolkit was assessed using the "Suitability Assessment of Materials (SAM)", which can be applied to evaluate new materials and cover a range of health-related education materials.

The modules were assessed in two phases: The first phase involved evaluating and rating the content suitability of the seven modules by 10 dietitians,who represent half of the dietitians working in the field of clinical nutrition in Mizoram.In the second phase, the seven modules were rated using SAM by two independent raters experts in Information Communication Education (IEC). Each of the seven modules was rated for six categories; "content, literacy demand, graphics, layout, learning stimulation and motivation, and cultural appropriateness, which make up to 22 variables. The material is rated 2, 1, or 0, depending on how good it is. All factors points are added up, and the result is a percentage score that can be "superior" (70% to 100%), "adequate" (40% to 69%), or "not suitable" (0% to 39%).

Table 2. Results of readability testing of seven online tools

S.No.	Nutrition Education Module	Flesch Reading Ease score:	Gunning Fog:	Flesch-Kincaid Grade Level:	The Coleman-Liau Index:	The SMOG Index:	Linsear Write Formula	Automated Readability Index:	Overall readability score
1.	Introduction to diabetes nutrition	77.7	6.6	6.3	4	4.2	7	3.7	Grade 5
2.	What to eat?	80.8	6.9	5.5	4	5.1	6.8	2.8	Grade 5
3.	When to eat?	82.8	7.7	5.9	2	4.2	7.8	2.7	Grade 5
4.	How much to eat ?	79.2	6.6	5	4	5.1	5.3	1.5	Grade 4
5.	Understanding high carbohydrate serving size	77.8	5.2	5	5	4.8	4.8	2.1	Grade 4
6.	Using a plate to control portion	77.8	7	5.7	5	5.2	6.4	3.7	Grade 5
7.	Snack sand diabetes	83.5	6.3	5.1	4	4	6.4	2.9	Grade 4

Interrater agreement

Cohen's kappa in SPSS Statistics 24.0 software was used to analyse the degree of agreement between two raters who independently rated the toolkit using SAM. The minimum value assumed by this coefficient is 0, and the maximum is 1.

RESULTS AND DISCUSSION

Readability testing result

Each of the seven diabetes nutrition education module underwent individual testing for readability using the school grade level. Table 2 shows that for all the materials, the overall readability score from seven online tools was "superior".The findings verify that the readability for all the modules meets the literacy demand (<_grade 5) of the intended population,adding greater value to the material to delivermore effective information.

SAM results

SAM is a good and reliable measure of the suitability of health-related educational tools for adults. It assesses the suitability of the modules for the target population grounded on the content, cultural relevance, literacy demand, and how they suits the learning stimulation and motivationalong with layout and graphics. Table 3 shows the total SAM score of the toolkit, ranging from 93.5% to 100%.The mean score of the seven modules was 97 percent. The modules "What to Eat?" and "Using Plate to control portion size" have perfect scores (100%), with "When to Eat" having the lowest score of 93.5%.

Inter-rater reliability

Inter-rater reliability was computed independently for each of the seven modules using the SPSS software version 24.0 . Inter-rater reliability for the kappa value for two modules was "Substantial" (0.778). The remaining five modules had a weighted kappa value of 1.00, indicating "perfect agreement" between raters. The results revealed a consistency among the evaluators, enhancing the assessments reliability. Table 4 presents individual kappa values.

In terms of categorical assessment all seven modules were rated "superior" for "learning, stimulation, and motivation." Making dietary decisions and sticking to a meal is challenging for individual with diabetes. The

Table 3. Total SAM results of seven modules in percentages

S.No.	Nutrition Education Module	Rater 1	Rater 2	Total Sam score
1.	Introduction to diabetes nutrition	97.5%	100%	98%
2.	What to eat?	100%	100%	100%
3.	When to eat?	92.5%	95%	93.5%
4.	How much to eat?	97.5%	97.5%	97.5%
5.	Understanding high carbohydrate serving size	97.5%	97.5%	97.5%
6.	Using a plate to control portion	100%	100%	100%
7.	Snack and diabetes	95%	95%	95%

Table 4. Strength of agreement of inter-raters

S.No.	Categories	Kappa value	Strength of agreement
1.	Introduction to diabetes nutrition	.778	Substantial
2.	What to eat?	1.000	Almost perfect
3.	When to eat?	.778	Substantial
4.	How much to eat?	1.000	Almost perfect
5.	Understanding high carbohydrate serving size	1.000	Almost perfect
6.	Using a plate to control portion	1.000	Almost perfect
7.	Snack and diabetes	1.000	Almost perfect

(Kappa value indication: 0.81-0.99 = "almost perfect," 0.61-0.80 = "substantial agreement", 0.41-0.60 = "moderate agreement," 0.21-0.40 = "fair agreement", 0.01-0.20 = "slight agreement).

toolkit focuses on solving problems, behaviour change, and providing how-to information to enhance learning and motivation. Problems and questions are raised for reader response, and instructions are given to enhance education and behavioural change.

Information anxiety isassociated with an excessive amount of information, which adds up to avoiding information(Soroya *et al.*, 2021). Therefore, each module must provide behaviour information directly related to solving immediate

health problems. It conveys key messages, keeping the text simpleand using behaviour-oriented images and illustrations. The "content" of the toolkit was rated "superior" for four modules, with two modules rated "adequate'.

Health literacy should not be limited to the patient's ability to interpret health information; the healthcare provider should also provide material to meet the demands of the target population. Individuals with low health literacy smisunderstand diabetes care and are proactive while seeking health information; to improve self-care, education needs to be tailored to patients' health literacy levels (Kim *et al.,* 2020). The toolkit was made to enhance nutrition literacy for low-literacy patients, and use conversation styles and active voice to increase understanding. The reading grade level was rated "superior" (< grade level 5) by school standards for all the modules.

In health care contexts, determining which types of illustrations and graphics best convey health information is valid(Schubbe *et al.*, 2020). Illustrations of foods, portion sizes, and utensils familiar to the target population are used to help the readers easily comprehend the key knowledge from the illustration alone. The toolkit score for "graphics" shows that two modules are rated "adequate" and the rest of the four modules are rated "superior." Simple lines and drawings appropriate for adults are used throughout, and for "layout and topography," all seven modules were rated "superior."

According to the American Diabetic Association (2019), addressing individual nutrition needs regarding personal preferences, cultural norms, and the willingness and capacity to modify behaviour is crucial.The toolkit is created to be interactive and provides individualised meal plans considering individual differences and cultural perspectives. Regarding "cultural appropriateness," all the modules were rated "superior." The average rating of SAM for all the modules was superior, with a mean of 97 percent.

Clinical implications

The Diabetic Nutrition Education Toolkit is applicable for 1:1 interaction through written or digital tools to assist diabetic nutrition therapy. Future studies can use the Toolkit for intervention to assesswhether cultural targets in nutrition education materials will lead to improved diet adherenceor not.

CONCLUSIONS

The Interactive Diabetic Nutrition Education Toolkit contained seven modules proposed for use among Mizo diabetics to improve dietary adherence. Suitability assessment was conducted for each module using the SAM instrument, and all the modules were rated "superior" and found suitable for the target Mizo population.

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A COMPARATIVE STUDY ON CHARACTERIZATION OF PROCESSED AND UNPROCESSED SORGHUM FLOURS

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ABSTRACT

The effect of physical, technological and functional properties of unprocessed, germinated and dry roasted grains and flours of sorghum were studied in the year 2023. The physical properties of the grains such as length, breadth, length/breadth ratio, thousand grain weight showed that, germinated grains measured minimal results as the stores were utilized for sprouting. Germinated sorghum yielded 94.6%, whereas, unprocessed and roasted yielded 91% of the total flour yield. The technological properties of the flours were analyzed as quality indicator. Bulk density, oil and water holding capacity, and swelling capacity of the roasted flour were higher in quality when compared to unprocessed flour. Optical property indicated that roasted flour was darker and germinated sorghum flour was lighter than unprocessed flour. FTIR of germinated sorghum flour showed stronger peaks indicating the presence of polysaccharides. TGA of germinated sorghum flour exhibited maximum reaction zone due to changes in saccharides and dextrin. Roasted sorghum flour showed better properties that increase hardness, whereas, germinated flour soften the food product. Sorghum flour showed less than 7 x 10^{-1} CFU/g at day 180.

Keywords*:* Germination, Property analysis, Roasting, Sorghum flour and grain

INTRODUCTION

India is the prime capital for consuming and producing millets and sixth largest producer of sorghum (*Sorghum bicolor* [L.] *Moench*) globally. Millet is a staple food in Indian food basket. Consumption of millet in daily diet have been decreased over the period of time due to lack of knowledge, increased cooking time, and insufficientnumber of processed food items (Llopart and Drago, 2016). Government and food manufacturers became more aware to increase the consumption and utilization of millets, particularly sorghum, to help farmers to cultivate more resilient crops and make population to overcome hunger, metabolic syndrome and climate change (Kane-Potaka *et al.* 2021). Sorghum is a gluten-free cereal substitute with high nutrient profile packed with micronutrients and a good source of fiber, protein, antioxidants, and bioactive compounds, but anti-nutritional factors such as phytic acid and tannin, prevent from reaching its full

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nutritional potential. The effects of several wet and dry processing techniques, including steeping, fermentation, germination, roasting, and popping at different temperature and time span on these antinutritional factors of sorghum flour were proved to have impact on the structural and chemical characteristics (Paliwal and Sharma, 2022 ; Anjitha *et al*., 2021). The primary phenolic compounds in sorghum are tannins, phenolic acids and 3-deoxyanthocyanidins with good potential of antioxidant activity. Consuming whole sorghum improves gut health, lowers the risk of chronic diseases, and is used as a promising natural multifunctional additive in a wide range of food applications. (Birhanu, 2021). This study was carried out with an attempt to improve the physical, technological, and functional qualities of white sorghum grains through soaking, germination, and roasting to utilize in food processing and production industry.

MATERIAL AND METHODS

Selection of sorghum grains

White variety of sorghum is highly available and consumed largely as compared to red or yellow colored sorghum. Good quality of white variety sorghum grains were purchased from supermarket in Coimbatore, Tamil Nadu.

Processing of selected sorghum grains

The whole sorghum grains were washed, cleaned and sorted to remove husk and other impurities and dried below 12 ºC. Conventional preliminary processing like soaking, germination and roasting were done to enrich the physical, technological and functional properties. Sorted sorghum grains were soaked for eight hours and dried by spreading in muslin cloth to remove the excess moisture. Then dried sorghum grains were tied in white muslin cloth for germination for 35 h as suggested by Olamiti *et al.* (2020). Then the germinated grains were shade dried for 6 h till the moisture attained 12 percent and ground into fine flour in milling machine and kept in air-tight container for further investigation. Clean dried white sorghum grains were dry roasted in iron pan for 12 minutes at 90 ^{o}C till few grains popped and the roasted grains were kept undisturbed till it reaches 25 ^{o}C to facilitate grinding. Then the roasted grain was milled and sieved through 3 mm mesh as recommended by Ranganathan *et al.* (2014) and preserved in an airtight container for future study.

Physical properties of grains

Physical properties of the selected and processed grains were analyzed to measure the quality. Thickness (mm), length (mm), breadth (mm), length / breadth ratio, thousand grain volume (ml) and thousand grain weight (g) were measured by the standardized procedure of Khatoniar and Das (2020).

Technological properties of flour

Technological properties of flour was determined to identify the difference among unprocessed, germinated and roasted sorghum flour. Bulk density (g/ml), swelling capacity (ml/g), water holding capacity (ml/g) andoil holding capacity (ml/g) were determined by usingthe procedure that was followed by Akinola*et al.* (2017). Standard AOAC procedure was used to analyze moisture by AOAC 930.15 and ash by AOAC 942.05 standards.

Optical properties of sorghum flour

The colour of unprocessed, germinated and roasted sorghum flour was analyzed through Laboratory Scale Food Colorimeterand different values of L*, a* and b* were obtained. L" values, 0 - 100 indicates lighter to darker shades of the taken sample. Positive value of a* refers to greenness and negative shows the presence of redness, whereas, positive and negative values of b* indicates yellowness and blueness.

Functional properties of the flour

The functional groups of the unprocessed and processed sorghum flour were determined through the peaks obtained from Shimadzu Miracle 10 Fourier Transform Infrared spectrophotometer. 0.5 g of sample was utilized to determine the spectra and the peaks were found between 4000 cm^{-1} to 450 cm^{-1} wave numbers at 16 runs per scan.

The crystalline or amorphous nature of sorghum flour was measured by patterns through X – Pert Pro, PANalytical model X-Ray Diffraction (XRD). 5 g of fine sample was loaded and operated at 30 mA and 45 kV between the scanning regionsfrom 10° to 79° 2 theta with continuous step size of 0.01 at 5.71 seconds. The Debye-Scherrer formula, D = K ë / â Cosè, has been applied to estimate the average crystallite size of sorghum flour, where, D is the crystalline size, ë is X-ray wave length of Cu, â is full width at its half maximum and k is dimension less shape factor with fixed value of 0.94.

Thermal properties of the flour

Thermal property analysis of unprocessed, germinated and roasted sorghum flour was identified by Thermo Gravimetric Analyser (EXSTAR/C300) to analyze the percentage of thermal degradation as the flour undergoes certain physiochemical changes with change in temperature. Sample taken was treated from 20 ºC to 1000 ºC with increase in 20 ºC per minute in alumina pan.

Microbial evaluation of the flour

Total Plate or microbial count was analyzed by modified procedure of Makawi *et al*. (2019). 9 ml of sterile peptone water (0.1%) and 1 g of sample were mixed. To create sequential 10 fold dilutions, 1 ml of the homogenate was diluted with 9.0 ml of water that contained 0.1% peptone, by pour plate method suitable serial dilutions in duplicate with plate count agar to make 10^1 to 10^5 dilutions and incubated at 35 °C for 48 ± 2 h. Colonies were counted when less than one-quarter of the dish is overgrown by spreading that is unaffected and multiply that number by the dish's total area.

RESULTS AND DISCUSSION

Grain yield of sorghum

500 g of cleaned and sorted sorghum were milled which yielded 91 percent of fine flour. Ninepercent were coarse flour when sieved in 3 mm mesh. 500 g of sorghum were germinated and the sprouts were grown upto 1.5 to 1.8 cm after 35 h of germinating and the weight of the grains were increased by absorbing water in the initial stage. After shade drying for six hours, the weight was reduced as the stores were utilized during germination and yielded 94.6 percent. Whereas, 500 g of roasted sorghum

Table 1. Grain yield of sorghum

S.No.	Sample	Sample Taken (g)	Processing Time	After Proces-sing (g)	Flour yield after drying(g)	% of Yield
1	Sorghum - Unprocessed	500	Unprocessed	500	475	91
2	Sorghum – Germinated	500	8 h soaking and 35 h Germination	548	473	94.6
3	Sorghum – Roasted	500	12 mins	450	455	91

yielded 91 percent due to increase in hardness of endosperm while browning and change in gelatinization of starch while popping. (Anjitha *et al*. 2021). After milling, germinated sorghum flour was soft in nature as compared to unprocessed and roasted flour and the total grain yield was depicted in Table 1.

Physical properties of sorghum grains

Physical properties of sorghum were determined through standardized procedures to check the quality of selected and processed sorghum grains and the results were presented (Table 2). The length, breadth and length to breadthratio of unprocessed and roasted sorghum were similar and those of germinated sorghum were reduced in size. Slight loss in thickness of the grains during germination was also noticed. Maximum thousand grain weight was observed in unprocessed sorghum, whereas, minimum was obtained in germinated sorghum. Thousand grain volume was ranged between 36 ml and 44 ml and the highest was noticed in germinated sorghum. Khatoniar and Das (2020) observed the physical dimensions of major millets and among them proso millet exhibited better properties.

Technological properties of flour

Technological properties namely, bulk density, swelling capacity, water and oil holding capacity of the flours were analysed and tabulated (Table 3). Bulk density and swelling capacity washigher in unprocessed sorghum flour than germinated and roasted flour. Water and oil holding capacity of roasted sorghum flour is higher followed by germinated and unprocessed flour as it is considered as the important factor for the formulation offood products as well as a thickening agent. Akinola *et al*. (2017) found that bulk density and water holding capacity was reduced during malting and fermentation as compared to unprocessed pearl millet. Germinated and roasted sorghum flour exhibited better technological properties as compared to other major millets (Khatoniar and Das, 2020). Germinated sorghum flour contains more moisture and ash content followed by roasted and unprocessed flour.

Optical properties of the sorghum flour

Optical property of sorghum flours were evaluated(Table 4). The properties were evaluated thrice, and presented as mean and standard deviation.Analysis was done in triplicates, reported as mean ± standard deviation and the results were tabulated in Table 4. The *L were ranging from 42.63 to 47.83, whereas, a* and b* were 4.81 to 9.5 and 3.05 to 10.49, respectively. The lightness (*L) is highest

Table 2. Physical properties of the grain (n=3)

S.No.	Quality Parameters	Sorghum Unprocessed	Sorghum Germinated	Sorghum Roasted
1	Length (mm)	0.61±0.012	0.52±0.12	0.61±0.20
2	Breadth (mm)	0.69±0.01	0.57±0.20	0.69±0.04
3	Length / Breadth ratio	0.88±0.40	0.91±0.54	0.88±0.40
4	Thickness (mm)	0.59±0.02	0.57±0.03	0.59±0.10
5	Thousand grain Weight (g)	29.6±0.32	23.2±1.09	26.4±0.82
6	Thousand Grain Volume (ml)	36±1.12	44±2.32	41±2.01

* Mean ± SD Values

Table 3. Technological properties of the sorghum flour (n=3)

S.No.	Parameters	Sorghum Unprocessed	Sorghum Germinated	Sorghum Roasted
1	Bulk Density (g/ml)	0.17 ± 0.01	0.14 ± 0.04	0.16 ± 0.07
2	Swelling Capacity (ml/g)	3.6 ± 0.17	2.4 ± 0.09	3.2 ± 0.12
3	Water Holding Capacity (ml/g)	1.2 ± 0.02	1.4 ± 0.05	1.8 ± 0.04
4	Oil Holding Capacity (ml/g)	0.8 ± 0.01	1.3 ± 0.12	1.0 ± 0.08
5	Moisture (%)	10.3 ± 1.15	15.7 ± 1.57	9.3 ± 0.98
6	Ash (%)	19.6 ± 1.28	20.0 ± 1.92	19.8 ± 1.03

Mean ± SD

in sorghum geminated flour with 47.83 ± 0.35 value which showed lighter than unprocessed and roasted flour. Germinated sorghum flour showed the most reddish and yellowish shade with 4.83 and 3.05 values, respectively. The product developed with roasted sorghum flour provides intense optical property as compared to unprocessed and germinated flour. Olamiti *et al*. (2020) declared that prolonged malting and fermentation times resulted in lighter flour, while shorter malting and fermentation times resulted in denser flour. Sorghum exhibited high L* content around 72.02 and 73.72, a* content within 2.50 and 3.30, and chrome content between 13.10 and 14.82.

Functional properties of the sorghum flour

Functional properties of the unprocessed, germinated and roasted sorghum flours were determined by Fourier transform infrared (FTIR) spectroscopy from 4000 cm^{-1} to 450 cm^{-1}. Unprocessed sorghum flour contains medium O-H peaks at 3873.06 to 3718.76 cm^{-1} and weak C –H band at 3502.08 to 3363.86 cm^{-1} , whereas, geminated sorghum flour contains strong peaks at 3834.40 cm^{-1} and roasted sorghum flour shows medium peaks at 3718.76 to 3834.40 cm^{-1} showed the presence of vibrational peaks due to bound water and moisture content in flour (Olamiti *et al*. (2020). Medium C–H peaks at 2306.86 to 2970.38 cm^{-1} region were present in all the three flours. Medium carbonyl stretch peak at 1697 to 1643 cm^{-1} for unprocessed andweak peak at 1689 cm^{-1} region shows the presence of lipids and germinated flour doesn't contain carbonyl stretch. Strong C=C stretching peaks at 1519 cm^{-1} for unprocessed and 1527 cm^{-1} for germinated and roasted sorghum flour shows the presence of the amide II from N – H bending that confirms the presence of protein. Unprocessed sorghum flour contains strong peak at 1519 cm^{-1} and weak peaks at 1080 to 1458 cm^{-1} shows the

Table 4. Optical properties of the sorghum flour (n=3)

S.No.	Sample	L*(0 - black, 100 - white)	a*(+ red, - green)	b*(+ yellow, - blue)
1	Sorghum Unprocessed	45.77 ± 1.48	4.81 ± 0.71	4.59 ± 0.75
2	Sorghum Germinated	47.83 ± 0.35	4.83 ± 0.5	3.05 ± 0.21
3	Sorghum Roasted	42.63 ± 0.18	9.05 ± 0.22	10.49 ± 0.65

Mean ± SD

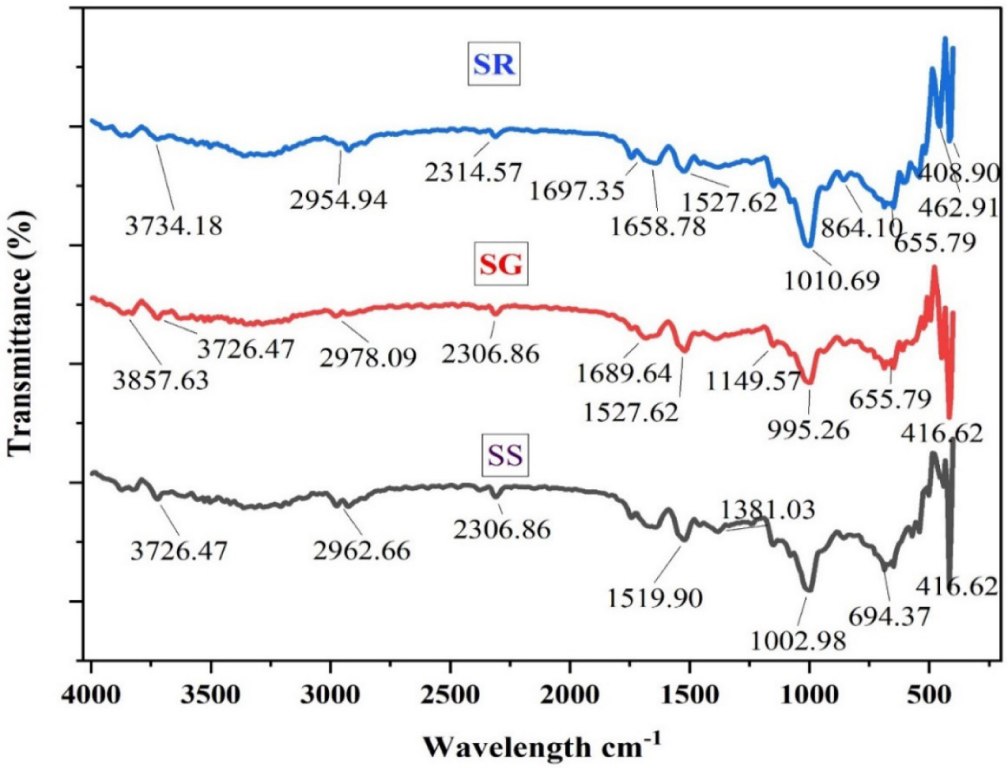

Figure 1. FTIR Peak of the sorghum flours

***SS – Unprocessed Sorghum Flour, SG – Germinated Sorghum Flour, SR – Roasted Sorghum Flour**

presence of O - H, C - O and C - C bonds which is saturated primary alcohol ring and germinated and roasted flour showed minimum weak peak at these region shows the denaturation of organic compounds. Unprocessed sorghum flour showed more peaks at 416 to 995 cm^{-1} , whereas, geminated flour showed at 416 to 725 cm^{-1} and roasted showed between 424 and 810 cm^{-1} confirming the presence of amylose and amylopectin.

A medium peak at 1743.65 $cm^{-1.}$ present in unprocessed sorghum flour showed the presence of tannin and there are no peaks at 1700 to 1400 cm^{-1} confirming the absence of tannin in germinated and roasted flour (Lin *et al.,* 2021). Steeping and fermentation of sorghum flour does not have impact on structural property but phytic acid and tannin content were reduced upto 21–52 percent due to first-order degradation kinetics due to conventional processing (Paliwal and Sharma, 2022). Malted and fermented pearl millet flour showed increased protein content due to protein synthesis as compared to unprocessed flour (Akinola *et al*., 2017).

Crystalline nature and crystal size of the processed and unprocessed sorghum flour was determined through X-Ray diffraction. Unprocessed sorghum flour showed main reflection at 11.8, 13.6, 15.3.8, 16.1, 19.1, 20.9, 21.3, 22.7, 23.7, 25.1, 31.2, 38.7, 40.6, 41.7, 44.0, 60.8, 61.0 and exhibited sharp peaks that indicated crystalline structure of the flour with a crystal size of 88.92 nm calculated by Debye-Scherrer formula, whereas, germinated sorghum flour exhibited peaks at 12.0, 15.3, 19.1, 21.1, 22.4, 24.9, 25.5, 27.6, 31.3, 32.1, 36.3, 38.5, 54.3regions with 55.91 nm crystal size and the

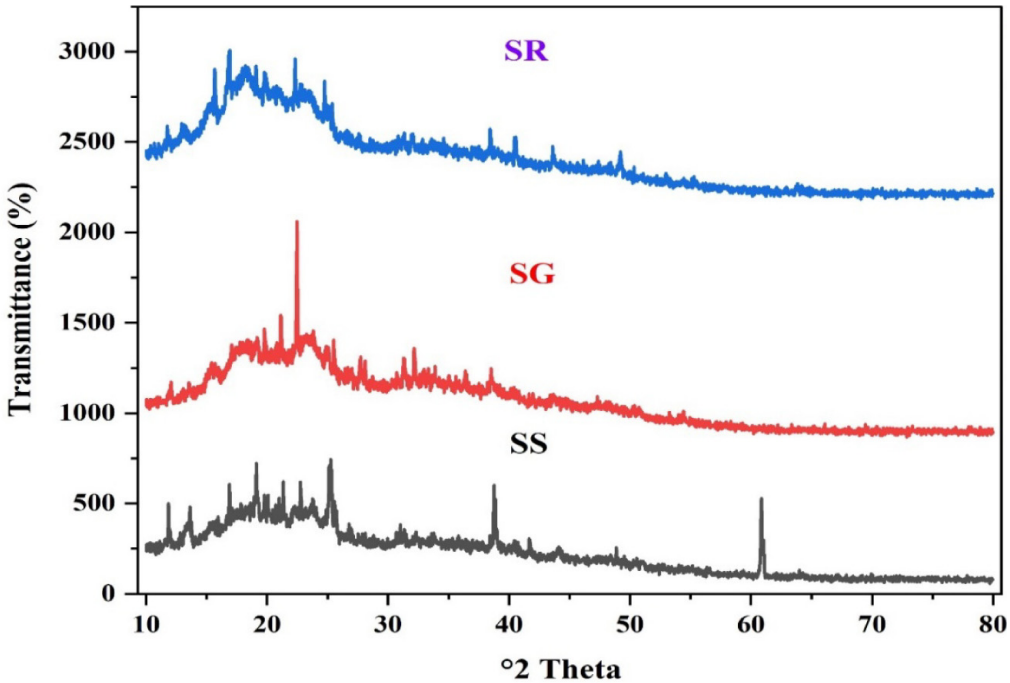

Figure 2. X-Ray Diffraction peak of the sorghum flours

***SS – Unprocessed Sorghum Flour, SG – Germinated Sorghum Flour, SR – Roasted Sorghum Flour**

peaks were low and diffused due to crystalline disruption of the double helices that influences the nature of flour.

Roasted sorghum flour showed sharp peaks at 13.1, 15.6, 16.8, 19.8, 22.3, 24.7, 25.3, 32.0, 38.4, 40.4, 43.6, 40.1 exhibited 65.25 nm crystal size. The changes in peak was noted in fermentation and malting of sorghum and pearl millet (Olamiti *et al*., 2020). Inclusion of water and other ingredients is determined by the particle size of flour. The smaller particle size, increases the water absorption property (Akinola *et al*., 2017).

Thermal properties of the flour

Thermal properties of unprocessed, germinated and roasted sorghum flours were analyzed through Thermo Gravimetric analyzer to evaluate the physicochemical changes and thermal degradation. 3.66 mg, 5.42 mg and 4.67 mg of unprocessed, germinated and roasted sorghum flour was taken in the alumina pan and thermally degraded from 24 ºC to 1000 ºC. No change was found till 50 ºC. Gradual weight loss was found till 100 ºC shows the thermal degradation of moisture. At 300 ºC, first zone of thermal degradationin unprocessed, germinated and roasted sorghum flour wereobserved as a weight loss of 40.4 percent, 33.3 percent and 33.1 percent, respectively. The second zone of changes were enormous which showed a weight loss of 36.7 percent, 42.3 percent and 49.4 percent, respectively in unprocessed, germinated and roasted sorghum flour. A total loss of 94.3 percent, 91.5 percent and 93.8 percent was noticed with a residue of 0.20 mg, 0.46 mg and 0.28 mg, respectively in unprocessed, germinated and roasted sorghum flour. Wang *et al*. (2021) observed the physicochemical changes in cereal and tubers starch between 100 °C to 320 °C due to changes in saccharides and dextrin compounds.

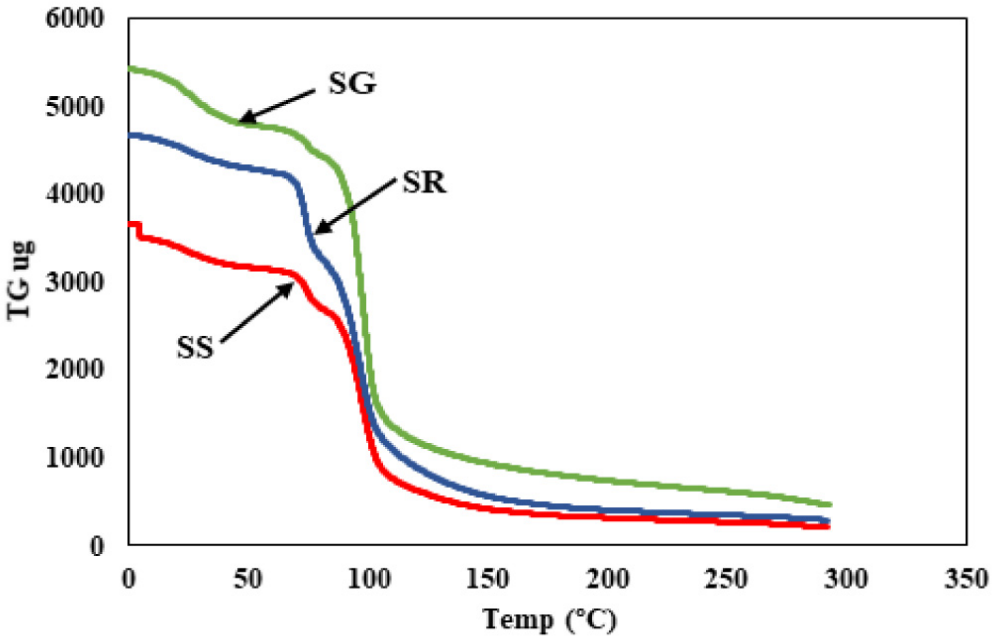

Figure 3.TGA of sorghum flour

***SS – Unprocessed Sorghum Flour, SG – Germinated Sorghum Flour, SR – Roasted Sorghum Flour**

Microbial evaluation of the flour

Microbial evaluation was done by total plate count for 180 days in the interval of 45 days. Sorghum flour grind in sterile and aseptic environment showed zero CFU/g at day 1 and minimal colony counts at 180 day. Unprocessed, germinated and roasted sorghum flour showed 1×10^{-1}, 2×10^{-1} and 0×10^{-1} on the day 45, 2×10^{-1}, 4×10^{-1} and 1×10^{-1} on the day 90 and 4×10^{-1}, 5×10^{-1} and 1×10^{-1} on the day 135. Unprocessed, germinated and roasted sorghum flour showed 5×10^{-1} CFU/g, 7×10^{-1} CFU/g and 3×10^{-1} CFU/g at the end of the 180 days. Germinated sorghum flour showed gradual increase in colony count as per the increased level of moisture content and water activity, whereas, roasted sorghum flour showed the minimal colony count. According to the Food Safety and Standards (Labelling and Display) Regulations (2020) total plate count of millet and millet products should not be more than 1,000 CFU/g.

CONCLUSIONS

This research studied the properties of sorghum flour by conventional processing methods so that one can develop products and increase the usage of this underutilized millet. Processed sorghum grains and flours influenced the functional property, optical property and technological property to reinforce food processing companies for standardizing baked and extruded products. Germinated flour might endure the preparation of smooth baked products, whereas, roasted flour can be used in the preparation of extruded crispy products when compared to unprocessed sorghum flour.

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PROPERTY ANALYSIS OF SODIUM ALGINATE FROM BROWN MACROALGAE (*Sargassum cinctum* J.Agardh)

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ABSTRACT

The purpose of the study was to examine the characteristics of sodium alginate that was collected in November 2019 from *Sargassum cinctum* J. Agardh in the Indian Gulf of Mannar locations. Using a standardized extraction procedure, sodium alginate was extracted from *Sargassum cinctum* J. Agardh and was subjected to elemental composition profile analysis with Energy Dispersive X-ray spectroscopy (EDX) and morphological analysis through a Field Emission Scanning Electron Microscope (FESEM) along with 3-dimensional optical profile analysis. Heavy metal residue analysis of mercury, lead, cadmium, chromium, and arsenic done through an Atomic Absorption Spectrophotometer (AAS) at ppm level, revealed the absence (lower than the detectable limit) of arsenic and lead indicating the safety of the extracted biopolymer sodium alginate.Lethality assessment conducted with brine shrimps for 24 h revealed a higher mortality rate on increased sample content after 8 h of exposure.The outcome of the study indicated that sodium alginate from *Sargassum cinctum* possesses higher application potential in various sectors including food, agriculture,textile, pharmaceutical, and cosmetic industries.

Keywords: Brine shrimp lethality assay, Characterization, *Sargassum cinctum,* Seaweed, Sodium alginate

INTRODUCTION

Alginates consist of linear polymers of saccharides abundantly found in brown macroalgae, especially from species *Sargassum, Laminaria* and *Ascophyllum*. These seaweed-based anionic polymers are repeated blocks of b-D-mannuronic linked at 1,4 and guluronic acid arranged in different proportions (Latifi *et al.,*2015; Jayasinghe *et al.,* 2016; Fertah,2017; Roy and Anantharaman, 2017; Ruslan *et al*., 2019; Giyatmi *et al.,* 2020; You *et al*., 2020; Mantri *et al.,* 2020). All species of brown algae are commercially known for their valuable cell wall component 'algin' found as a precursor material for Sodium alginate. Thus, this group of algae is scientifically addressed as 'Alginophyte' (Dave *et al.,* 2017; Wang *et al*., 2019). While the majority of alginate that is sold on the market comes from brown seaweed, soil bacterium capsules also contain some polysaccharides that are comparable.

According to Jayasinghe *et al.* (2016) alginates are known for their potential

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applications in the food industry as texture stabilizers and potential additive properties. It is widely used as an efficient encapsulation material. Its application areas include pharmaceuticals, cosmetics, agriculture, paper, and textile industries apart from environmental engineering and allied sectors. Due to its hydrophilic nature and biocompatible capacity alginate is widely used in medical applications as wound dressings, scaffold material in hepatocyte culture, and surgical or dental impression material, even in skin allergy conditions.

While brown algae are well-known for their potential phytochemical compounds alginates in the extracted form do not have any nutritional advantages because of their indigestible form. Experiments have revealed a marked decrease in total cholesterol, triglycerides,and low-density lipoprotein in rats due to the administration of polysaccharides from *Sargassum fusiforme* (Roy and Anantharaman, 2017). Similar findings from the crude extract of *Sargassum polycystum* indicated the same. Alginates are non-toxic and are approved by the US Food and Drug Administration. The Acceptable Daily Intake (ADI) for alginates is neither specified nor prescribed which forms it as the highest possible classification as a food additive. United States Food and Drug Administration classifies alginate under (GRAS) Generally Recognized As Safe criteria.The FAO and WHO joint additive committee approve 0-50 mg of sodium alginate per kg body weight as a daily permissible dose forhumans. Also, FAO and WHO removed the limitations in 1990, for the daily human consumption of alginates.*S. cinctum*is widely found in the tropical marine regions of the Pacific Ocean, especially around the Indian subcontinent.Understanding the fundamental variations in properties through the profiling of sodium alginate isolated from this particular type of algae can prove advantageous in various application industries.

The primary goal of the research is to understand the characteristic profiles of sodium alginate separated from *Sargassum cinctum* a brown algae (Phaeophyceae) obtained through a standardized and featured extraction technique (Pragatheeswari and Raajeswari, 2021). To analyze the morphology and energy-dispersive properties of the sodium alginate separated from *S.cinctum*, energy-dispersive X-ray analysis was performed through a field emission scanning electron microscope. To assess the surface topography with a 3D-Optical profilometer.Thermal degradation and destruction properties were analyzed through thermogravimetric analysis and DSC techniques. Heavy metal toxicity and lethality assessment through brine shrimps were done for safety analysis.

MATERIAL AND METHODS

Sargassum cinctum J. Agardh, samples from November 2019 taken from the coastal regions surrounding the Gulf of Mannar in India, are displayed in Fig. 1a. The samples were first carefully cleansed in seawater and then twice more under running water to get rid of any epiphytes and debris that had adhered. Following 48 h of shade drying at room temperature and 24 h of cabinet drying at 45 °C, cleaned samples were dried until their weight remained constant. Scientist-E of the Botanical Survey of India, Southern Reginal Centre, India, identified and verified the samples. Using an electrical blender, dried samples of *S.cinctum* were ground into a powder as indicated in Fig. 1b. The powder was then sieved and kept in an airtight container until further analysis.

The extraction process of Sodium Alginate: The extraction process of sodium alginate from *Sargassum cinctum* was carried out by the modified procedure standardized in comparison with different extraction techniques (Pragatheeswari and Raajeswari, 2021).

Where 5 g of powdered sample was soaked in 100 ml of 2% formaldehyde (Merck, Germany) at room temperature for a duration of 24 h and the samples were initially rinsed by distilled water followed by which they were soaked in 100 ml of 0.2M HCl (Thermo Fisher Scientific, USA) at room temperature for 24 h and washed again with distilled water. It was subjected to extraction with 500 ml of 2% Sodium Carbonate (2% Na_2Co_3) (Merck, Germany) at 100 °C for 1 h. The extracted contents were centrifuged for 30 minutes at 3000 rpm. The supernatant was separated and the soluble fractions were precipitated by two volumes of 85% Ethanol (Merck, Germany) with continuous stirring. The precipitate was collected and dried in a hot air oven at 65 °C until it reached a constant weight.

Profile Analysis: The morphological structure and composition of elementals inthe sodium alginate were understood with (i) Field Emission Scanning Electron Microscope (FESEM) with (ii) Energy Dispersive X-Ray Spectroscopy of model Tescan-Mira3 XMU enhanced with gold sputtering.(iii) The surface characteristics, topography, and optical profile of the extracted seaweed hydrocolloid sodium alginate were assessed with a Zeta 20 3D-Optical profilometer that provides a non-destructive analysis (iv) Thermal stability and thermal properties of the sodium alginatewere assessed with TGA/DSC - EXSTAR/6300 (Thermo Gravimetric Analyser) from a temperature range from 30 °C to 1000 °C at the speed of 20 °C per minute. The mass difference in the sample is measured as the function of temperature in TG, Wherein, the temperature variation needed to raise the same is compared with the reference material in differential scanning calorimetry. Alumina is used as standard reference material in this experiment (Kaidi *et al*., 2021).

Toxicity Assessment: The Thermo Scientific ICE 3000 Series model Atomic Absorption Spectrophotometer (AAS) with SOLAAR software was used to determine the heavy metal content of the sodium alginate.The sample preparation technique suggested by Mohammed *et al*. (2017) was adopted with slight modifications. The hydrocolloid sample of 0.5 g was predigested with 5 N Nitric acid at room temperature for 24 h. Predigested samples were digested at 100 °C until they dissolved completely and made up to 50 ml with distilled water. The obtained clear solution was used for the analysis of heavy metals such as mercury, lead, cadmium, chromium, and arsenic at parts per million (ppm) level.

A lethality assessment was carried out with brine shrimps to understand the acute toxicity of the extracted sodium alginatefrom *S. cinctum*.The given sample was weighed and dissolved in 25 ml double distilled water to get solutions of different concentrations (250, 500,1000,1500, 2000 µg/ml) and the motility of the shrimps was assessed for 24 h (Sarah *et al*., 2017).Statistical analysis for the interpretation of mean values and one-way ANOVA was carried out using SPSS Software, version 1.0.0.140.

RESULTS AND DISCUSSION

FESEM analysis: The morphology of the sodium alginate obtained was examined with FESEM. Fig. 2a and 2b reveal the surface morphology and microscopic view of the polysaccharide. The obtained sodium alginate was noted to be powdery to somewhat granular in appearance. An irregular-shaped surface was depicted in 200 ì m and 10 ì m resolution microscopic analysis. Dangar *et al*. (2021) also revealed similar findings in the organoleptic character evaluation indicating sodium alginate as powder and granule at the same time which differed relative to the species of origin.

EDX analysis: The composition of elements found in the extracted sodium alginate identified using energy dispersive X-ray depicts (Table 1) various elements present in the sample in peaks with corresponding atomic concentrations. Sodium alginate was found to have Carbon, Oxygen, Sodium, Molybdenum, Chloride, Potassium, and Calcium with different percentages of atomic weight 37.88%, 48.99%, 8.04%, 0.98%, 3.19%, 0.38% and 0.54%, respectively. Dangar *et al*. (2021) revealed a higher proportion of water-soluble ash content was noted in *Sargassum tenerrium*, which was found to be less in other compared species such as *Dictyotadichotoma, Spathoglossum asperum,* and *Iyengaria stellata.*

3-D Optical Profile: Non destructive surface topography and optical profile of the obtained sodium alginate assessed with Zeta 20 3D-Optical profilometer. True-colour images and software-enhanced, 3-dimensional images of sodium alginate are depicted in Fig. 4A and 4B indicating a pale yellowish-white powder. The surface roughness of the powder sample is visible with the difference in colour intensity of the software-enhanced image. These outcomes correspond with the findings of Kusumawati *et al*.(2018) and Dangar *et al*. (2021) who reported the whiteness degree of sodium alginate from *Sargassum sp.* was found to be higher than *Turbinaria sp.* as well as commercially available sodium alginate. Dangar *et al.*(2021) indicated sodium alginate from *Sargassum tenerrium* as a yellowish-grey powder.

Thermal Property: The thermal property of the thus obtained sodium alginate is given in Figures 5A and 5B. The difference in load of the sample is measured as the basis of temperature. Sodium alginate on the increase of temperature undergoes an exothermic reaction and chars above the temperature of 100 ^{o}C. The ambient temperature was raised gradually from 30 ^{0}C to 1000 ^{o}C at the rate of 20 ^{o}C per minute and a total weight loss of 72.9% was achieved at around 980 ^{o}C. A gradual weight loss was 28.3% at 300 ^{o}C and 32.5% at 800 ^{o}C was noted.The initial weight of the sample decreased from 10.44 mg to 2.829 mg as residual mass when the experiment was over. A study by Dangar *et al*. (2021) revealed a similar ashing potential of *Sargassum sp.*on direct heat.

Heavy Metal Content: Assessment of the heavy metal content of the extracted sodium alginate revealed the absence (lower than the detectable limit) of arsenic and lead as indicated in Table 2. It was shown that chromium was in merge amounts of 0.021 ppm. Wherein, cadmium was present at the level of 0.034 ppm and mercury was found at 0.882 ppm level. These results meet the toxicity data given for alginates by FAO/WHO expert Committee on Food Additives and heavy metals limit by Indian Pharmacopoeia (<40.0 ppm) as given by Sachan *et al*. (2009).

Brine Shrimp Lethality Assessment: An evaluation of the survivability of brine shrimp was conducted to determine the acute toxicity of the extracted sodium alginate from *S.cinctum.* The study shows a higher mortality rate on increasing the concentration of the sample. Sodium Alginate showed less toxicity compared to control Potassium dichromate, as presented in the given Table 3 and Fig 6. By the conclusion of 8 h, one shrimp was found to be dead at the peak concentration indicating shorter exposure time is completely safe. By the conclusion of 24-hour treatment mortality rate drastically increases to 87% for the higher concentration.One-way ANOVA done between and within the sample, blank and control showed no significant difference at $p < 0.05$; where the f-ratio value is 0.3462. A study by Ramu *et al*.(2020) revealed "oral administration of compounds extracted from *Sargassum wightii* does not cause any significant organ toxicity in Wistar rats"

supporting the results of the study. Indeed, a study by Hira *et al*.(2019) revealed that "crude sulfated polysaccharides from *Sargassum swartzii* (Turn.) C. Ag. showed a protective effect against acetaminophen-induced liver toxicity in rats".

CONCLUSIONS

The main aim of the studies on seaweed is to analyze the potential of application in the industrial sector. Previous studies on seaweed have demonstrated notable differences in the characteristics of the extracted compounds concerning the method of extraction and species of origin. In this study, thermal analysis of sodium alginate depicted an exothermic degradation with significant residual mass, andanalysis of heavy metals revealed noticeably decreased quantitiesof mercury, lead, cadmium, chromium, and arsenic. Lethality Assay through brine shrimp for the analysis of acute toxicity of the extracted sodium alginateshoweda higher mortality rate only on the increased concentration of the sample after 8 hours of exposure.These findings demonstrate that the isolated component is suitable for use as a food source for humans and other relevant applications in the pharmaceutical industries. From the results, we can put forward that the mariculture of brown seaweed of *Sargassum cinctum* should be promoted for the sustainable development of coastal regions of the country. Increasing the magnitude of marine agronomy encourages the identification and utilization of new species of seaweeds with interesting compounds for wide industrial applications.

Table 1. Elemental composition of acquired Sodium Alginate from *Sargassum cinctum*

S.No.	Element	Atom concentration (at%)
1.	Carbon	37.88
2.	Oxygen	48.99
3.	Sodium	8.04
4.	Molybdenum	0.98
5.	Chlorine	3.19
6.	Potassium	0.38
7.	Calcium	0.54

Table 2. Heavy metal content of acquired Sodium Alginate from *Sargassum cinctum*

S.No.	Heavy Metals	Sodium Alginate (*ppm)
1.	Arsenic	Nil
2.	Cadmium	0.034
3.	Chromium	0.021
4.	Lead	Nil
5.	Mercury	0.882

***ppm-parts per million**

Table 3. Lethality assessment of acquiredSodium Alginate from *Sargassum cinctum* (n=30)

S.No.	Sample Code	Concentration (µg/ml)	Mortality of Brine shrimp (no. of shrimps dead) (n=30)					
			1h	2h	4h	8h	24h	% Mortality
1.	**Blank**	**Saline water**	**0**	0	0	0	0	0
2.	**Control $K_2Cr_2O_7$**	**1000**	**30**	-	-	-	-	100
3.	**SA**	250	0	0	0	0	15	50
		500	0	0	0	0	12	40
		1000	0	0	0	0	6	20
		1500	0	0	0	0	25	83
		2000	0	0	0	1	26	87
	F Value 0.3462^{NS}							

P <0.05; NS- Not Significant

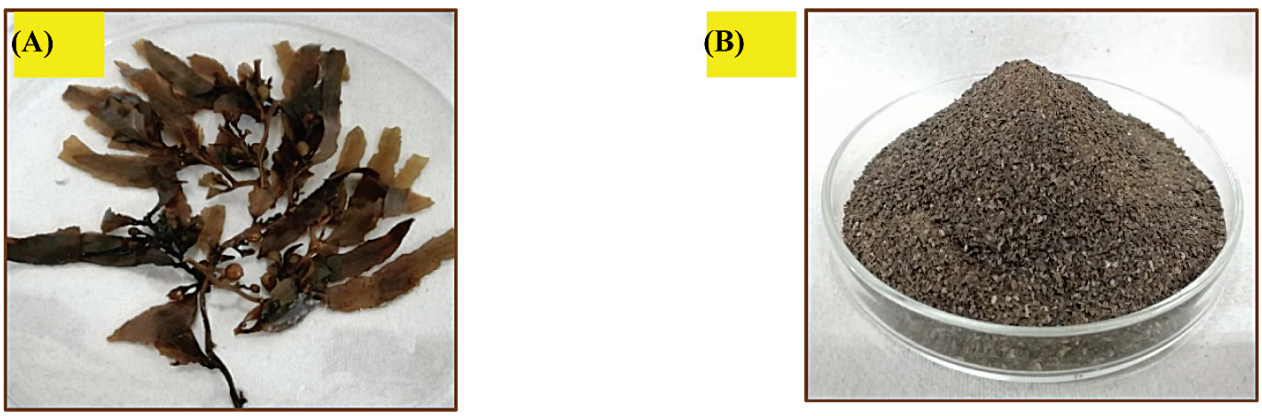

Figure 1. (A) Sargassum cinctum (B) Powdered Sargassum cinctum

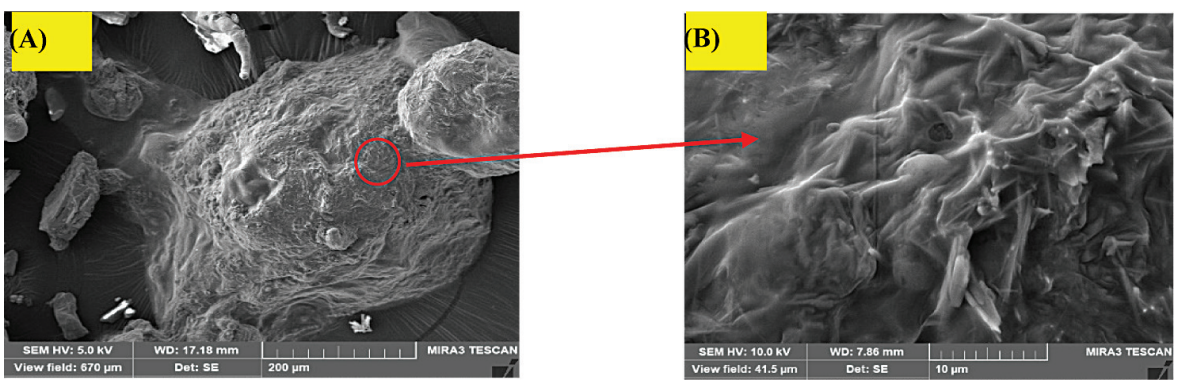

Figure 2. (A) and Fig. (B) FESEM Structure of acquired Sodium Alginate from *Sargassum cinctum*

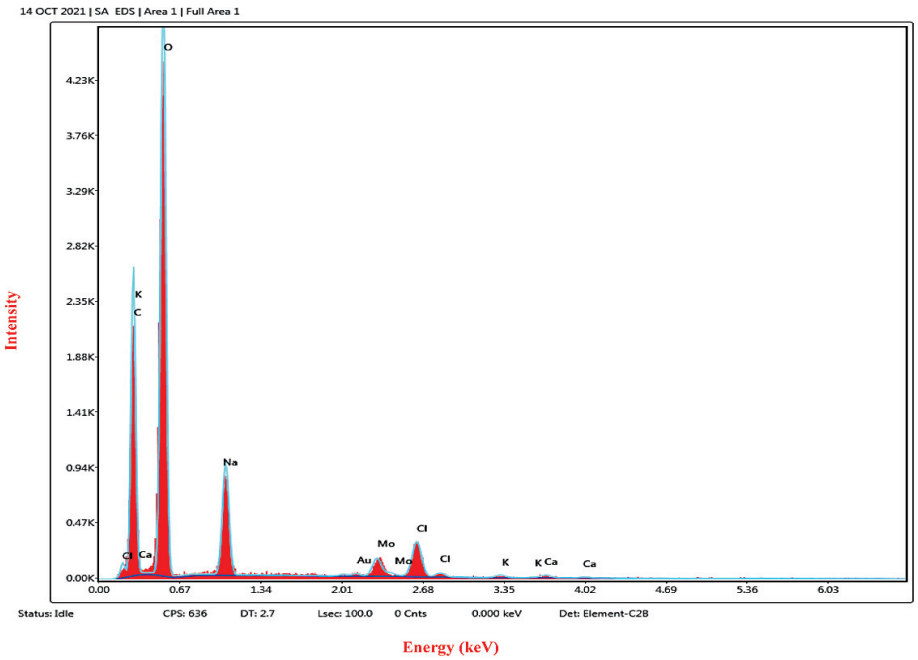

Figure 3. EDX spectrum of acquired Sodium Alginate from Sargassum cinctum

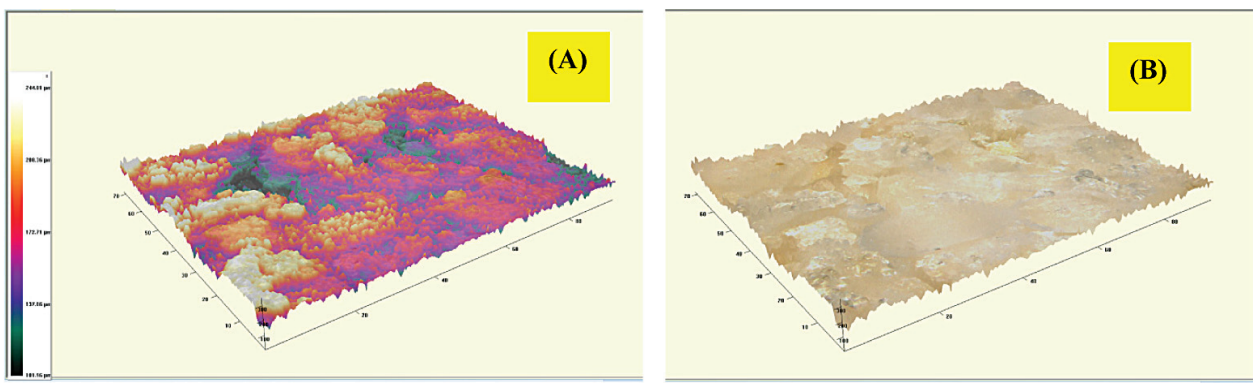

Figure 4. 3D image of acquired Sodium Alginate (A) Software Enhance Image(B) True Color Image

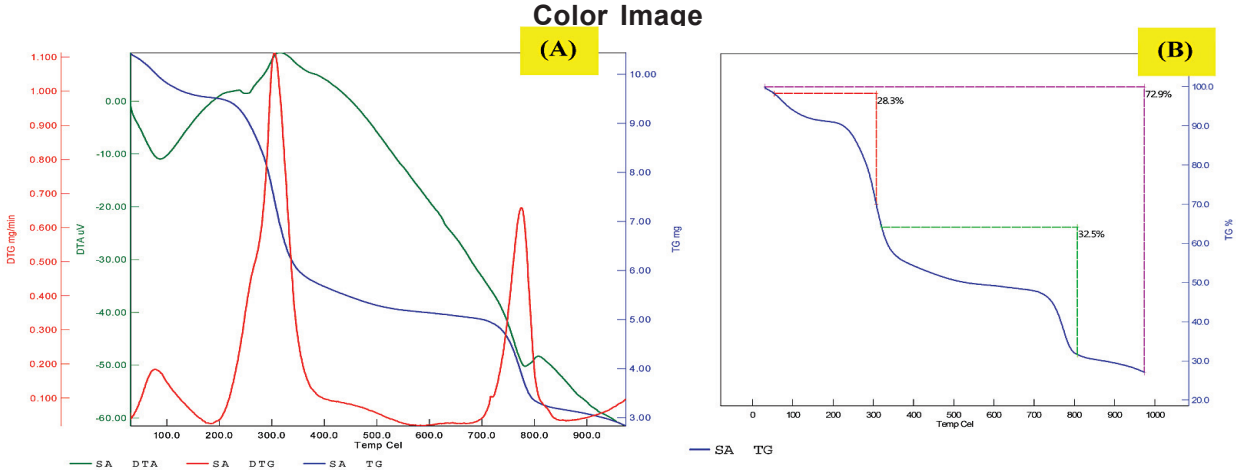

Figure 5. Thermal Property of acquired Sodium Alginate fromSargassum cinctum (A) DSC curve (B) TGA% curve

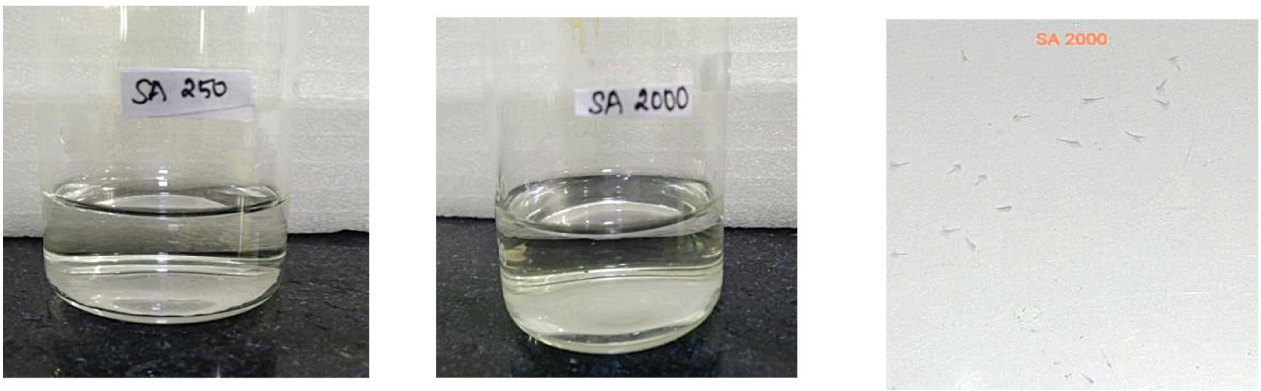

Figure 6. Movement of shrimp in samples of acquired sodium alginate from *Sargassum cinctum* at its lowest and higher concentrations

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STANDARDIZATION AND QUALITY EVALUATION OF WHEAT FLOUR BASED EDIBLE TABLEWARE

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ABSTRACT

Wheat flour was used in varying proportions ranging between 90% and 40% along with 10% to 60% maize flour for the preparation of edible tableware. The best treatment was selected through organoleptic evaluation and textural properties. Among the various combinations, 90 percent wheat flour and 10 percent maize flour was found to be better than other combinations in regards to organoleptic and textural properties. The selected edible tableware was subjected to proximate analysis and observed to have moisture (2.92%), energy (405.98 kcal), protein (13.58 g 100 g^{-1}), fat (3.18 g 100 g^{-1}), carbohydrate (80.76 g 100 g^{-1}), fibre (0.93 g 100 g^{-1}) and total ash (1.56 g 100 g^{-1}). The selected edible tableware was subjected to physico-chemical analysis and observed to have pH (5.28), water absorption index (5.24%), water solubility index (7.76%) and oil absorption capacity (2.20%). The cost of the selected edible tableware was Rs.30.56 per 100 g, which was less than the price of similar products in the market. Biodegradable tableware plays vital role in green marketing for sustainable environment.

Key Words: Cost, Edible tableware, Maize flour, Physico-chemical, Wheat flour

INTRODUCTION

Packaging is vital to preserve the properties of food and drinks. Plastics are widely used in the food packaging sector due to its attributes including low manufacturing costs, longevity, strong mechanical qualities, and resistance to oil and chemicals. Earthenware, wood and glass pots have been used for thousands of years and over the last two hundred years, metals including steel have been used for storing and preserving foods. Plasticshave become available for use in the food industry only over the last century. The demand for food packages and containers is increasing over the past few decades. The sale of plastic tableware is growing at a rate of 30 percent per year. Most of the street shops and public eating places use substandard plastics and thermoform plates for serving foods. These are unhealthy because of the presence of toxins and carcinogens. Edible tableware is trending way to overcome reversal of these problems caused by plastic food packages and other unhealthy packaging materials (Gaspar and Braga, 2023).

Edible cutlery is a natural and biodegradable commodity which can be

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concocted to be nutritious and replace the usage of plastic cutlery (Mukherjee and Raju, 2023). Edible cutlery offers the same convenience as disposable forks, spoons, chopsticks and bowls without causing environment problems. Edible tableware is a new concept in which the utensils such asplates, bowls and spoons are eaten along with the meal. Even if they are not consumed, they are able to degrade in the outside environment as they do not need any specific conditions for degradation. This idea has been used for long but has not got enough impetus in the developing world. Biodegradable films and tableware are prepared by using biological materials such as starch, proteins of animal and vegetable origin, fat, resins, polysaccharides, proteins and their derivatives, which are renewable and natural (Tanwar and Modgil, 2012). Edible cutlery is an upcoming line of plantbased utensils that are completely safe to eat and are considered as a boon to the ecosystem. These edible cutleries are a perfect alternative to harmful plastic cutlery. The edible cutlery are not only safe for the environment but are also enriched with nutritious ingredients (Ghosh, 2016).

Edible tableware is designed as ready to eat products. Edible tableware can be used as utensils and also to servesolid and semisolid things. The textural properties and water retention capacity are the important parameters tomeasure the strength of edible tableware. These are environment friendly as they can be easily discarded and eaten by animals. Unlike the plastic cutlery that are always thrown after use, the edible cutlery can just dissolve in the soil as they are made of food grains. Every food sector service can use it to make the environment cleaner. The edible cutlery can be made from edible ingredients such as rice, wheat, millets, vegetables and tubers.

Wheat (*Triticumaestivum* L.) has been used since time immemorial for the manufacture of several bakery items because of the presence of unique gluten protein which is responsible for providing the viscoelastic characteristics to dough. The two fractions of gluten, gliadins and glutenins give elastic and extensible properties to dough which is essential for producing good quality bakery products (Hamdani *et al*., 2020).

Maize or corn is a cereal crop that is grown widely throughout the world in a range of agroecological environments. It is the most important cereal in the worldafter wheat and rice (Jalali *et al*., 2020). Corn flour also has vast potential in the bakery industry for the development of glutenfree baked products like composite bread, crackers and biscuits (Sun *et al*., 2019).

It is pertinent to mention that scanty data is available on the standardised protocols for the production of edible tableware using commonly available and cost effective food resources. Hence, the study was proposed to develop healthy and environment friendly edible tableware in the year 2019.

MATERIALAND METHODS

Materials

The experiment was conducted between 2019 and 2023 at theDepartment of Community Science, College of Agriculture, Kerala Agricultural University, Thrissur. The edible tablewarewas formulated by using wheat flour and maize flour as the major ingredients. Wheat flour, maize flour and all other raw ingredientneeded for the study were purchased from the local market.

Formulation of edible tableware

Six variants of wheat flour based dough were prepared by combining different proportions. Wheat flour was used in varying

proportions ranging from 90 to 40 percent along with 10 to 60 percent maize flour. The flours were blended well and thendough was prepared using water and oil. Table 1 depicts the experimental design of the variations for the preparation of wheat flour based edible tableware.

Thick dough was prepared by uniformly blending the raw materials and was sheeted by dough rolling pin. Then, sheets were cut into a specific shape by dough moulders and were placed on a tray and subjected to baking in a conventional oven for 90 min at 180 °C temperature, as depicted in Fig.1.

Organoleptic evaluation

A series of acceptability trials were carried out using simple triangle test at the laboratory level and a panel of fifteen judges between the age group of 18- 35 years were selected as suggested by Jellineck (1985).The organoleptic evaluation of the edible tablewarewas carried out using a 9-point hedonic scalefor each quality parameter *viz*., colour, appearance, texture, flavour, taste and overall acceptability was recorded.

Texture analysis of edible tableware

Edible tableware wasanalysed for their textural profile (hardness) through TA TX2 Texture Analyser (Stable Micro Systems Ltd., UK) equipped with a 5mm diameter cylindrical probe. The settings for tests were 500 N load cell, pre-test speed of 2 mm/s, test speed of 1 mm/s, post-test speed of 2 mm/s. Each sample was subjected to compression test and the texture was indicated by the first maximum peak force in the force-distance curve.

Proximate composition determination

Proximate composition (moisture, crudeprotein, crude fat, and crude ash contents) of selected edible tableware was analysed asper the recommended AOAC (1990) methods. Carbohydratecontent was calculated by subtracting the weight ofmoisture, crude protein, crude fat, and ash contents fromtotal sample weight. Energy value of edible tableware (kcal/100 g) was calculated onthe basis of their caloric value which consists of caloriccoefficients corresponding to the contents of proteins,carbohydrates and fats according to the formula: Caloric value kcal/ 100 g = (g protein x 4) + (g fat x 9) + (g carbohydrates x 4)

Physico-chemical properties

The pH value of edible tableware was determined using apH meter (Methrohm AG, Herisau, Switzerland). The pH of the products were measured using food grade pH meter. The solution for reading pH was prepared in a ratio of 1:10 (Berwal *et al*., 2004). Water absorption index (WAI) and water soluble index (WSI) were determined by the method of Anderson *et al*. (1969). Oil absorption capacity (OAC) was determined by standard procedure of Ranganna (1986). WAI, WSI and OAC were calculated by the formulas below:

$$\text{WAI} = \frac{\text{Weight of the sediment}}{\text{Weight of the dry solids}}$$

$$\text{WSI} = \frac{\text{Weight of the dissolved solids in supernatant}}{\text{Weight of the dry solids}}$$

OAC = Initital volume of oil added to the sample - Volume of the supernatant

Statistical analysis

The scores obtained for organoleptic evaluation were evaluated by Kendall's Coefficient of Concordance (W).

Cost of Production

The cost of production of the most acceptable combination of wheat flourbased edible tablewarewere computed based on the

market price of procured ingredients used for preparation of products along with labour charge, fuel charge, electricity charge and packaging cost. The cost was calculated for 100 g of the product and compared with similar products available in the market.

RESULTS AND DISCUSSION

Organoleptic evaluation

Sensory evaluation was used to measure, analyse and interpret how sensory attributes of a product are perceived by people. The suitability of wheat flour in combination with maize flour for the development of edible tableware was assessed. The mean scores were statistically analysed and mean rank scores were obtained for organoleptic qualities of each treatment. Kendall's value showed that there was significant agreement between the judges at 1% level.

The wheat flour incorporated edible tableware were standardised with different proportions of wheat flour and maize flours (Fig.2).

In the study, the mean score for the appearance of edible tableware varied between 8.06 and9.00 with mean rank scores in the range of 2.10 to 4.70. Among different treatments, the highest mean score of 9.00 for appearance was noticed in $WT_1$and $WT_3$and the lowest in WT_5 and WT_6(8.06).

The highest mean score for colour (8.83) was noticed in $WT_3$and the lowest mean score for colour was observed in WT_5 (8.23). The mean rank scores of various treatments ranged between 2.40 and 4.60. The mean score for flavour of edible tableware was 6.56 to 8.60 with the maximum in WT_1 (8.60).

The mean score for the texture of wheat flour based edible tableware varied between 6.16 and 7.33 with mean rank scores in the range of 1.95 to 5.15. The mean score for taste of different wheat flour based edible tableware ranged from 5.86 to 7.50.

The highest mean score of 8.13for overall acceptability was noticed in WT_1. The lowest mean score (6.68) with mean ranking score of 2.20 was noticed for treatment WT_6. The mean scores and mean rank scores for sensory parameters (appearance, colour, flavour, texture, taste and overall acceptability) of edible tablewareprepared with wheat flour and maize flour was the highest for treatment WT_1(90% WF + 10% MF). The highest total score of 48.49 was noticed in WT_1 followed by 47.56 (WT_2), 46.85 (WT_3), 44.35 (WT_4), 41.65 (WT_5) and 42 (WT_6), respectively. Kendall's value showed that there was significant agreement between the judges at 1% level. Stuti and Virginia (2022) found that wheat flour, semolina, ragi, gram flour, orange peel powder and mosambi peel powder incorporated edible bowl secured overall acceptability of 8.83 and for all other quality attributes like appearance, colour, flavour, texture and taste were scored above 8. A similar study was conducted by Sood and Deepshikha (2018), who reported that the rice flour and sorghum flour incorporated edible plate scored 7.2 for overall acceptability.

Texture analysis of edible tableware

The textural property of the edible tablewarewas studied using texture analyser. The hardness values for edible spoons are depicted in Table 3.

The effect of incorporation levels of wheat flour on the hardness of the edible tableware were analysed. The variation in the texture of the edible tableware may be attributed to the change in the gluten strength. The replacement of wheat flour with maize flour adversely affected the formation of gluten network (Gull *et al*., 2015). As shown in Table 3, the hardness of wheat flour based samples,

Table 1. Different levels of wheat flour and maize flour for standardization of edible tableware

S.No.	Ingredients (%)	Variations					
		WT_1	WT_2	WT_3	WT_4	WT_5	WT_6
1	Wheat flour	90	80	70	60	50	40
2	Maize flour	10	20	30	40	50	60
3	Water (ml)	125	125	110	100	100	90
4	Oil (ml)	5	5	5	5	5	5

peaked at 90 g/100 g and 80 g/100 g inclusion levels respectively and then dropped for the next inclusion level.The highest hardness of 98.71 N was noticed in WT_1 followed by 94.97 N (WT_2), 67.70 N (WT_3), 66.59N (WT_4), 60.72N (WT_5) and 44.61N (WT_6) respectively. Sindhu *et al*. (2023) found that hardness for sample is 7.95 N, which contain 40 g of wheat flour, 40 g of sorghum flour, 20 g of rice flour and 2 g of guar gum.

Proximate composition of selected edible tableware

Based on sensory evaluation, in edible tableware based on wheat flourflour, the treatment WT_1(90%WF+10%MF)was found to be the best. The nutritive value of selected wheat flour based edible tableware was observed to have moisture (2.92%), energy (405.98 Kcal), carbohydrate (80.76 g 100 g^{-1}), protein (13.58 g 100 g^{-1}), fat (3.18 g 100 g^{-1}), crude fibre (0.93g 100 g-1), starch (75.01 g 100 g-1) and total ash (1.56 g 100 g^{-1}) as shown in Table 4. Sood and Deepshikha (2018) reported that the edible plate contains

Table 2. Mean scores for organoleptic evaluation of wheat flour based edible tableware

S. No.	Treatments	Sensory attributes						Total score
		Appearance	Colour	Flavour	Texture	Taste	Overall acceptability	
1	WT_1	9.00	8.70	8.60	7.26	6.80	8.13	48.49
	(90% WF + 10% MF)	(4.70)	(4.50)	(5.40)	(4.40)	(3.75)	(6.00)	
2	WT_2	8.83	8.50	8.00	7.33	7.5	7.40	47.56
	(80% WF + 20% MF)	(3.70)	(3.20)	(4.40)	(5.15)	(5.45)	(4.25)	
3	WT_3	9.00	8.83	7.50	7.00	7.16	7.36	46.85
	(70% WF + 30% MF)	(4.70)	(4.60)	(3.80)	(3.90)	(4.85)	(3.10)	
4	WT_4	8.83	8.33	7.00	6.5	6.33	7.36	44.35
	(60% WF + 40% MF)	(3.70)	(2.55)	(2.55)	(2.95)	(2.80)	(3.05)	
5	WT_5	8.06	8.23	6.76	6.36	5.90	6.69	42
	(50% WF + 50% MF)	(2.10)	(2.40)	(2.45)	(2.65)	(2.20)	(2.50)	
6	WT_6	8.06	8.33	6.56	6.16	5.86	6.68	41.65
	(40% WF + 60% MF)	(2.10)	(3.10)	(2.40)	(1.95)	(1.95)	(2.20)	
7	Kendall's W value	.649**	.262**	.631**	.570**	.680**	.588**	

WF - Wheat flour, MF - Maize flour; Values in parentheses is mean rank score based on Kendall's W;
** Significant at 1% level

Table 3. Textural property (hardness) of wheat flour based edible tableware

S.No.	Treatments	Hardness (N)
1	WT_1	98.71
2	WT_2	94.97
3	WT_3	67.70
4	WT_4	66.59
5	WT_5	60.72
6	WT_6	44.61

Table 4. Proximate composition and physico-chemical properties of wheat flour based edible tableware

S.No.	Parameters	WT_1(90% WF+10% MF)
1	Moisture (%)	2.92
2	Energy (Kcal)	405.98
3	Protein (g/100 g)	13.58
4	Fat (g/100 g)	3.18
5	CHO (g/100 g)	80.76
6	Crude fibre (g/100 g)	0.93
7	Starch (g/100 g)	75.01
8	Total ash (g/100 g)	1.56
9	pH	5.28
10	Water absorption index (%)	5.24
11	Water solubility index (%)	7.76
12	oil absorption capacity (%)	2.20

* WF -Wheat flour, MF - Maize flour

2.57 ,1.60, 1.72 ,4.81 and 0.64 percent moisture, ash, crude fat, crude protein and crude fibre respectively. Sindhu *et al*. (2023) observed that edible spoon (40 g wheat flour, 40 g sorghum flour and 20g rice flour) to have moisture (5.32%), protein (3.85 g 100 g^{-1}), fat (1.6 %)and total ash (1.38%).

Physico-chemical properties of selected edible tableware

The selected edible tableware was subjected to physico-chemical analysis and observed to have pH (5.28), water absorption index (5.24%), water solubility index (7.76%) and oil absorption capacity (2.2%) as shown in Table 4. The pH is slightly similar to the

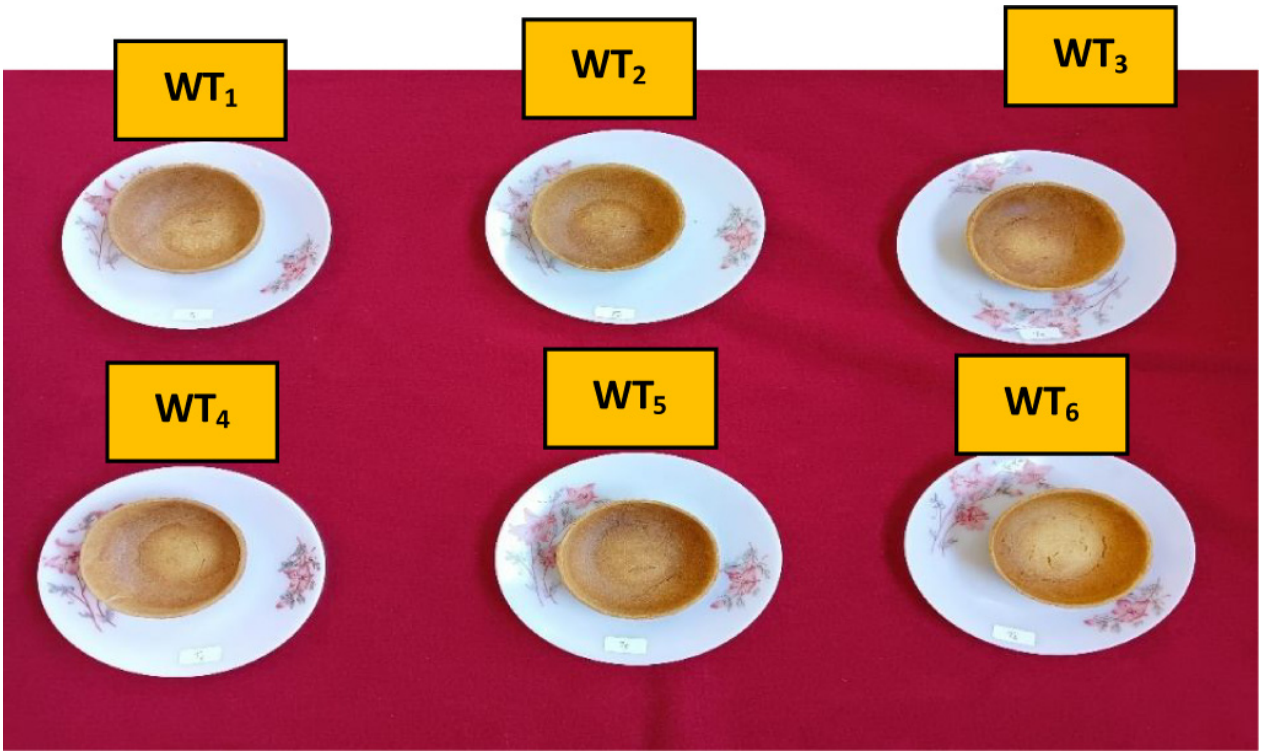

Fig. 2. Wheat flour based edible tableware

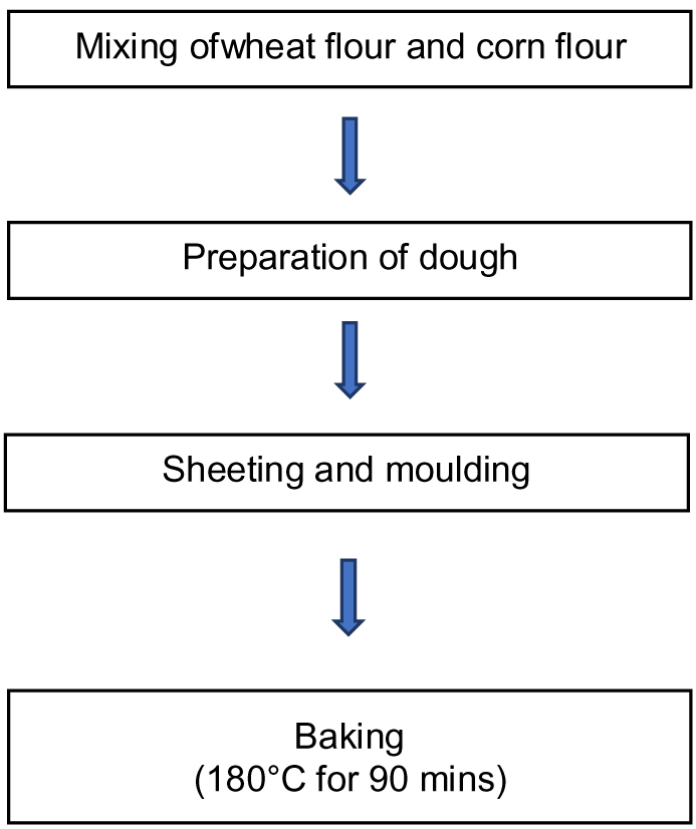

Figure 1. Flowchart for the preparation of edible tableware

experiment conducted by Rumler *et al*. (2023), who observed that the sorghum and wheat flour incorporated noodle dough mixhad a pH of 6.24, water solubility index of 2.76% and oil absorption capacity of 1.46%. According to Shrestha and Srivastava (2017), water absorption index is ability of a product to absorb water within its structural boundaries. Hazra and Sontakke (2023) reported that *Withania somnifera* root powder supplemented edible spoon have 9.70% of water absorption index.

Cost of wheat flour based edible tableware

The cost of edible tableware was Rs.15.28/ 100 g. The market price of edible tableware was observed as Rs.84.6/piece. The cost of prepared edible tableware was lower compared to the market price.

CONCLUSIONS

This research highlighted the development of edible tableware using wheat flour and maize flour for nutritional enrichment. The edible tableware developed in this study, as a sustainable alternative to single-use plastic and these tableware were nutritious that can be eaten or disposed in a compost pit, or they can be eaten by animals. From the study it is evident that highly acceptable edible tableware could be prepared from wheat flour and maize flour. The treatment WT_1(90% WF+ 10% MF) was the most acceptable combination. Although the idea of edible tableware is a relatively recent one, it can be advantageous for both people and the ecosystem as a whole.

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PROBIOTIC POTENTIAL OF *PICHIA KUDRIAVZEVII* Y01 AND PREPARATION OF FERMENTED WHEY BEVERAGE BY RESPONSE SURFACE METHODOLOGY

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ABSTRACT

This study conducted in the year 2022 aimed to the evaluation of probiotic potential of yeast *Pichia kudriavzevii* Y01 obtained from traditionally prepared fermented milk and develop a probiotic functional whey beverage using this lactose-fermenting yeast by employing response surface methodology (RSM). The probiotic properties of *Pichia kudriavzevii* Y01, including bile tolerance, acid tolerance, temperature tolerance, autoaggregation, and bacterial cell hydrophobicity, were assessed to determine its suitability for probiotic applications. Yeast strain exhibited remarkable acid, bile and temperature tolerance and showed good auto-adhering potentiality with a value index of greater than 85%. Additionally, the study focused on the development of a probiotic whey beverage enriched with ginger extract to enhance its sensory appeal. Response surface methodology was usedto optimize the fermentation conditions, ensuring a well-balanced flavour profile that meets consumer expectations. Significant correlation models were established with the coefficient of correlation (R^2) greater than 0.9. The study revealed that *Pichia kudriavzevii* Y01 exhibited excellent probiotic potential, making it an ideal candidate for delivering probiotic benefits. Furthermore, the developed probiotic whey beverage, enriched with ginger extract, was sensorially acceptable and met the consumers' preferences.

Keywords: ginger, *Pichia Kudrivzevii,* probiotic, whey, yeast

INTRODUCTION

Consumers today are mindful of their health and well-being. There is a growing significance placed on probiotic drinks in recent times, and the food and beverage industries are investing more in research and development in the realm of health-promoting beverages. As the consumption of milk and dairy products continues to rise, the production of dairy waste also increases. Neglecting proper management of these waste products leads to significant environmental problems. Whey, which is the primary liquid by-product derived from cheese production, holds immense importance (Skryplonek & Mituniewicz-Maek, 2019). Utilizing whey to create a probiotic beverage would represent a groundbreaking advancement in the beverage industry.

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On average, whey consists of approximately 93% water and contains about 50% of the total solids found in milk, with lactose being the primary component. Whey proteins make up less than 1% of the dry matter in whey (Beucler *et al*., 2005) The characteristics of cheese whey pose a threat to the environment. It has a significant impact due to its high levels of biochemical oxygen demand (BOD) and chemical oxygen demand (COD), surpassing 35,000 and 60,000 parts per million (ppm) respectively (Smithers, 2008).

Yeasts are eukaryotic microorganisms consisting of single cells that are extensively spread outin the environment. They can be found in numerous traditional fermented foods and beverages, serving both as beneficial microorganisms and potential agents of food spoilage (Riesute *et al*., 2021). Various species, including *S. boulardii*, as well as those belonging to the genera *Kluyveromyces*, *Debaryomyces*, *Candida*, *Pichia*, *Hanseniaspora*, and *Metschnikowia*, are acknowledged as probiotics (Agarbati *et al*., 2020; Karim *et al.,* 2020; Zahoor *et al*., 2021).*Pichia kudriavzevii* (*Candida krusei*) is emerging as an intriguing yeast due to its metabolic activities and impact on flavour development. *P.kurdiavzevii* Y33 isolated from mango pickle improvedthe flavour and exhibited probiotic potential (Latha *et al.,* 2022). The presence of this species has been previously detected in a traditional fermented milk,'Thairu' and was found to be lactose fermenting (Chandran *et al.,* 2022).

The fermentation of whey offers various other benefits, including a reduction in lactose content, partial hydrolysis of whey protein (which can be linked to allergies), an extended shelf life due to lactic acid, and the generation of aroma compounds that enhance sensory characteristics (Skryplonek & Mituniewicz-Maek, 2019).

Ginger has a rich historical background in both conventional and complementary therapies. Its various applications include aiding digestion, alleviating motion sickness, and combating the flu and common cold. However, direct consumption of ginger is relatively low. To increase the intake of this natural antioxidant and nutrient-rich herb, it is possible to incorporateit into beverages. By adding ginger juice to whey, the taste, aroma, palatability, and nutritional value can be improved, making it a more appealing and nutritious option (Sharma *et al.,* 2020).

Therefore, the main objective of this study was to evaluate the probiotic potential of yeast *Pichia kudriavzevii* Y01 obtained from traditionally prepared fermented milk and the development of functional whey beverage with probiotic potentialusing this lactose fermenting yeast.

MATERIAL AND METHODS

Whey was obtained as a by-product after the production of cheese from College of Dairy Science and Technology, Pookode Wayanad. Ginger and sugar purchased from local market. Pichia kudriavzevii Y01 was obtained from the culture stock of Department of Dairy Microbiology, CDST, Pookode. Yeasts were activated by inoculating 1% of inoculum in 10 ml of YEPD growth medium(2% yeast extract, 1% peptone, and 2% glucose (w/v)) andit wasincubated at 25 °C for 24 h. The study was conducted in the year of 2022.

Evaluation of probiotic properties of *Pichia Kudriavzevii* Y01

Bile tolerance

YEPD broth with different concentration of bile 0.3%, 0.6% was sterilized and then inoculated with 1% active yeast culture and incubation done at 37 °C.The optical density was measured in each hour for 4 h and 24 h

at 620nm in UV-Visible spectrophotometer (Agilent, USA)

Acid tolerance

YEPD broth was made in pH of 1.5 and 3. Active culture (1%) was inoculated in five tubes with varying pH (1.5, 3) and incubation done at 37 °C for 4 hours and optical density was measured at 600 nm in UV-Visible spectrophotometer(Agilent, USA).

Temperature tolerance test

Tubes containing YPD broth was sterilized and inoculated with 1% active culture and incubation done for 24 h at different temperature 4 °C, 10 °C, 25 °C, 45 °C. Optical density was measured at 600 nm (Costa *et al.,* 2014).

Adhesion potential

Adhesion potential of isolate was evaluated based on thecell surface hydrophobicity and auto aggregation (%). Auto aggregation was measured as per the procedure described by Del Re *et al*.(2000).Measurement of adhesion potential wasin terms of cell surface hydrophobicity by BHA (Bacterial Adhesion to Hydrocarbons) assay (Collado *et al*., 2008).

Development of fermented whey ginger beverage

In this study, whey obtained after cheese pressing was pasteurized at 75°C for 10 minutes and then clarified and cooled. Ginger extract and sugar were subsequently added, followed by cooling to 25°C before inoculating with a yeast culture. The whey sample prepared was inoculated with yeast culture at one percent level and was incubated at 25°C for 24 h, allowing fermentation to occur. Finally, the probiotic whey beverage was cooled and stored at 4°C.

Sensory analysis

Sensory evaluation of the prepared beverages was done by five panels of judges. Sensory attributes such as colour, appearance, consistency, taste and flavour and overall acceptability of the prepared beverages was done by adopting nine-point hedonic scale (Saikia *et al.,* 2020). The samples were placed before the judges.

Statistical analysis

A central composite rotatable design (CCRD) coming underresponse surface methodology (RSM) technique was employed for the optimization of ginger and sugar in fermented whey beverage. The CCRD of two factors contained 13 combinations, including lower and upper limits, along with their responses for sensory parameters are displayed (Table 1). The data generated for different responses were analyzed using Design Expert® software (13.0.2 version) (Stat-Ease, Inc., 2021 E. Hennepin Avenue, Minnepolis, USA).

RESULTSAND DISCUSSION

Evaluation of probiotic potential

The acid environment of stomach presents a formidable challenge for many probiotic strains, as it can lead to reduced viability and effectiveness. However, *Pichia Kudriavzevii* Y01 has demonstrated exceptional acid tolerance, enabling it to survive the harsh gastric conditions and reach the intestines alive (Table 2). Studies by (Greppi *et al*., 2017) have mentioned that *Pichia Kudriavzevii* demonstrated exceptional survival even in extremely acidic environments (pH 2.0), making it a promising candidate for oral probiotic formulations. Bile salts released into the small intestine play a crucial role in digestion but can also be detrimental to probiotic viability. *Pichia Kudriavzevii* has been found to withstand the inhibitory effects of bile

salts effectively (Table 1), as indicated by research conducted by Wulan *et al.* (2021). This trait enhances its potential to survive and exert beneficial effects in the intestinal environment.Probiotic strains with the ability to withstand temperature fluctuations are highly favorable for both storage stability and gastrointestinal survival. *Pichia Kudriavzevii* has been shown to exhibit robust temperature tolerance (Table 3), as evident from the study by (Chamnipa *et al.*, 2018). This characteristic enables the strain to maintain viability during manufacturing processes and storage, enhancing its suitability for commercial probiotic applications.

Effective adhesion to the gut epithelium is essential for probiotics to exert their beneficial effects by interacting with host cells and modulating immune responses. *Pichia Kudriavzevii* has demonstrated significant adhesion potential (Table 4 and Table 5), as revealed by the research conducted by (Chen *et al*., 2010). The ability of this isolate to adhere to intestinal surfaces suggests its potential for colonization and persistence in the gut, which are most critical factors for sustained probiotic activity.

Development of fermented whey ginger beverage by RSM

Optimization of levels of ginger extract and sugar by response surface methodology.

The levels of incorporation of ingredientsfor the fermented whey beverage was selected based on thepreliminary trials. The selected levels of each ingredient were optimized using Central Composite Rotatable Design (2 Factors) of Response Surface Methodology. Table 6 contains the actual and codedlevels of the two factors (design factors) and a design matrix of representation of different combinations of the two factors are given in Table 7.

Effect of different levels of ginger and sugar on sensory attributes of fermented whey beverage

The sensory scores of the different combinations obtainedbythe proposed experimental design are summarized (Table 8).The quadratic models for sensory attributes such as flavour, body and texture, overall acceptability and colour and appearance were obtained through continuous regression analysis. The partial coefficients of regression of linear, quadratic andterms of interaction for each model and their R^2 values are shown (Table 9).

The F-value of the model for all the aspects are significant at five per cent level ($p<0.05$) declaring that the given model is significant. The coefficient of determination (R2) for flavour, colour and appearance, consistency, sweeteness and overall acceptability was found to be 0.96, 0.94, 0.96, 0.90 and 0.97 which showed that the quadratic model which is fitted, indicate more than 80 per cent of the variation in the experiment data. The non-significant lack of fit test (Table 9) specified that the given model is accurate satisfactorily for forecasting the sensory properties.

Effect on flavour

The scores for flavour are summarised in Table 8 and Table 9 contains the partial regression coefficients.The followingequation from response surface methodologywas generated to forecast the change in flavour with different level of factors:

$$\text{Flavour} = 8.42 + 0.306954 * A + 0.0978553 * B + 0.075 * AB - 0.4225 * A^2 - 0.3475 * B^2$$

Effect of colour and appearance

The yellowish green colour and appearance is the ideal characteristics for fermented ginger incorporated whey beverage.

Table 1. Bile tolerance of yeast culture

S.No.	Bile salt (%)	OD at various hours			
		0 h	1 h	2 h	3 h
1	0.6	0.0218 ± 0.07	0.0673 ± 0.01	0.1392 ± 0.00	0.1682 ± 0.03
2	0.3	0.053 ± 0.03	0.0987 ± 0.03	0.1912 ± 0.02	0.2113 ± 0.01

Figures are mean ± standard error of three replications

Table 2. Acid tolerance of yeast culture

S.No.	pH	OD at various hours				
		0 h	1 h	2 h	3 h	4 h
1	3	0.098 ± 0.02	0.131 ±0.07	0.155 ±0.00	0.174 ± 0.10	0.293 ±0.03
2	1.5	0.143 ± 0.04	0.156 ± 0.04	0.178 ± 0.01	0.199 ± 0.05	0.238 ± 0.03

Figures are mean ± standard error of three replications

Table 3. Temperature tolerance of yeast culture

S.No.	Time	Temperature				
	(h)	4 °C	10 °C	25 °C	37 °C	48 °C
1	0	0.0133 ± 0.002	0.0211 ± 0.025	0.0183 ± 0.009	0.0196 ± 0.034	0.0200 ± 0.056
2	1	0.0199 ± 0.010	0.0243 ± 0.002	0.0254 ±0.003	0.0432 ± 0.044	0.0218 ± 0.002
3	2	0.0260 ± 0.008	0.0425 ± 0.002	0.0369 ± 0.023	0.0481 ± 0.042	0.0291 ± 0.012
4	3	0.0493 ± 0.031	0.0441 ± 0.043	0.0586 ± 0.014	0.0653 ± 0.011	0.0586 ± 0.007
5	4	0.0476 ± 0.022	0.0579 ± 0.032	0.0613 ± 0.035	0.1258 ± 0.211	0.2186 ± 0.001
6	5	0.0498 ± 0.012	0.0639 ± 0.728	0.1813 ± 0.055	0.1336 ± 0.090	0.5841 ± 0.045
7	24	0.0763 ± 0.009	0.0813 ± 0.043	1.2426 ± 0.032	1.4178 ± 0.083	1.1170 ± 0.120
8	48	0.0770 ± 0.001	0.0800 ± 0.033	1.7379 ± 0.134	1.8133 ± 0.065	1.7933 ± 0.022

Figures are mean ± standard error of three replications

Table 4. auto aggregation results of yeast culture

S.No.	Culture	Initial (A1)	Final (A2)	$= \frac{A1 - A2}{A1} \times 100$
1	Y 01	0.0095 ± 0.004	0.0013 ± 0.00	86.13%

Figures are mean ± standard error of three replications

Table 5. Cell hydrophobicity of yeast culture

S.No.	Culture	Initial (A1)	Final(A2)	$=\frac{A1-A2}{A1}\times 100$
1	Y 01	1.133 ± 0.002	0.811 ± 0.007	28.48%

Figures are mean ± standard error of three replications

Table 6. The coded and actual levels of Ginger and Sugar

S.No.	Coded level Factor	Lower limit	Factorial point	Centre coordinate	Factorial point	Upper limit
		-2	-1	0	+1	+2
1	Ginger (%)	0.5	0.625	0.75	0.875	1
2	Sugar (%)	6	7	8	9	10

Table 7. The Central Composite Rotatable design of the two factors

Run	Factor 1	Factor 2
	A: sugar	B: ginger
1	8	0.75
2	8	0.75
3	6	0.5
4	10	1
5	8	0.4
6	8	1.1
7	8	0.75
8	8	0.75
9	6	1
10	5.2	0.75
11	8	0.75
12	10.8	0.75
13	10	0.5

The scores of colour and appearance are summarised in the Table 8 and Table 9. The following equation from response surface methodology was generated to forecast the change in colour and appearance with different level of factors:

Colour and appearance = 8.06 + 0.0728553 * A + 0.0301777 * B -0.025 * AB -0.13 * A^2 - 0.105 * B^2

Effect on consistency

The characteristic feature of paneer whey. The scores obtained for consistency are summarized Table 8 and the partial coefficients are given in Table 9.The following equation from response surface methodologywas generated to forecast the change in body and texture with different level of factors

Table 8. Sensory characteristics of yeast fermented whey beverage

Run	Response 1	Response 2	Response 3	Response 4	Response 5
	flavour	colour and appearance	consistency	sweetness	overall acceptability
1	8.5	8	8.2	8.4	7.8
2	8.4	8	8.3	8.4	7.8
3	7.4	7.7	7.8	7.6	7.2
4	8.1	7.9	8.1	8.3	7.6
5	7.6	7.8	7.9	7.8	7.3
6	7.8	7.9	8	7.7	7.4
7	8.4	8.1	8.3	8.4	7.7
8	8.3	8.1	8.2	8.3	7.9
9	7.5	7.8	7.8	7.5	7.2
10	7	7.7	7.6	7.3	7
11	8.5	8.1	8.2	8	7.8
12	8.1	7.9	8.1	8.2	7.5
13	7.7	7.9	8	8.2	7.4

Table 9. ANOVA and Regression coefficients of quadratic model fitted

S.No.	Partial Coefficients	Sensory characteristics				
		Flavour	Colour and appearance	consist-ency	Sweet-ness	Overall acceptability
1	Intercept	8.42	8.06	8.24	8.3	7.8
2	A- Sugar	0.306**	0.072**	0.150**	0.334**	0.163**
3	B- Ginger	0.097**	0.0301*	0.0301^{ns}	-0.0176^{ns}	0.0426*
4	AB	0.075^{ns}	-0.025^{ns}	0.025^{ns}	0.05^{ns}	0.05^{ns}
5	A^2	-0.422**	-0.13**	-0.188**	-0.237**	-0.262**
6	B^2	-0.347**	-0.105**	-0.138**	-0.237**	-0.212**
7	Lack of fit	3.08^{ns}	0.0279^{ns}	0.758^{ns}	0.550^{ns}	0.457^{ns}
8	Model F value	40.80*	25.67*	39.56*	13.21*	49.11*
9	R^2	0.96	0.94	0.96	0.90	0.97
10	Adeq. Press	16.367	12.773	16.771	8.962	17.965

*- Significant at five percent level ($p<0.05$), **- Significant at one percent level ($p<0.01$), ns- non significant ($p >0.05$)

$$\text{Consistency} = 8.24 + 0.150888 * A + 0.0301777 * B + 0.025 * AB - 0.18875 * A^2 - 0.13875 * B^2$$

Effect on sweetness

The scores for sweetness are summarized in Table 8 and the partial coefficients are given in Table 9. The following equation from response surface methodology was generated to forecast the change in overall acceptability with different level of factors.

$$\text{Sweetness} = 8.3 + 0.334099 * A - 0.0176777 * B + 0.05 * AB - 0.2375 * A^2 - 0.2375 * B^2$$

Table 10. Criteria and constraints for optimization of yeast fermented whey beverage

S.No	Constraint	Goal	Lower limit	Upper limit
1	Sugar (%)	In range	6	10
2	Ginger (%)	In range	0.5	1
3	Flavour	Maximize	7	8.5
4	Colour and appearance	Maximize	7.7	8.1
5	consistency	Maximize	7.6	8.3
6	sweetness	Maximize	7.3	8.4
7	Overall acceptability	Maximize	7	7.9

Table 11. Obtained solutions after response surface analysis

Solution No	Sugar (%)	Ginger (%)	Desirability
1.	8.8	0.8	0.95

Effect of overall acceptability

The scores of overall acceptability are summarized in Table 8 and the partial coefficients are given in Table 9.The following equation from response surface methodologywas generated to forecast the change in overall acceptability with different level of factors:

$$\text{Overall acceptability} = 7.8 + 0.163388*A + 0.0426777 * B + 0.05 * AB - 0.2625 * A^2 - 0.2125 * B^2$$

Optimized solutions and their validation

The criteria for optimization and solutions generated through Response Surface Methodology are outlined in Table 10 and Table 11. The levels of sugar and ginger were kept in range. The sensory scores were kept maximum during the optimization process. Table 11 shows the solution suggestedfor preparation of yeast fermented whey beverage with ginger extract. It was also noted that the solution had high desirability value of 0.95.

CONCLUSIONS

This study demonstrated the successful development of a sensorially acceptable probiotic whey-based beverage enriched with ginger extract. The optimization process using response surface methodology ensured a well-balanced flavour profile, meeting the consumer's expectations. Furthermore, the yeast *Pichia kudriavzveii* Y01 used in the formulation exhibited excellent probiotic potential, making it an ideal organism for delivering probiotic functions. This novel probiotic whey beverage holds promise as a health-promoting and flavourful beverage option for consumers, with potential applications in the functional beverage industry.

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FACTORS IMPACTING FINANCIAL LITERACY OF FARMERS IN KALABURAGI, KARNATAKA

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ABSTRACT

The empirical study was conducted in 2023 in Kalaburagi, Karnataka. This reseacrh evaluated farmers' ''financial literacy'' levels and their impact on financial management. The financial literacy of farmers was assessed through a standardized knowledge index. Financial literacy was measured according to the OECD Toolkit (Organization for Economic Co-operation and Development). In the study area, 29 percent of farmers were high financial literate, 32 percent were moderate, and 39 percent were low financial literate. The financial literacy of farmers needed to improve. It was noted that R^2 was significant, implying 67 percent of the variation. Moreover, the significant F ratio (18.52) indicated that thefarmers' financial literacy was impacted by age, education, farm income, and bank accounts. Farmer's financial literacy significantly affected crop insurance, investment, KCC, mobile banking, crop loans, and other agriculture loans. To reach the farmers, better coordination should be with financial literacy centers and banks.

Key Words: Finance, Financial Literature, Financial Management, Sustainable Agriculture

INTRODUCTION

Financial literacy (FL) is educating and promoting knowledge about financial products. It concerns understanding and using various financial skills, including personal financial management, budgeting, and investment. It is necessary for financial well-being, economic stability, and informed decision-making. It enables individuals to take control of their financial destinies, make responsible decisions, and contribute to a more financially secure and prosperous society. FL is the ability and knowledge to understand and manage your finances effectively. According to the Organization for Economic Co-operation and Development (OECD), three significant elements constitute financial literacy, *i.e.,* knowledge, behaviour and attitude. Financial knowledge deals with a basic understanding of critical financial concepts; financial behaviour deals with how a person behaves with various financial products; financial attitude is concerned with a person's preferences and attitudes while dealing with financial products and services. FL affects multiple factors. It is pivotal in the present time because of the introduction of complex, sophisticated financial products by financial institutions.

A study in Nigeria showed that FL helps people determine their saving patterns in formal and informal financial institutions (Adetunjiand David-West, 2019). Recently, the Indian government has implemented a program to make a cashless economy. Digitalizing the Banking

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sector requires people's knowledge of financial products and services. Standard and Poor Survey (2017) of the Global Financial Literacy Excellence Centre (GFLEC) states over 75 percent of Indian adults are illiterate in financial concepts adequately. Only 27 percent of people in India are financially literate (NCFE 2019). Seventy-two percent of Indians are illiterate to save or invest their money to reach financial independence. Real estate and other tangible assets account for 84 percent of the average Indian household's wealth. Hence, FL is more than just knowledge. Farmers' FL is crucial because more than 45.5 percent of workers have been employed in agriculture. Hence, FL is the backbone of achieving the goal of financial inclusion in the context of India. Farm profitability must be increased through sound financial management. Effective financial planning, purchasing, and finance allocation are essential for crops requiring significant initial investment and outstanding working capital. Effective farm financial management would result in using critical input and adopting cutting-edge technology, positively affecting the farm's profitability. This would be accomplished by adequate financial planning, acquisition, and distribution. For effective agricultural finance management, a farmer's knowledge of finance is essential. Farmers must know the cash flows, purchases, credit management, inventory management, debt management, market awareness, etc. (Patel, 2020). Young farmers must manage their earnings from agriculture more effectively (Koigiand Mwangi, 2017). However, most Indian farmers lack FL, and it among Indian farmers varies significantly by geographical region (Maji andLaha, 2023). Lack of comprehension of financial products and an incapacity to digest financial information restrict millions of developing-world rural families from making informed financial decisions. Farmers need financial awareness to improve borrowing decisions.

FL helps farmers avail themselves of credit facilities from a financial institution, allowing them to invest in agricultural activities and expect high returns from farming. FL Impacts the Credit Utilization Behavior of Farmers (Gunawardhana and Silva, 2021). The credit of farming purposes aids resource allocation, which results in increased food availability for the population. Before such possibilities can be pursued, farmers must be informed of the financial options. Formal credit should be made available to rural regions, particularly to people engaged in small-scale farming (Moahid and Maharjan, 2020). Few studies indicated that the government should create a village or community group of various bankers and government representatives to assist rural residents in developing their FL (AnkrahTwumasi *et al.,* 2022). To access banking products and services for the benefit of the community and to promote the strengthening of the national economy through the financial sector, adequate FL will lead to better public financial behavior (Widhiyanto *et al.,* 2018). A farmer's financial behaviour and attitude are strongly influenced by his level of financial education, which may aid him in making the best decisions for his farming and allied activities. The study aimed to assess the farmers' level of FL and its impact on his/her farm financial management.

MATERIAL AND METHODS

The study area was Kalaburagi district of Karnataka, North of Karnataka. Kalaburagi is the second biggest district in area and population, *i.e.*, 8.49 percent and 5.9 percent, respectively, and the total population is 25,66326, with a population density of 234. The district consists of 4,69,020 households. The agricultural cultivators of the district are 2,20198 and the agricultural labourers are 4,15,267 (GoI, 2011). The projected population for 2022 is 3080566. Multistage and Random sampling was used to select the samples. The study used a structured

schedule to collect data. The study locations were the Kalyana- Karnataka (HK) region of Karnataka. It is the most backward region of Karnataka. Five Talukas were selected from a total of 11 Talukas, *i.e.,* Aland, Afzalpur, Chincholi, Sedam and Chittapur. From each Taluk, the researcher chose around 30 farmers, and a total of 154 farmers were selected randomly and interviewed randomly. The study was carried out in 2023, Kalaburagi. FL is the ability of an individual to make informed and 35 judicious choices in managing their finances. This is the knowledge about financial products and services, understanding and evaluating their implications, and making well-informed decisions that contribute to personal financial well-being. The FL of farmers is assessed through a standardized knowledge index (Ravikumar, 2013) -

i.e., K/P *100

Where, K = Knowledge scores obtained by an individual respondent; P= Maximum possible scores for all items. FL is measured as per the OECD Toolkit (Organization for Economic Co-operation and Development), 2022, and the scores are into three categories: High (>75), Moderate (75 to 50), and Low (<50). The OECD Toolkit was developed in 2010 and welcomed by G20 leaders in 2013. According to the OECD and NCFE (2019), an individual's FL ranges from a minimum of 0 to a maximum of 22 (100 %) points, including 22 statements. Regression analysis was used to find the factors affecting the farmer's FL -

$$Z = f(X_1, X_2, ...X_6)$$

$$Z = b_0 + b_1X_1 + b_2X_2 + ...+ b_5X_5+U$$

Where,Z = Total discriminant score; X_1= Age; X_2= Education; X_3= Farm income; X_4 = Size of land holding; X_5 = Bank account (1 if yes, 0 otherwise); b_0, b_1,.....b_5= Coefficients to be estimated, U= Error term. We have limited the age, education, income, and land holdings based on the findings from the literature.

RESULTS AND DISCUSSION

The agriculture sector is significant. It needs finance, too, just like any other sector. Agriculture practices begin with soil preparation, sowing, adding manure and fertilizer, irrigation, protecting from weeds, harvesting, and storage. The amount of capital requirement depends on these activities. Each activity needs a little or much capital for a short or a long time. Bank loans, financing from cooperative organizations, and other unorganized sources help farmers meet their capital needs. The farmer decides on several financial issues related to each agricultural activity, including the credit period, interest rate, acceptance source of capital, amount of capital, investment in agricultural machinery, and payment of agri-labor. The Minimum Support Price (MSP) for crops, agricultural insurance (Pradhan Mantri Fasal Bima Yojana -PMFBY), and the farmer Pension Scheme (Pradhan Mantri Kisan Maan-Dhan Yojana) are all examples of farmer-focused government programs that assist farmers. Therefore, having financial literacy means that a farmer can comprehend the fundamentals of agricultural finance.

Farm finance needs and management are significantly impacted by the land holding size. It affects the source of financing and its use. It was observed that all the farmers have bank accounts. Hence, the data on land holdings from the sample farmers are tabulated and presented (Table 1).

Table 1 shows that most of the sample farmers are categorized as medium landholding with a land size of 47 acres, *i.e.,* 30.51 percent. The distribution of small and marginal farmers is 22.07 percent and 11.68 percent, respectively. The share of farmers in different land holding sizes was significantly different. The average land holding size is eight acres, and 7.8 percent of the farmers have irrigated land. The region is famous for its black soil.

Table 1.Share of agricultural land (n=154)

S.No.	Type of Landholding	Land Size(Acres)	Frequency	Percentage
1	Marginal	<1	18	11.68
2	Small	1 to 2	34	22.07
3	Semi-medium	2 to 42	33	21.42
4	Medium	4-10	47	30.51
5	Large	**>10**	22	14.28
	Total		**154**	**100**

Source: Survey data, 2023

Socio-economic and demographic profile of the farmers

Most of the respondents (93.5%) are male, implying high participation of males in agricultural activity. The FL score of male farmers was 93 percent, and the same of the female farmers was 7%, implying a substantial gender gap. The majority of the farmers (80%) were married. 76.32 percent of the farmers were noticed to be farm workers, and the balance was farm owners. TheFL of 76.32 percent of farmers is merely 37.77 percent, whereas, the FL score of 37.77 percent of owners is 50.15 percent. Such a difference in their FL score is statistically notable.

The results indicated a significant gender divide in FL among farmers, in line with the existing theoretical conviction. Women must catch up in financial planning. Women have lower FL levels and financial confidence. Women are reported to struggle more than males to complete financial calculations, and they also need more awareness of basic concepts in finance and better knowledge, preventing them from making appropriate financial choices and decisions. They have traditionally held a role that males oversee,

Table 2. Gender-wise and age-wise distribution of the respondents (n=154)

S. No.	Gender	Frequency	Percentage
1	Male	144	93.5
2	Female	10	6.5
	Age-wise Distribution		
S. No	**Age Group**	**Frequency**	**Percentage**
1	Below Age 25	3	2.0
2	25 to 34	28	18.2
3	35 to 44	41	26.6
4	45 to 54	40	26.0
5	55 to 64	34	22.1
6	65 above	08	5.2

Source: Survey data, 2023

Table 3. Overall FL of farmers (n=154)

S. No.	Category	Frequency	Percentage
1	High	45	29
2	Moderate	49	32
3	Low	60	39

Source: Survey data, 2023

making financial decisions.71.23 percent of the sample farmers fall below the poverty line category, with an FL score of only 37.86 percent. The average FL score of above-poverty-line farmers was noticed to be 55.71 percent. However, such a difference in FL scores amongst the APL and BPL farmers was not statistically notable. In this study, people from different age groups were equally engaged. Of the total, 2 percent are from below 25 years, 18.2 percent are from 25 and 34 years, 27 percent are from 35 and 44 years, 26 percent are from 45 to 54 years, and the remaining 22.1 percent are from 55 and 64 years, moreover, above 60 years is 5.2 percent. The differences in FL scores amongst various age cohorts are also not statistically significant.

Overall FL of farmers

Proper management of farm finance is necessary for the commercialization of agricultural activities. The success of farm finance management may be significantly influenced by FL. The FL assessment findings, FL components, and distribution of FL of farmers are presented in this section.

Extant literature suggested that the FL amongst people across the globe is noticed to be poor. Most farmers, *i.e.,* 39 percent of the sample farmers in Kalaburagi, have low FL in line with the FL trend worldwide. Thirty-two percent are moderate, and 29 percent have high FL. The average FL score of the farmers in Kalaburagi is 38.74 percent, implying a low level of FL. The agriculturally advanced villages possess a higher FL than those not advanced in agriculture. The mean FL scores of the farmers in the agriculturally advanced and backward areas are 45.20 percent and 32.29 percent, respectively. Agricultural advancement favourably affects the FL of the farmers. Farmers from agriculturally productive Indian states have a higher FL rate than farmers from agriculturally backward states. Typically, farmers from agriculturally developed areas have higher education and skills, and their income opportunities are also higher, leading to increased FL. Farmers from the agriculturally advanced districts exhibited more FL in this study.

Distribution of FL across educational status, age group, and income

Their educational level could significantly impact FL among farmers. Farmers with varying educational backgrounds would exhibit a range of FL and financial practices. Age is a significant factor that affects farmers' level of FL. As a result, information on FL levels across age groups and educational levels is analyzed. The result is shown in Table 4.

Table 4 provides a breakdown of the distribution of FL across different categories, including educational status, age groups, and income levels. The table shows a clear trend in FL is based on educational status. Individuals with higher levels of education (such as high school or graduate/postgraduate) tend to have a higher percentage of moderate to high FL than those with lower education levels. This suggests a positive correlation between educational attainment and FL. The data indicates that FL varies across different age groups. For instance, individuals in the age group of 55 to 64 tend to

Table 4. Distribution of FL across educational status, age group, and income (n=154)

	S.No.	Category	Financial Literacy					
			Low		Moderate		High	
			Frequency(f)	Percentage (%)	Frequency(f)	Percentage (%)	Frequency(f)	Percentage (%)
Education	1	No Formal Education	28	47	4	8	3	7
	2	Upper Primary and Secondary	20	33	15	31	11	24
	3	High School	10	17	28	57	17	38
	4	Graduation and PG	2	3	2	4	14	31
		Total	**60**	**100**	**49**	**100**	**45**	**100**
Age(Year)	1	Below Age 25	01	2	00	0	02	04
	2	26 to 34	08	13	10	20	10	22
	3	35 to 44	10	17	15	31	16	36
	4	45 to 54	19	32	11	22	10	22
	5	55 to 64	20	33	10	20	04	09
	6	65 & above	02	3	03	06	03	07
		Total	**60**	**100**	**49**	**100**	**45**	**100**
Income (Rs.)	1	Less than 2,50,000	49	81.7	35	71.4	18	40
	2	More than 2,50,000	11	18.3	14	28.6	27	60
		Total	**60**	**100**	**49**	**100**	**45**	**100**

Source: Survey data, 2023

have the highest percentage of low FL, while those in the age group of 35 to 44 and 45 to 54 have higher percentages of moderate FL. The distribution of FL by income level reveals an interesting pattern. Individuals with higher income levels (more than Rs. 2,50,000) show a higher percentage of moderate to high FL, while those with lower income levels (less than Rs.2,50,000) have a higher percentage of low FL. This suggests a potential correlation between income level and FL. Overall, the results indicated that educational status, age, and income all play significant roles in influencing FL. Higher educational attainment, higher income levels, and certain age groups are associated with a higher percentage of moderate to high FL. This suggests that targeted FL programs and interventions may be particularly beneficial for specific demographic groups, such as those with lower educational attainment, younger or older individuals, and those with lower income levels. Understanding the distribution of FL across these categories can help design more effective and tailored financial education initiatives to address the varying levels of FL within different demographic groups.

Overall, the results of the analysis indicated that several key factors significantly influence the FL of farmers. Specifically, age,

Table 5. Factor influencing the FL of framers (n=154)

S.No.	Variables	Co-efficient	Standard Error	T-ratio	P value
1	Age	0.462***	0.114	3.321	0.000
2	Education	0.671**	0.321	3.242	0.014
3	Farm Income	0.574***	0.432	2.425	0.002
4	Size of land holding	0.585*	0.440	1.712	0. 013
5	Bank account	0.512**	0.321	2.324	0.018

R^2 value = 0.687, F-ratio = 18.52 n=154; Note: ***1%, **5% and *10% level of significance

Source: Survey data, 2023

education, farm income, size of land holding, and having a bank account all show statistically significant positive effects on FL among the farmers in the sample. The findings suggest that older age, higher levels of education, greater farm income, larger land holdings, and the presence of a bank account are associated with higher levels of FL among farmers. These results could provide valuable insights for policymakers, financial institutions, and other stakeholders interested in promoting FL and inclusion among farming communities. With a relatively high R-squared value of 0.687, the included variables explain a considerable portion of the variation in FL observed among the sampled farmers, further highlighting the importance of these factors in understanding and potentially enhancing financial literacy within the agricultural sector.

FL and farm financial facilities

Digital payments are significant because, nowadays, the Indian government focuses mainly on online payments. Hence, the Government has launched the BHIM APP for mobile banking. The

Table 6. FL, awareness, and banking products (n= 154)

S.No.	Particulars	Low (60)		Moderate (49)		High (45)	
		Aware	Practice	Aware	Practice	Aware	Practice
1	Saving Bank Account	60	15	49	20	45	29
2	Fixed Deposit	23	02	25	03	32	06
3	Insurance	60	15	49	18	45	17
4	Investment	56	04	49	06	45	10
5	Kisan Credit Card	25	02	35	06	40	12
6	Debit Card/ATM	56	15	49	16	45	21
7	Mobile Banking	11	03	20	05	45	07
8	Crop Loan	55	45	45	43	45	44
9	Other Agri Loans	40	14	35	15	40	36

Source: Survey data, 2023

Table 7. The regression coefficient of FL and accessibility of banking products (n=154)

S.No.	Financial Product	â	R^2	t-value	P-value
1	Fixed Deposit	0.34	0.11	4.385	***p<.001
2	Crop Insurance	0.41	0.18	5.759	***p<.001
3	Investment	0.21	0.37	3.620	***p<.001
4	KCC	0.44	0.19	3.429	***p<.001
5	Mobile Banking	0.33	0.30	6.128	***p<.001
6	Crop Loan	0.29	0.22	6.222	***p<.001
7	Other Agricultural Loan	0.45	0.26	5.124	***p<.001

Source: Survey data, 2023

question relies on farmers' use of these digital payments, agricultural loans, etc.How many of them are aware of these facilities?.

It is evident from the above table that financially literate respondents influence the access to financial products the banks offer. It can also be accessed. the impact of FL on the accessibility of banking products. Fixed Deposit R^2 value of 0.11 revealed that the predictor variable explained 11 percent variance in the outcome variable. One can conclude that FL positively predicted access to fixed deposits (â=0.34, p<.001). As shown in theTable 7, crop insurance, Investment, KCC, Mobile Banking, Crop Loans, and other Agriculture Loans are significantly impacted by FL.

CONCLUSIONS

High FL accounted for 29 percent, 32 percent were moderately literate, and 39 percent were low financially literate. The respondents with high FL are more aware of all the financial products. A statistically significant difference in awareness level is found for bank fixed deposits, crop insurance, investment, KCC, Mobile banking, Crop Loans, and other agriculture loans.There is a dire need for campaign mode awareness on financial products and FL. The significant F ratio (18.52) indicated the best fit of the regression model. The farmers' FL is impacted by age, education, farm income, land holding size, and bank accounts. Farmer's FL significantly affects crop insurance, investment, KCC, mobile banking, crop loans, and other agriculture loans. There should be more coordination with banks and Financial Literacy Centers (FLC) to reach farmers effectively. While conducting FL programs, the Gram panchayat should cooperate with FLCs. Farmers should also cooperate and show interest in attending FL programs. RBI can provide more staffing in FLCs to cover more number of villages. RBI should ensure that there is good cooperation between banks and FLCs.

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TRENDS IN MILLETS PRODUCTION, CONSUMPTION AND EXPORT FROM INDIA

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ABSTRACT

In the year 2021, total area under millets cultivation in India was 9.76 million hectares accounting for 31.55 percent of world's total area.Total production of millets in India was 13.21 million tonnes in 2021 contributing about 43.90 percent to world's total millets production. The objective of this study was to analyse the growth performance of millets production in India and world, and exports of millets (quantity and value) from India. The study was based on secondary data and the analysis was carried out using compound growth trend and decomposition analysis. In India, millets area and production reduced during 1966-67 to 2019-20, whereas, per hectare yield increased with compound growth rate of 1.08 percent per annum. Decomposition analysis suggested that yield was negatively affecting the overall growth in production of finger millet, minor millet and sorghum, whereas, it was positive for pearl millet. Total export of millets from India was 27.42 million USD in 2021 and it was growing with a compound growth rate of 15.67 percent per annum during 1981-2021.The index value of revealed comparative advantage, revealed symmetric comparative advantage, revealed competitive advantage and trade specification coefficient index suggested that India has comparative advantage and competitiveness in export of millets to different parts of the world.

Keywords: Millet, revealed comparative advantage, revealed competitive advantage, revealed symmetric comparative advantages, trade specification coefficient

INTRODUCTION

Millets cultivation is as old as human civilisation and evidences of growing millets were found in early 3000 BC in Indus valley civilization (Yes Bank and APEDA, 2022). Millets are grown in 131 countries of the world and it is traditional food for 590 million people in Asia and Africa continent. Millets are drought tolerant, climate resilience, short to medium duration, survive in nutritional degraded soils, low inputs requirement, and resistance to pests and diseases. Millets are the carbon neutral andenergy efficient crop andit is climate adaptable crops in harsh hot condition (up to 64^{o}C) and drought environments.Wheat and paddy provide food security only, while millets provide food, fodder, health, nutrition, livelihood and ecological security (Millet

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Network of India, Undated). Millets are C4 crops which has ability to carbon sequestration that leads to reduce greenhouse gas (Rao *et al*., 2021).

In 2021, area under millets cultivation in the world was 30.94 million hectares with production of 30.09 million tonnes**.** In 2021, area under millets cultivation in India was 9.76 million hectares with total production of 13.21 million tonnes and average yield was 13.53 quintal per hectare. In 2021, quantity of millets exports from India was 91287 tonnes worth 27.42 million USD (FAO, s2023).

Considering the importance of millets,Indian government led awareness schemes for recognition of millets as nutri-cereal including declaration of 2018 as National Year of Millets in 2018 (Jadaun and Khan, 2023). Indian Government's proposal for commemoration of 2023 as 'International Year of Millets' (IYM-2023) at United Nations General Assembly was accepted and declared.

The objectives of this study was: [1] to estimate the growth trend of area, production and yield of millets in India and the world and to study the consumption pattern of millets, wheat and rice in India; [2] to find out the impact of area, yield and interaction (area and yield) on overall millets production in India and the world; and [3] to calculate the growth performance, comparative advantage and competitiveness of millets export from India.

MATERIAL AND METHODS

This study was based on the secondary data and it was collected from different data bases like FAOSTAT and Indian Institute of Millet Research (IIMR), Hyderabad.

To study the temporal growth in area, production and yield of milletsexponential function was used(Singh *et al*., 2023). The algebraic model is described below:

$$Y = a\, xb^{t}$$

Where,

Y is the dependent variable (area/ production/ yield),

t is the independent variable (it is rank given to the year concerned),

a is the functional coefficient used in exponential function, and

b is the compounding coefficient

To study the contribution of area, yield and its interaction (area and yield) effect towards the overall millets production, a decomposition technique was used (Singh *et al.,* 2016). The algebraic model is described below:

$$P = Y_0 (A_n - A_0) + A_0 (Y_n - Y_0) + \triangle A \triangle Y$$

$$1 = \{(Y_0 \triangle A) / P\} + \{(A_0 \triangle Y) / P)\} + \{(\triangle A \triangle Y) / P)\}$$

Where,

P is the change in millets production, A_0 is the area in base year,

A_n is the area in current year, Y_0 is the yield in base year,

Y_n is the yield in current year,rA is the change in area ($A_n - A_0$),

$\triangle Y$ is the change in yield ($Y_n - Y_0$)

Here, on right hand side of equation, first term is area contribution, second term is yield contribution and last term is interaction effect of area and yield.

To measure the Revealed Comparative Advantage (RCA) of India's millets trade pattern, Balassa method was used (Singh *et al*. 2021). The mathematical expression is as:

$$RCA_{ij} = \{(X_{ij}\ \Sigma X_{ij}) / (\Sigma j\ \Sigma i X_{ij})\}$$

Where,

X_{ij} is the exports of commodity imillets in country j(India),

ΣiX_{ij} is the total agricultural export from India,

ΣjX_{ij} is the world's exports of millets, and

$\Sigma jSIX_{ij}$ is the total agricultural exports of world

If value of RCA index is < 1, it indicates that the share of Indian exports in the country's overall export trade is lower than its share in world's trade. If RCA >1 it means the share of millet export, export in different products is more than world's shares hence it has a comparative advantage in millets export.

Another method suggested by Vollrath and Dalum to measures the competitiveness of a particular country to avoid the problem of double counting was Revealed Symmetric Comparative Advantages (Singh *et al*., 2021). The RSCA can be expressed as:

$$RSCA_{ij} = [\{(X_{ij} / \Sigma X_{ij}) / (\Sigma j\, X_{ij} / \Sigma j\, \Sigma iX_{ij})\}-1] / [\{(X_{ij} / \Sigma X_{ij}) / (\Sigma j\, X_{ij} / \Sigma j\, \Sigma iX_{ij})\}+1]$$

Where,

Xij is the exports of millets from India,

ΣiX_{ij} is total agricultural export from India,

ΣjX_{ij} is the world's export of millets, and

$\Sigma j\Sigma iX_{ij}$ is the world's total agricultural export

The value of RSCA index ranges between -1 to +1 to avoid the problem of zero. Positive values of the index indicate the stability as well as the competitiveness of India's millets export.

Revealed Competitive Advantage (RC) index measures the balances in supply and trade by using the values of export. The RC index can be estimated by using following method (Singh *et al*. 2021).

$$RC_{ij} = \{(X_{ij} / \Sigma X_{ij}) / (\Sigma j\, X_{ij} / \Sigma j\, \Sigma iX_{ij})\} - \{(M_{ij} / \Sigma M_{ij}) / (\Sigma j\, M_{ij} / \Sigma j\, \Sigma iM_{ij})\}$$

Where,

X_{ij} is the exports of millets from India,

ΣiX_{ij} is total agricultural export from India,

ΣjX_{ij} is the world's exports of millets,

$\Sigma jSiX_{ij}$ is total agricultural export of world,

M_{ij} is the imports of millets in India,

ΣiM_{ij} is total agricultural import of India;

ΣjM_{ij} is the world's import of millets, and

$\Sigma j\Sigma iM_{ij}$ is total agricultural imports of world

The values of index must be either positive or negative. If the index values are positive, the commodity of the country is competitive,whereas, if the index value is negative means it will not competitive internationally in that commodity trade.

Trade specification coefficient index (TSC Index) has been employed to understand the export competitiveness of Indian exports during the study periods. The mathematical model of the TSC index represented as follows (Borisagar *et al*., 2023):

$$TSC = \frac{(X_{ij} - M_{ij})}{(X_{ij} + M_{ij})}$$

Where,

TSC is the trade specification coefficient,

X_{ij} is total exports of the millets, and

M_{ij} is total imports of the millets

TSC index represents the ratio of the trade balance of a particular commodity in a country to the total value of the trade for that particular commodity. The value of the index ranges between -1 and +1. The value of this index equals 'zero' when a commodity's exports are equal to its imports. A positive index values

indicates that the country's exports of a particular commodity are higher than the imports of the commodity.

RESULT AND DISCUSSION

Growth performance of millets production in the world

Table 1 depicted Africa (60.11 percent) and Asia (37.69 percent) continents dominated in area allocation under millets cultivation during 2021. The percentage share of production of millets in the continents of Africaand Asia was 40.23 and 56.49 percent, respectively during 2021 (Table 1). The positive growth in area under millets cultivation was observed in Africa, Oceania and Australia & New Zealand, whereas, rest of the continents *viz.,* America, Asia and Europe registered declining trendduring 1961-2021. In case of millets production, positive growth was observed in Africa, Oceania and Australia & New Zealand, whereas highest negative growth in millets production was observed in Europeduring 1961-2021. Augmentation in millets' yield was an important indicator that is reflected by the development of high yielding variety (HYV) seed and improved agronomic practices in the world. But, the pace of adoption of HYV seeds and improved agronomic practices by the farmers for millets cultivation was varied from one continent to another continents.Meena*et al.* (2021) reported that area under millet at world level reduced by 25 percent in 2016-18 compared to 1961-63.

Table 1. Growth performance of millets in world and continents, 1961-2021

Particulars	Year	World	Africa	America	Asia	Europe	Oceania	Australia & New Zealand
Area								
1. Area (Million Hectares)	1961	43.40	11.43	0.30	27.78	3.87	0.022	0.022
	2021	30.93	18.60	0.27	11.66	0.37	0.036	0.036
2. Share to world's total (%)	1961	100.00	26.34	0.69	64.02	8.91	0.05	0.05
	2021	100.00	60.11	0.89	37.69	1.19	0.12	0.12
3. CAGR (% per year)	1961-2021	-0.60	1.14	-0.90	-1.80	-3.40	0.49	0.49
Production								
1. Area (Million Hectares)	1961	25.71	6.59	0.373	16.00	2.74	0.022	0.022
	2021	30.09	12.11	0.359	17.00	0.59	0.037	0.037
2. Share to world's total (%)	1961	100.00	25.62	1.45	62.21	10.64	0.08	0.08
	2021	100.00	40.23	1.19	56.49	1.97	0.12	0.12
3. CAGR (% per year)	1961-2021	0.18	1.49	-0.30	-0.40	-3.00	0.59	0.59
Yield								
1. Yield (Quintal/Ha)	1961	5.93	5.76	12.49	5.76	7.08	10.09	10.09
	2021	9.73	6.51	13.08	14.58	16.07	10.21	10.21
2. CAGR (% per year)	1961-2021	0.78	0.35	0.62	1.42	1.33	0.10	0.10

CAGR: Compound Annual Growth Rate

Table 2. Decomposition analysis (Percent)

Particulars	World	Africa	America	Asia	Europe	Oceania	Australia & New Zealand
1. Yield effect	352.10	18.83	-671.43	6037.15	-194.99	-36.58	-36.58
2. Area effect	-150.53	69.64	647.09	-2359.74	121.45	145.40	145.40
3. Yield & area Effect	-101.57	11.53	124.34	-3577.40	173.53	-8.83	-8.83

Decomposition analysis suggests that yield was an important factor for growth of total millets production in the world, area became secondary importance (Table 2). In case of Africa, America, Oceania and Australia & New Zealand, area under millets was an influencing factor for overall growth of millets production, whereas in Asia, millets' yield was influencing total production of millets. In case of Europe, interaction effect was positively associated with total millets production (Table 2).

Growth performance of millets production in India

In the last few decades, India has experienced a sharp decline in the area under millets cultivation which was 80, 46, 59 and 23 percent for small millets, finger millets, sorghum and pearl millet, respectively (Behera, 2017).Total area under finger millet in the country was 1.984 million hectares in 1966-67 and it was declined to 1.005 million hectares by the year 2019-20 (Figure 1). Gowri and Shivakumar (2020) similarly found that decrease in the area of millets was 16.21 percent between 1950 and 2019.

Area under finger millet was declining with a compound growth rate of -1.90 percent per annum. Area under minor millets cultivation was 4.584 million hectares in 1966-67 and it was droppedto 0.458 million hectares by the year 2019-20. It was declining with a compound growth rate of -4.70 percent per annum. In 1966-67, area under pearl millet cultivation in the country was 12.240 million hectares and it was dropped to 7.543 million hectares by 2019-20, which registered a negative compound growth rate of -1.00 percent per annum during same period of the study. Rani *et al.* (2023) also reported the negative per annum growth rate in area of millet after 1970-71. In case of sorghum, total area was 18.054 million hectares in 1966-67 and it was declined to 4.824 million hectares by the year 2019-20. Area under

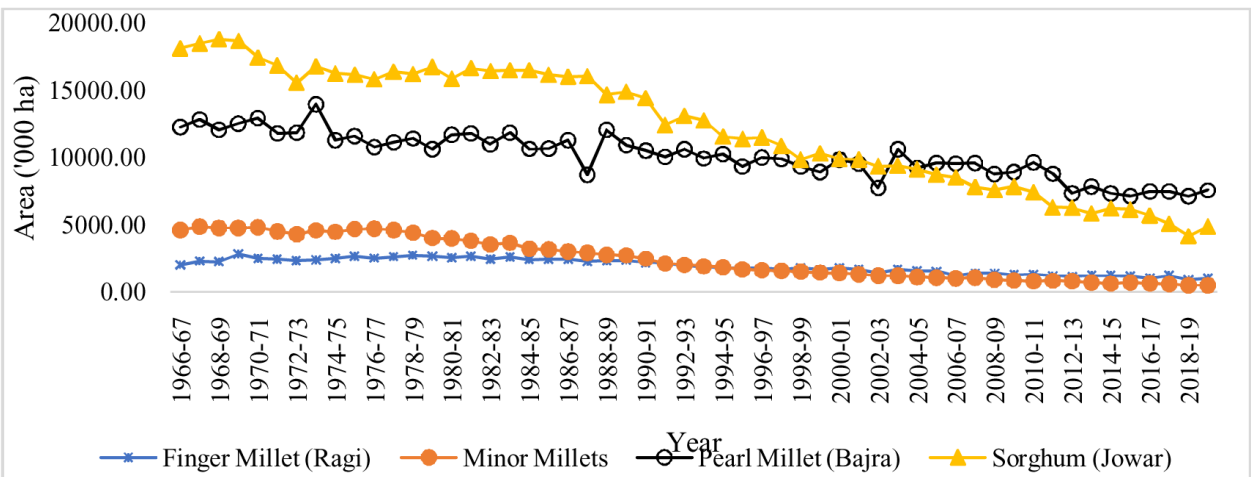

Figure 1. Area under millets cultivation in India ('000 ha)

sorghum cultivation was declining with a compound growth rate of -2.60 percent per annum. Thus, it is clear from above discussion that area under all the millets was declined, but pace of declinein area under millets was varying during the study period.Sukumaran *et al*. (2023) also found that during 1970 to 2019, the area for above mentioned millets was decreased, whereas productivity was increased. The decline in production for above mentioned millets due to reduction in area couldn't be fully compensated with increase in yield, except for pearl millet.

In 1966-67, production of finger millets in the country was 1.631 million tonnes and it was augmented to 1.755 million tonnes by the year 2019-20 registering a compound growth rate of -0.50 percent per annum (Figure 2). Minor millets production in the country was 1.489 million tonnes during 1966-67 and it was declined to 0.371 million tonnes by the year 2019-20. Pearl millet production was 4.468 million tonnes in 1966-67 and it was augmented to 10.363 million tonnes by the year 2019-20. Pearl millet production was reducing with a compound growth rate of -1.46 percent per annum. In 1966-67, sorghum production was 9.224 million tonnes and it was reduced to 4.772 million tonnes by the year 2019-20. Sorghum production was declining with a compound growth rate of -1.50 percent per annum.

Per hectare yield of finger millets in the country was 8.22 quintal in 1966-67 and it was

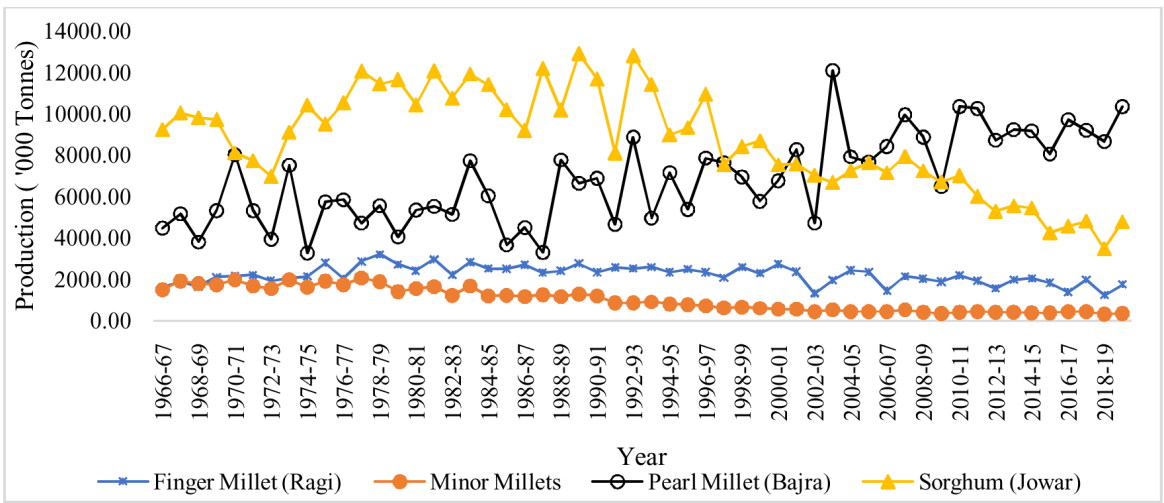

Figure 2. Millets production in India ('000 Tonnes)

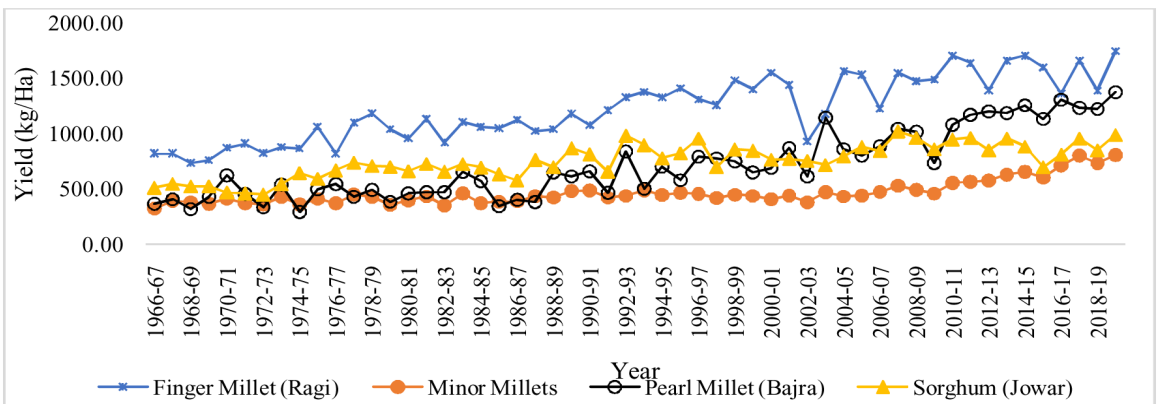

Figure 3. Millets yield in India (kg/ha)

Table 3. Decomposition analysis

Particulars	Finger millet	Minor millets	Pearl millet	Sorghum
1. Yield effect (percent)	-2299.81	-145.90	229.07	-138.96
2. Area effect (percent)	1190.90	115.00	-36.63	135.09
3.Yield & area effect (percent)	1208.90	130.91	-92.44	103.87

increased to 17.47 quintal by the year 2019-20. Finger millets was growing with a compound growth rate of 1.37 percentper annum during same period of time (Figure 3). In case of minor millets, per hectare yield was 3.25 quintal in 1966-67 and it was increased to the level of 8.09 quintal by the year 2019-20. Minor millets' yield was enhancing with compound growth rate of 1.08 percent per annum during study period. Per hectare yield of pearl millet was 3.65 quintal in 1966-67 and it was augmented to the level of 13.74 quintal by the year 2019-20. Per hectare yield of pearl millet was growing with compound growth rate of 2.44 percent per annum. Per hectare yield of sorghum in India was 5.11 quintal in 1966-67 and it was increased to the level of 9.89 quintal during 2019-20 registering a compound growth rate of 1.11 percent per annum.

The decomposition analysis was carried out to find the yield effect, area effect and interaction (area and yield) effect on total millets production in country. In case of finger millets, minor millets and sorghum, area and interaction (area and yield) effect was positively associated with overall production of these millets (Table 3). In case of pearl millet, yield was positively associate with the overall growth of pearl millet production in the country, whereas, area and interaction effect was negatively associated with the overall production of pearl millets in the country.

It was concluded that area and production of finger millets, minor millets, pearl millet and sorghum was declined after introduction of green revolution technologies in the country and at the same time productivity of these millets have increased over the period of time. The rate of decline in area under millets was not fully compensated with the increased yield of these crop.The millets area has been diverted largely to soybean (Madhya Pradesh, Maharashtra, Rajasthan, Andhra Pradesh and Karnataka), maize (Andhra Pradesh, Gujarat, Karnataka, Maharashtra and Tamil Nadu), cotton (Andhra Pradesh, Gujarat

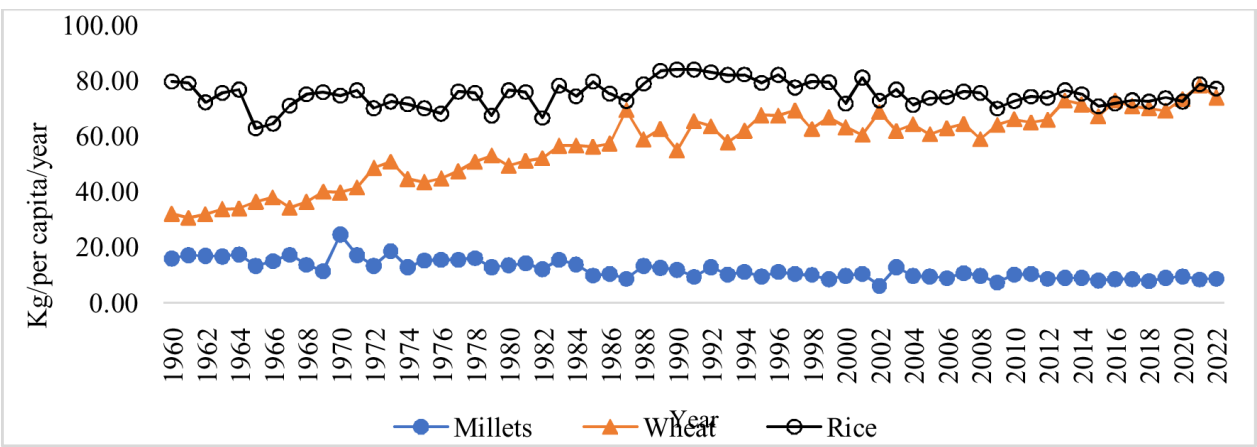

Figure 4. Per capita consumption of millets, wheat and rice in India

Table 4. Per capita consumption of millets, wheat and rice, India during 1960 - 2022 (kg/ capita/year)

Year	Millets	Wheat	Rice
1960	15.89	31.88	79.54
1965	13.26	36.22	62.73
1970	24.52	39.47	74.46
1975	15.17	43.31	70.00
1980	13.43	49.26	76.49
1985	9.74	56.03	79.57
1990	11.86	54.68	83.97
1995	9.30	67.39	79.11
2000	9.53	63.06	71.72
2005	9.44	60.61	73.69
2010	10.08	65.91	72.70
2015	7.94	66.94	70.64
2020	9.45	73.20	72.38
2022	8.54	73.74	77.09

and Haryana), sugarcane (Maharashtra, Tamil Nadu and Uttar Pradesh) and sunflower in Karnataka (Singh *et al*., 2010).

Per capita consumption of millets, wheat and rice in India

For estimation of per capita per year consumption of millets, wheat and rice during 1960-2022, data related to millets, wheat and rice consumption was collected from the United States Department of Agriculture (2023) and India's demographic data was collected from Macrotrends (2023). Per capita per year millets, wheat and rice consumption during 1960 to 2022 is presented in Figure 4 and Table 4.

In 1960, per capita per year millets consumption in India was 15.89 kg and it declined to 8.54 kg by the year 2022 (Table 4). Millets consumption declined with a compound growth rate of -1.30 percent per annum during 1960-2022. Per capita per year wheat consumption was 31.88 kg in 1960 and it increased to 73.75 kg by the year 2022 (Table 4). Per capita wheat consumption in India depicted compound growth rate of 1.26 percentper annum. Per capita per year rice consumption in the country was 79.54 kg in 1960 and it reduced to 77.09 kg a compound growth rate of 0.04 percent per annum (Figure 4). It is evident from the above discussion that the per capita per year millets consumption in India was decline and peoples' dependency on wheat and rice consumption was increased.

Growth performance of millets export from India

Total quantity of millets export from India was 30 tonnes in 1981 and it was increased to the level of 91287 tonnes by the year 2021(Figure 5 and Table 5). Millets export from India to different destinations was growing

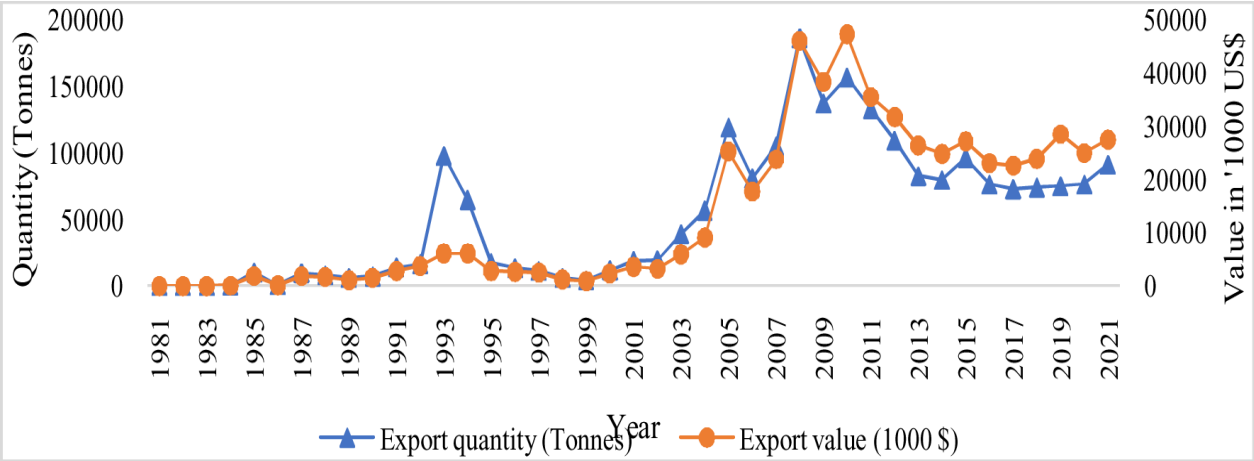

Figure 5. Total millets export from India

Table 5. Total millets export from India

Year	Total millets export		Quantity of Millets export (Tonnes)		
	Quantity (Tonnes)	Value (1000 $)	Pearl millet	Finger millet	Sorghum
1981	30.00	5.00	-	-	-
1985	10148.00	1874.00	-	-	-
1990	7310.00	1551.00	-	-	-
1995	17358.00	2823.00	-	-	-
2000	12025.00	2423.00	-	-	-
2005	119320.00	25310.00	-	-	-
2010	157004.00	47245.00	5466.28	564.75	4329.20
2011	132958.00	35531.00	8423.85	426.52	3088.45
2012	109480.00	31676.00	6644.93	803.02	24378.85
2013	82602.00	26318.00	2913.51	1059.25	5399.47
2014	79886.24	24794.00	5585.71	939.96	12102.19
2015	96263.71	27089.00	6051.97	559.00	5238.21
2016	76340.00	23040.00	4883.28	643.99	5337.29
2017	72849.23	22664.00	4549.72	827.29	3305.38
2018	74237.16	23871.00	3886.36	1053.44	8674.14
2019	75026.28	28491.00	4065.25	964.51	1354.85
2020	76481.47	24956.00	-	-	-
2021	91287.36	27421.00	-	-	-

with a compound growth rate of 14.10 percent per annum during study period. In 1981, total value of millets export from India was 5000 USD and it was increased to the level of 27.421 million USD by the year 2021 registering a compound growth rate of 15.67 percentper annum (Figure 5 and Table 5).

In 2010-11, India exported 564.75 tonnes of finger millet and it was increased to 964.51 tonnes by the year 2019-20 (Figure 6 and Table 5). The export of finger millets from India was growing with a compound growth rate of 5.78 percent per annum. The major export destination for finger millet was Nepal, Sri Lanka DSR, Malaysia, Arab Emirate, USA, Kuwait, Oman, Maldives, Bahrain, Saudi Arab, Australia, Canada and Singapore, etc.

In case of sorghum, export from India was 4329.20 tonnes in 2010-11 and it was reduced to the level of 1354.85 tonnes by the year 2019-20 (Figure 6 and Table 5). The export of sorghum was declining with a compound growth rate of -8.18 percent per annum during same period. India's major sorghum export destination was Philippines, Saudi Arab, Kuwait, UAE, Japan, Taiwan, Bahrain, Oman, Yemen Republic, Qatar, New Zealand, Sri Lanka, Israel and Kenya etc. Kumar *et al*. (2023) also found that there was positive growth in millets export from India during 2003-04 to 2021-22.

Comparative advantage and competitiveness of India's millet export

To study the comparative advantage and competitiveness of Indian millets trade, Revealed Comparative Advantage (RCA),Revealed Symmetric Comparative Advantage (RSCA), Revealed Competitive Advantage (RC) and Trade Specification Coefficient (TSC) index was analysed for the period of 1990 to 2021 and index values are presented in Figure 7, 8, 9 and 10. The RCA index value for millets export was ranged between 1.84 to 25 during study period, which was more than one suggests that India has comparative advantage in the millets export (Figure 7).Singh *et al.* (2023) also calculated the RCA value for India's primary export markets and determined that there was substantial advantage for millets export from India during 2011 and 2020.

The revealed symmetric comparative advantage (RSCA) index was found to be between 0.29 and 0.92 with positive sign for all years which suggests that India has competitiveness in the export of millets to different parts of the world (Figure 8). The

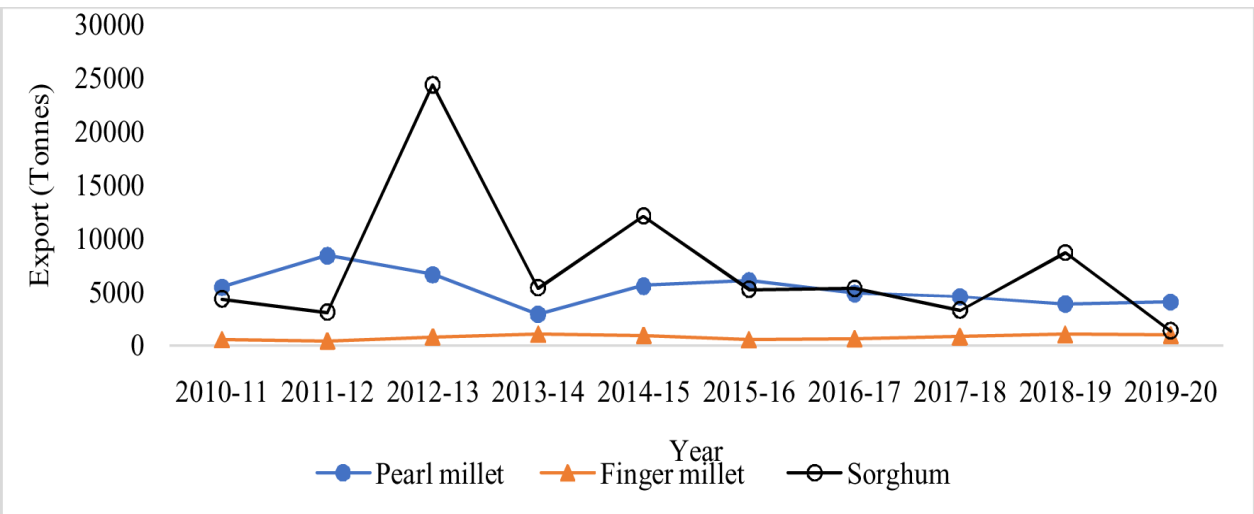

Figure 6. Export of pearl millet, finger millet and sorghum from India (Tonnes)

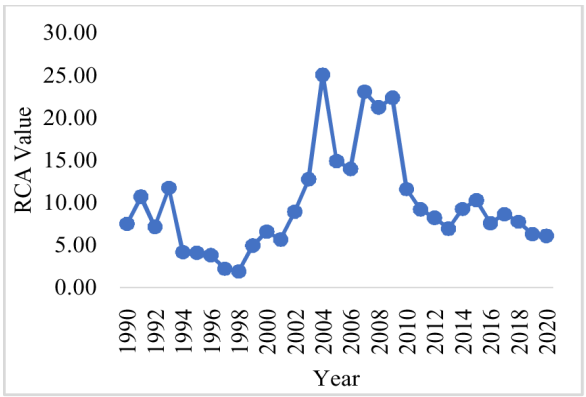

Figure 7. Revealed comparative advantage

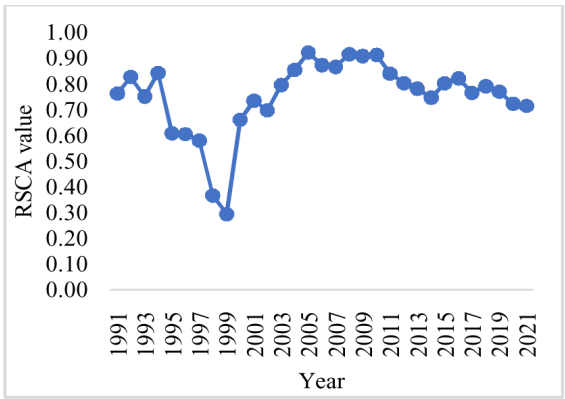

Figure 8. Revealed symmetric comparative advantage

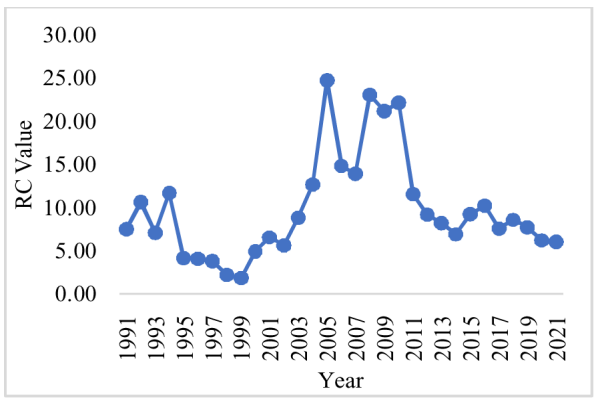

Figure 9. Revealed competitive advantage

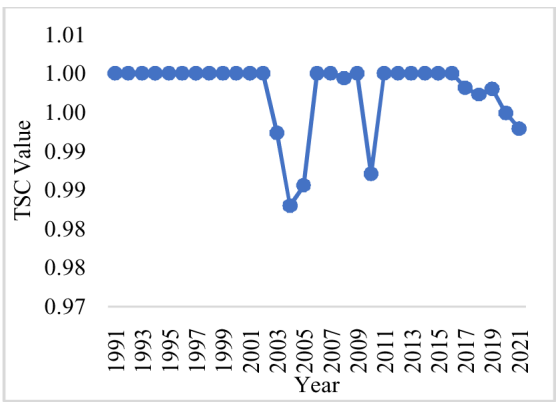

Figure 10. Trade specific coefficient index

index value of Revealed competitive advantage (RC) was lying between 1.84 to 24.68 with positive sign for all the years of study, thus it can be suggested that India's millets export was competitive and India's millets import was less than the export (Figure 9). The trade specification coefficient (TSC) index has been employed to understand the export competitiveness of millets from India. The index value of trade specification coefficient was ranging between 0.98 to 1.00 with positive sign throughout the study period. The TSC value or millets which suggests that export of millets from India was higher than the import value and Indian millets export were export competitiveness (Figure 10).

CONCLUSIONS

During 1961-2021, area under millets cultivation was declined in the world, whereas, production of millets was increased. Per hectare yield of millets in the world was increased due to development and adoption of new HYV varieties of millets and agronomic practices. In case of India, area and production of finger millets, minor millets, pearl millet and sorghum was declined after introduction of green revolution technologies in the country, at the same time, productivity of these millets were increased during 1966-67 to 2019-20. It is due to varietal improvement and improved agronomic practices. Per capita per year millets consumption in India was declined and consumption of wheat and rice was increased

during study period. The millets export from India was growing with a compound growth rate of 14.10 percent per annum during 1981-2021. Similar results were also reported by Kumar *et al*. (2023). The export of pearl millet and sorghum from India was declining with a compound growth rate of -4.93 and -8.18 percent per annum respectively, whereas, export of finger millet was growing with a compound growth rate of 5.78 percent per annum during 2010-11 to 2019-20. India has comparative advantage and competitiveness in the export of millets. In order to increase the consumption of millets, awareness should be created explicitly among the population about the superior level of millets in nutritional chart. Government should frame effective policy to distribute millets through Public Distribution System (PDS) under National Food Security Mission (NFSM). Value addition and modernization of the processing sector based on millets may benefit the urban and high-income households to consume more millets or millets-based food products.

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INSTITUTIONAL FARM CREDIT– INPUT - OUTPUT TREND ANALYSIS: A STUDY FROM KALABURAGI DISTRICT, KARNATAKA

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ABSTRACT

This research (2022–2023) examined the impact of institutional credit on farm inputs and output by farmers in Kalaburagi district of Karnataka using the panel data at district level covering the period between 2001 and 2020. The farmers rely heavily on credit mechanisms to modernize their agricultural activities. To assess the functional relationship between agriculture input-output and institutional credit, correlation and multiple regression analysis was conducted. The results showed that modern farm inputs are highly responsive to formal farm credit. An attempt was made to measure the degree and intensity of usage of modern farm inputs with availed credit and its implication on agricultural output in the region. One of the notable results was that farmers' increased credit capacity has enabled them to procure inputs such as quality seeds, fertilizers, pesticides, and farm mechanization.

Keywords: Agricultural Credit, Farm Productivity, Input-output relationship, Karnataka Economy, and Mechanization of Farming

INTRODUCTION

Agricultural credit is a crucial resource for farmers to procure essential inputs such as seeds, pesticides, fertilizers, livestock, crop transportation vehicles, harvesters, and set-up irrigation facilities. Agricultural credit in this study refers to timely accessible funds for the farming community from sources other than the farm industry. In this respect, credit is a medium that facilitates the temporary transfer of purchasing power from surplus to deficit units. A considerable amount of farm credit denotes the loan or advance to the farmers with 'the entitlement to resources' (Ambaraya, 2022). It is considered one of the invaluableinputs in agriculture activities by farmers.It is commonly regarded as the lifeblood of any economic activity and is essential for improving efficiency, productivity, and marketing (Malik, 2021). Its impact can be seen in modernization, which ultimately increases agricultural productivity. Access to credit indirectly affects productivity through its positive and direct influence on agricultural technology (Awotide *et al*., 2015). The growth in agricultural productivity would be the single most prominent solution for reducing poverty and foodinsecurityinthe country. The need for agricultural and institutional credit has become imperative in recent years. Approximately 52 percent of all the farming households in India have been struggling to repay their non-institutional debt (NSSO, 2018). Hence, most of the small and marginal farmers have

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negative savings. In the state of negative savings and the vulnerable condition of agriculture in India, the present research paper aims to highlight the input and output trend analysis of agriculturalcredit system in Kalaburagi district, where, farmers see it as a necessary component for modernization and mechanization of farming. The agrarian credit analysis was undertaken to explore its impact on agricultural produce in Kalaburagi district. For this purpose, an agro-economic profile of Kalaburagi district is discussed, which forms the background of the research, was discussed.

Agro-economic Profile of Kalaburagi

Kalaburagi is widely known as a '*Tur Bowl*' of Karnataka. It is located in the state's northern region. It contributes almost 40 percent to the overall *tur* production in the state. The district is abundant with black soil suitable for crops such as 'tur' and *jowar*, groundnut, paddy, and legumes.The district houses a total population of 25,66,326 as per the Census of India (2011) , of which 33 percent are urban and 67 percent are rural. Around 80 percent depends primarily on agriculture for their livelihood. In terms ofindustrial development, Kalaburagi has scarce natural resources. However, most people rely on agriculture as their primary sustenance. As per the Government of Karnataka (2020), the District's Gross Domestic Product (GDP) constitutes 3 percent of the State's GDP. Therefore, the region's per capita income, Rs.67,886 is much lower than the state's already low per capita income. In this context, the agricultural credit system has been seen as a panacea for the vulnerable agriculture sector..The study of the farm credit support system in the district highlighted the nature of the farm input and output trend with farm credit in the study period.

Kalaburagi Farm Credit Support System

Before 1917,the concept of modern banking was virtually unknown in Kalaburagi. On the other hand, the foundation of the Central Cooperative Bank (now known as Gulbarga District Central Cooperative Bank) in 1917 marked the commencement of banking in the district. Several outside banking institutions were invited to open branches in the district. The Gulbarga Urban Cooperative Bank was founded in 1932. The Central Bank of India was the first joint stock bank outside the district to open a branch. In 1944, the State Bank of Hyderabad opened a branch in Kalaburagi. Canara Industrial and Banking Syndicate opened an additional branch in 1956, Canara Bank in 1957, and Punjab National Bank in 1960. Furthermore, establishing rural banks known as "Grameen Banks" effectively bridged the divide between urban and rural financial services (Kumar, 2023).

Farm credit is also provided by financial institutions such as cooperative societies. Establishing cooperative organizations was the first step in delivering institutional finance to ruralIndia. These venues include state, cooperative, commercial, and regional rural banks. Furthermore, non-institutional loan flows to the agricultural sector in India are significant. According to the All-India Debt and Investment Survey 2019, non-institutional credit accounted for 90.9 percent of total farm loans in 1951. However, by 2013, its share had dropped dramatically to 36 percent, and it has since fallen further to 32.9 percent in 2019, showing that a significant percentage of agricultural financing comes from the formal sources such as regional rural banks, microfinance institutions, government, and cooperatives. Non-institutional credit is

generally provided by moneylenders,dealers, relatives, commission agents, landlords, and local shopkeepers. It is often unproductive and harmful due to its high interest rate (Sahoo, 2021).

In India, agricultural finance is divided into three categories: short-term, medium-term, and long-term. Short-term credit refers to cash made available to farmers for a certain period, often 15 months, to cover input expenses and modernize machinery. It handles issues like sowing, fertilizer application, plant protection, and employee wages. The acquisition of bullock carts, milk animals, farming appliances, farm building construction, and well digging is supported by medium-term credit ranging from 15 months to 7 years. Long-term loans of seven to twenty years duration promote purchasing equipment as permanent property (Malik, 2021). According to the World Bank, lending is critical to agricultural development. The crop loans are significant because of their fiscal, social, and technical ramifications. Most Indian farmers, accounting for more than 86 percent of the total are small and marginal (Agricultural Census, 2016). Providing credit to farming or secondary activities raises their standard of living significantly. It increases crop yields and income levels and has a multiplier effect onfamilywelfare. Furthermore, it contributes to the expansion of agro-based enterprises (Paramasivan and Pasupathi, 2016)

Formal finance works like a lifeline and blood for agricultural activities. It goes into production in production process and formed into many shapes like used to purchase inputs during the cropping season, agrarian finance allows purchasing capital assets such as irrigation equipment, machinery, and draught animals (Fink *et al*., 2020). It also frequently replaces high-interest unsecured borrowing, resulting in a “consumption smoothening effect” for farmers. The objectives of this research was to assess the impact of farm financing on agricultural output, namely through its utilization for the acquisitionof fixed assets and modern tools; to analyze the utilization of farm credit and its subsequent impact on agricultural practices and to evaluate the efficiency of farm credit in the context of farming.

MATERIAL AND METHODS

Data Collection: The study carried out in the year 2022–2023 was entirely dependent on the secondary data found in the reports and sources listed below, which were released by the Karnataka government as late as 2019-20, with aparticular focus on economic aspects relevant to the examination of thefarm credit system, farm input and outputtrend within the district. It drew on records such as the Karnataka State Economic Survey, Kalaburagi district at a Glance (2016-17), and annual GESCOM and MVD Kalaburagi reports. Aside from these, the DACNET 2017 website is utilized for input surveys, the Directorate of Economics and Statistics, the Government of Karnataka website for agricultural statistics, the Centre for Monitoring Indian Economy (CMIE), the States of India database, and the ICRISAT District level database. The Input Survey, DACNET 2017, provides data on inputs such as fertilizers (N, P, K), hand-operated, animal-operated, power-operated, and quality seeds. Annual reports from GESCOM and the Kalaburagi Motor Vehicle Department were used to obtain time series data on several pump sets and tractors accordingly. DACNET 2017 data on crop cultivation area and total crop production were obtained. The ICRISAT area-level database wasused to collect the value of agricultural loan disbursed in the area. The Gross District Domestic Product and sectoral contribution were collected from DESs, Karnataka

publications, which can be found at CMIE States of India. The District demographics taken from the District at a Glance report published latest for the year 2016-17 by GoK.

Data Analysis Tools: The statistical method of correlation and log-log model of multiple regression analysis were used. Furthermore, rigorous tests for multicollinearity, autocorrelation, and stationarity were performed to provide more credible estimator estimations. Econometric model:log AGDDP = $\hat{a}_1$ + $\hat{a}_2$ log c + $\hat{a}_3$ log f + $\hat{a}_4$ log t + $\hat{a}_5$ log r + u, Where, AGDDP is the share of crops and livestock in Gross District Domestic Product (Note: the contribution of forestry and fisheries are not taken into account since their combined contribution is minimal. The 'c' is the notation used for formal farm credit, 'f' for fertilizer consumption in tones, 't' for the number of tractors, 'r' for

Table 1. Increasing trend of direct and indirect institutional credit for agriculture and allied activities in Kalaburagi over 15 years (Units in 1000 rupees)

S.No.	Year	Direct and Indirect total institutionalcredit	Growth Rate (%)
01	2002	2921318	-
02	2003	3308973	13
03	2004	3826076	16
04	2005	6958668	82
05	2006	10223343	47
06	2007	13483686	32
07	2008	15788255	17
08	2009	18979503	20
09	2010	21860089	15
10	2011	19425228	-11
11	2012	14561834	-25
12	2013	23630652	62
13	2014	18115801	-23
14	2015	31133503	72
15	2016	28870995	-7.27
16	2017	26608487	-7.84
17	2018	26730109	0.46
18	2019	12026500	-55.01
19	2020	41460100	244.74
Average Growth Rate		**27.33**	

Source: **Reserve Bank of India (RBI), 2022**

annual rainfall in the region (in mm) and 'u' for the error term which can be surrogates of all other unknown factors which affect the output.

Hypotheses: The first hypothesis of this study posits that the provision of institutional, agricultural loans has played a crucial role in facilitating the adoption of more efficient and advanced farming practices.The second hypothesis postulates that institutional farm loans are of utmost importance and play a pivotal role in promoting the modernization of agricultural practices, particularly the adoptionof machinery and equipment. The third one hypothesizes that the agricultural credit system has significantly enhanced the process of agro-capital building.

RESULTS AND DISCUSSION

Trend Analysis: Agriculture and Livestock; The credit in the district has increased by leaps and bounds due to the establishment of many financial institutions across the district through time. The priority sector lending (PSL) policy followedby banks and other financial institutions onthe instruction of the Government of India enabled banks to set a target for the number of loans entitled for agricultural purposes. Apart from these, a lead bank is established in every district to monitor the loan disbursement and flow to the farm sector. As a result, a rising trend in farm credit is depicted in the Figure 1.

Between 2002 and 2003, there was a notable rise of 13 percent in total farm loans distributed to the agricultural activities in the district. The growth rates experienced significant increasesfrom 16 percent to 47 percent between 2004 and 2007. These improvements can be attributed to the recognition of agriculture sector as the 'primary moving force' in the tenth five-year plan of the Central Govt. The growth rates slightly declined from 2008 to 2010 and in the last few successive years due to a global financial crisis. The growth rate experienced a significant upsurge in 2013 with the emergence of a new administration, which fulfilled its pre-election commitment by waiving

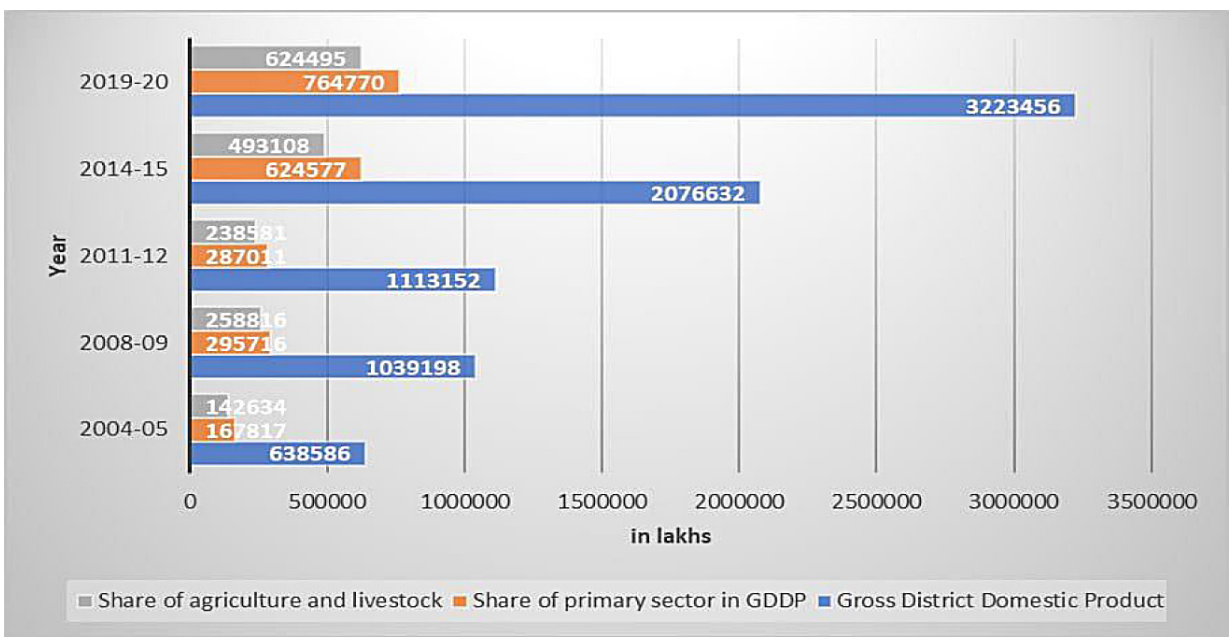

Figure 1. Share of agriculture and livestock in Gross District Domestic Product (GDDP) over 15 years (Source: Department ofEconomic and Statistics, Govt. of Karnataka, 2020)

farm loans.

Consequently, there was a notable surge in the demand for new loans, amounting to a 62 percent rise. In that, the percentage of direct credit was relatively higher than that of indirect credit as direct credit was proven to be more productive (Manoharan and Varkey, 2022). Hence, over time, the district had an upward trend and trajectory in agricultural credit. This phenomenon aligns with the increasing prevalence of the share ofthe primarysector inthe Gross District Domestic Product, as depicted in the Figure 1.

However, farm credit tends to positively impact the household income of better-off peasants who receivelarger credit volumes (Luan and Bauer, 2016). The amount of agricultural holdings positively correlated with credit borrowing (Brijesh, 2019). Borrower farmers have a larger cultivated area than non-borrowers since the financial help from agriculturalcredit allows them to increase their net sown area. Borrower farmers had higher cropping intensity than non-borrower farmers (Nakazi Florence, 2020). Therefore, relatively better-off farmers appropriate the larger share of the rising primary sector in alignment with farm credit.

Farm Machinery: A similar pattern can be seen in tools and machinery in Figure 2 below. Furthermore, the study observed a substantial link between farm loans and agro-capital in and per-hectare investment in farm structures among borrowers. This phenomenon also aligns with the increasing prevalence rate of farm credit over time.

Hand seed fertilizer drill, chaff cutter, and hand sprayer-duster are the three primary hand implementsthat were employed in agriculture. The use of chaff cutters experienced a decline of 99.9 percent; this phenomenon also aligns positively with the increasing prevalence of farm credit over time, whereas the change remained almost stagnant for hand seed-fertilizer drill and hand sprayer-duster.

The trend in the usage of animal-operated implements, steel plows, cultivator tripoli, seed cum fertilizer drills, and bullock carts was analyzed from 2001-2015. The

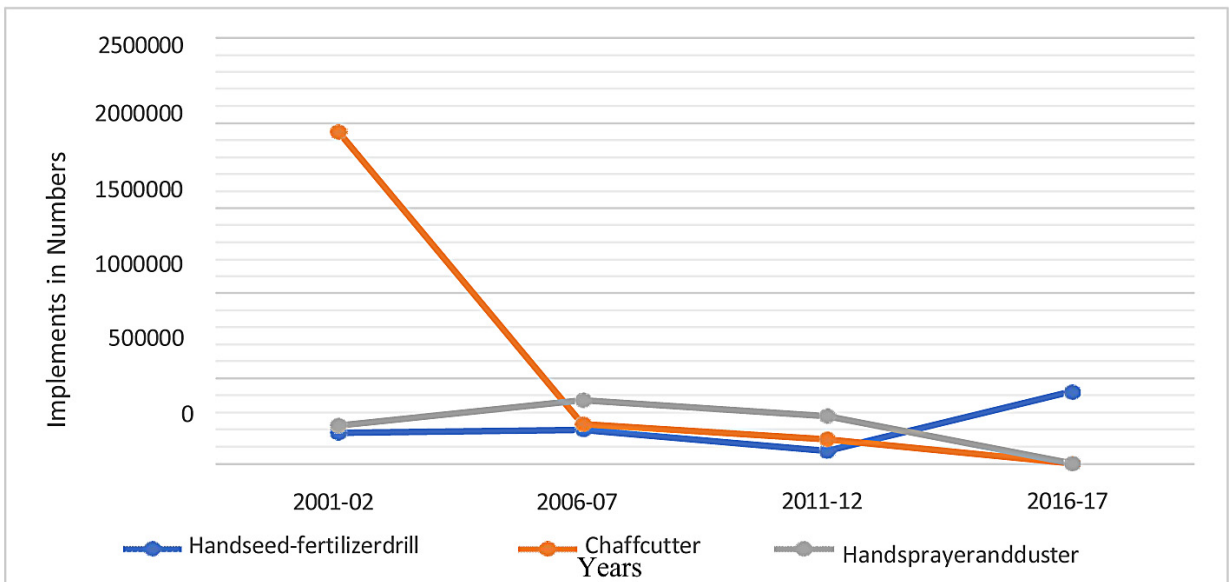

Figure 2. Trend in the use of major hand implements
Source: Input Survey, DACNET, 2017

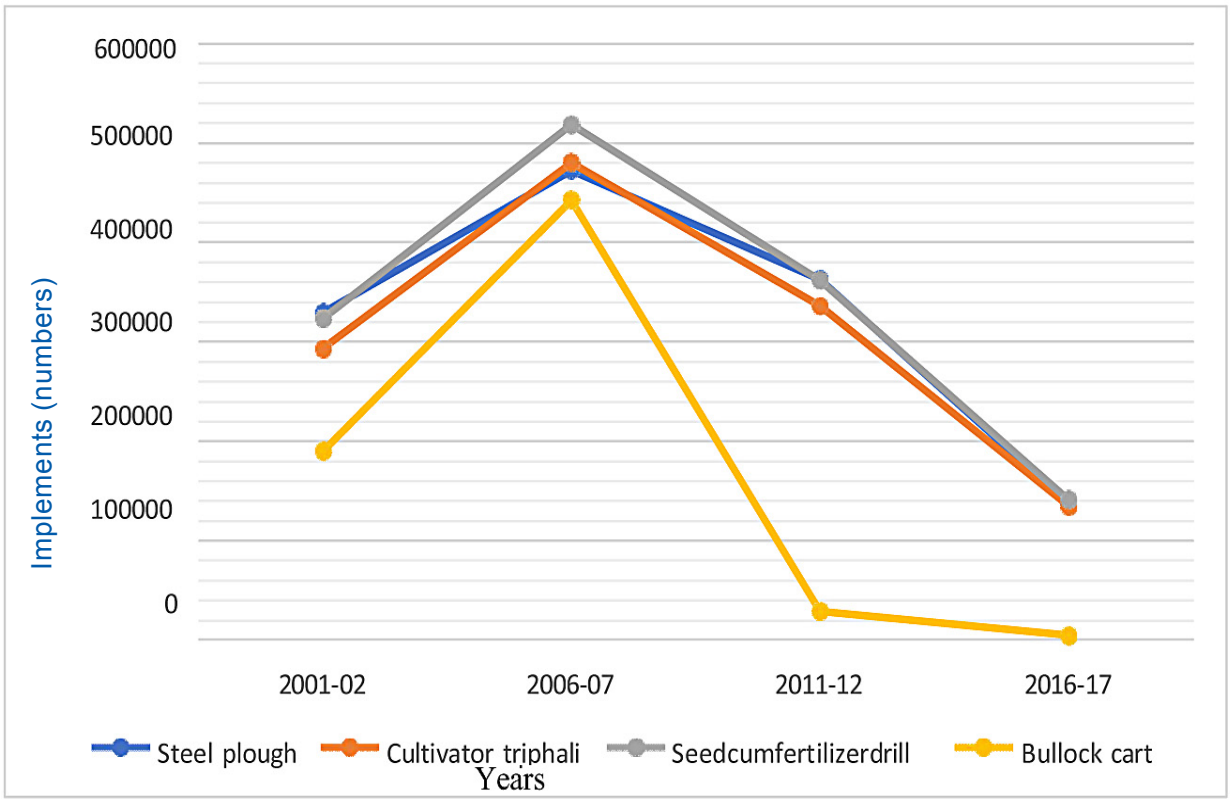

Figure 3. Trend in the use of animal-operated implements
Source: Input Survey, DACNET, 2017

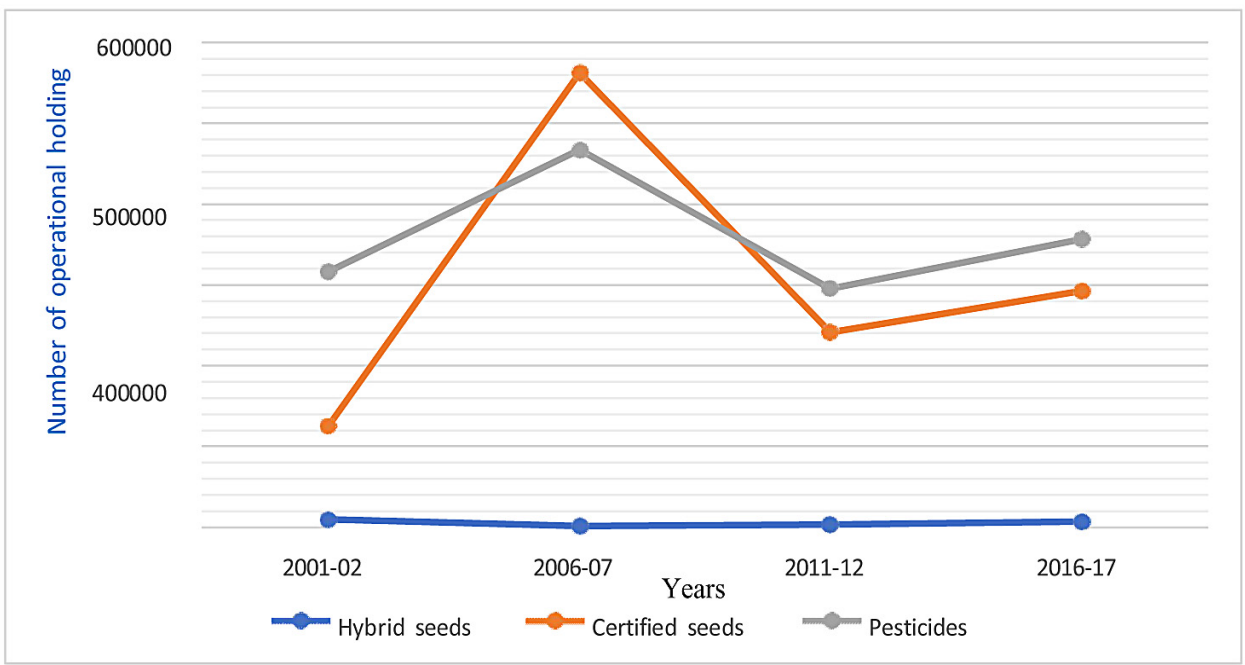

Figure 4. The trend in usage of seed sand pesticides
Source: Input Survey, DACNET 2017

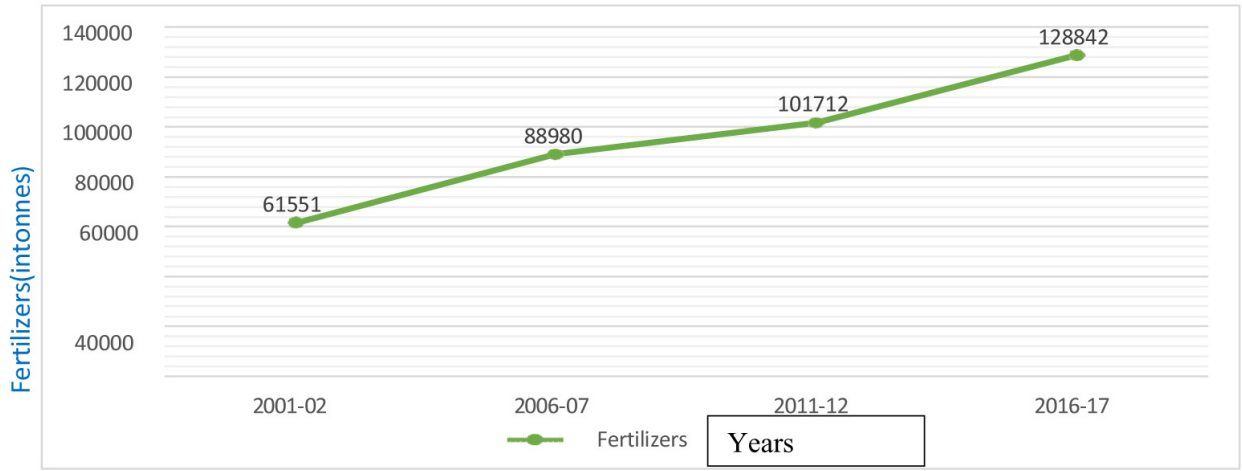

Figure 5. Trend in fertilizer usage; Source: Input Survey, DACNET 2017

number ofanimal-operated tools of the steelplow, cultivator tripoli, seed cumfertilizer drill, and bullock cart declined by 58.19 percent, 54.05 percent, 56.37 percent, and 97.66 percent, respectively. The decline is significantly attributed tothe provision and utilization of formal farm credit as itstrend also aligned with the trend of farmcredit with assumptions of other things remained the same.

The number of fertilizers (NPK) was 61551 tons in 2001, which further increased to 128842 tons in 2016-17, which indicated a hike of 65.2 percent in fertilizer consumption in the region. Thus, the credit for the modernization of agriculture goes to the

Table 2. Correlation analysis between farm credit, farm inputs, and farm equipment

S.No.	Category	Particulars	Correlation Coefficient with FC Significant
1	Farm Inputs	Fertilizer	+0.895
		Pesticides	+1
		Certified Seeds	0.692
		Hybrid Seeds	-0.370
		Handseed fertilizer drill	0.833
2	Obsolete Farm Equipment	Chaff cutter	-0.903
		Hand sprayer/duster	-0.719
		Steel plough	-0.557
		Cultivator triphali	-0.466
		Seed cum fertilizer drill	-0.442
		Bullock cart	-0.649
3	Modern Farm Inputs	Sprayer/duster	0.138
		Electric pumpsets	0.914

institutional credit established in the area. Installing modern farm inputs has emerged from the availability and accessibility of the credit system. Researcher have found a strong and significant association between farm credit and modern farm inputs.

A statistically significant correlation was shown between farmloans and agricultural output. Adirect and moderate correlation was computed between farm financing and agrarian inputs (Hemming *et al*., 2018). However, as shown in the Table 2, a more pronounced correlation between these agrarian inputs and farm loans was found, especially with pesticides, fertilizers, and electric pump sets. Hence, most borrowings are utilized to finance agricultural operations expenditures, particularly acquiring fertilizers and pesticides. The credit-deposit ratio of commercial banks in the Gulbarga district increased from 50 percent to 80 percent during the examination period. Most importantly, it has the effect of smoothing consumption by replacing informal credit borrowing. According to the study, borrowers have more efficient production than non-borrowers. Borrower farms have higher capital turnover than non-borrower farms, indicating that borrower farms provide higher returns on inputs used (Sangeetha *et al*., 2016).

The inclusion of formal farmcredit in the modelas an independent variable concedes the financial aspect of the district's agricultural and livestock production, which is used by farmers both to pay labor wages and to form capital. The variable 'f' captures the impact of fertilizer consumption on the agricultural output. Mechanization is an essential factor in increasing agricultural efficiency. The number of tractors is a proxy for the levelof mechanization in farming, and it is included in the model to capture the impact of modern farming practices. Also, rainfall as it isacritical factor for agricultural output, and the 'e' is for all other factors that affect the Agriculture GDP of the district.

Hypotheses tests: The coefficient of log c, denoted as â, has been estimated to be 0.664. Agricultural credit exhibits a positive impact of farm credit on AGDDP, as seen by a 0.664percent increase in AGDDP for every one percent increase in agricultural credit. Inthe Table 3 calculated t-value for agriculturalcredit is 5.461, which exceeds the criticalt-value of3.106, and also based on the obtained p-

Table 3 .Estimators of the Multivariate Regression Model

Coefficients								
		Unstandardized Coefficients		Standardized Coefficients			Colinearity Statistics	
S.No.	Model	Beta (â)	Std. Error	Beta (â2)	t-value	(p-value) Sig.	Tolerance	VIF
01	(Constant)	-10.222	9.048		-1.130	.285		
02	logc	0.664	0.122	1.267	5.461	<.001	0.178	5.606
03	logf	-0.292	0.328	-0.199	-0.890	0.394	0.191	5.226
04	logr	-0.572	0.289	-0.222	-1.983	0.075	0.766	1.305
05	logt	2.105	0.765	0.299	2.753	0.020	0.814	1.229

a. Dependent Variable: log AGDDP

value of 0.000 for the calculated t-value, one might conclude that the null hypothesis, which posits a weak positive relationship between agricultural loans and agricultural output, was rejected. This implies that the observed value of â= 0.664 of A GDDP and farm credit is not a random outcome. The p-value associated with the fertilizer data in the model is 0.394, indicating that â2 = -0.29 is not statistically significant at a 5 percent significance level; therefore,researcher couldn't reject the null hypothesis. This conclusion is supported by the t-statistic for fertilizer, which was 0.890, less than t-value of 2.201, hence, researcher couldn't reject the null hypothesis stating that fertilizer substantially positively affects agricultural productivity. The anticipated value of the third estimator â, which represents the coefficient of the logarithm of 'f', is -0.292. Therefore, this suggested a negative correlation between the application of fertilizer and the AGDDP within the region. The utilization of fertilizer exhibits a negative proportional relationship with the AGDDP, whereby a percentage rise (or reduction) in fertilizer usage results in a corresponding drop (or increase) in the GDDP by 0.292 percent. This phenomenon may arise as a result of

Table 4. Summary statistics of the model

S.No.	Model	R	R Square	Adjusted R Square	Error of Std.the Estimate
01	1	.951a	.904	.866	.161

a. Predictors:(Constant), logt, logr, logf, log c

Extremely low p-value (p<.001[b]) associated with the model, F-statistics support the value ofR-squared to be statistically significant, substantiating the overall significance of the regression model.

Table 5. Analysis of Variance (ANOVA)

N	Model	SumofSquares	df	MeanSquare	F	Sig.
01	Regression	2.462	4	.616	23.542	<.001b
02	Residual	.261	10	.026		
	Total	2.724	14			

a.Dependent Variable: log AGDDP

b.Predictors:(Constant), log t, log r,log f, log c

Table 6. Durbin-Watson Statistics*

N	Variable	DW- statistics
01	Log agricultural GDDP	1.318
02	Log agricultural credit	1.799
03	Log fertilizer	2.1133
04	Log tractor	1.96
05	Log rainfall	1.68

farmers' knowledge of appropriate fertilizer utilization practices within agricultural settings. The phenomena observed in the region, characterized bypoor fertilityof agricultural land, may be attributedto the excessive or insufficient use of fertilizers over the study period. The estimated fourth e stimator ß, which represents the co efficient of the logarithm of 't',is determined tobe 2.105. This indicates thata 1 percent increase in the number of tractorsused for agricultural purposes resultsin acorresponding rise in more than two percent in the AGDDP. The p-value associated with the tractor variable in the regression model is 0.020, in dicating statistical significance at a5 percent significance level. The computedt-value forthetractor variable is 2.753, exceeding the criticalt-value of 2.201. Hence, the research erconcluded that the null hypothesis, which posited that the quantity of tractors does not significantly affect agricultural productivity, is rejected and not supported by the data. Hence, it is evident that the modernization of farms serves a potent role in transforming the agricultural sector, with its effectiveness being positively related to the availability of loans. The calculated fifth estimator ß, which represents the coefficient of the logarithm of rainfall (logr), is determined to be 0.572. This value suggests that a one percent rise or fall in the amount of rainfall is associated with ß, percentage amount fall and rise in the AGDDP, suggesting an inverse relationship between the two. The potential causefor this phenomenon could be attributed toclimate changei.e,intensive rain or droughtseen during the researchperiod.The p-value associated withrainfall in the regression modelis 0.075, indicating a statistically significant relationship (substantiated via the given p-value in the Table 3) between both variables.

*Durbin Watson test statistics was run to check the autocorrelation in the regression model of output. It ranges from 0 to 4 with a value of 2 indicating zero autocorrelation. The values between 1.5 to 2.5 is considered to be normal. Hence, the model is free from autocorrelation and therefore, it is best fit.

CONCLUSIONS

The institutional farm credit was found to be one of the significant drivers of Agricultural Gross Domestic Product in the Kalaburagi district of Karnataka. A one percent increase in farm credit is associated with 0.66 percent increase in Agricultural Gross Domestic Product. This means that the degree of the responsiveness of the farm produce w.r.t farm credit is high and efficient. It is performing a dual role: firstly, modernization of agriculture through the purchase of moderninputs, and secondly, contributing to the growth of AGDP significantly and directly. Farm credit has enormous potential and leverage to reduce farmers' overreliance on non-institutional loans over the years. A notable association was shown between farm finance and agricultural inputs, particularly w.r.t fertilizers, pesticides, premiumseeds, and other farmimplements. The proposition suggeststhat the utilization of formal credit substantially influenced the process of capital formation and the modernization of agricultural practices. The research revealed an inverse relation between credit and traditional farming methods where ases a positive relation between credit and advanced farming tools and equipment with increasing utilization of modern farm inputs and machines. This resulted in significantly improved efficiency in cultivating and raising livestock and a movement from traditional to modern contemporary practices. This trend suggested that financial institutions had been progressively efficient in supporting the sector's transformation.

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CULTURAL VARIABILITY OF TRICHODERMA ISOLATES IN CHICKPEA GROWN SOILS OF GUNTUR DISTRICT

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Trichoderma spp. are omnipresent in soil acting as effective biological control agents in various crop ecosystems for the control of various soil borne diseases. They act as a part of eco-friendly practices to achieve higher yields (Kumari *et al*., 2023). High reproductive capacity, ability to survive under unfavorable conditions and hyper-parasitizing nature on phytopathogenic fungi led to the success of *Trichoderma* species asbiocontrol agents. Mycelial growth rate and colony appearance help to differentiate *Trichoderma* isolates before their exploitation in biocontrol. Taking into consideration of the above facts, the present investigation was carried to characterize the variability of *Trichoderma* isolates with respect to their cultural characters.

Variability studies of 13 *Trichoderma* isolates*viz*., T 19001, T 19002, T 19006, T 19012, T 19015, T 19020, T 19022, T 19023, T 19026, T 19027,T 19028, T 19029 and T 19030) collected during *rabi* 2021 from chickpea growing areas in Guntur district were carried out in the Department of Plant Pathology, Agricultural College, Bapatla. Confirmation, maintenance of pure cultures and other *in vitro* studies were continued during 2021 and 2022.

Isolation of *Trichoderma* isolates

Soil samples collected from the rhizosphere from seven different chickpea growing areas (Bapatla, Throvagunta, Gundlapalli, Venkupalem, Gundreddipalem, Jangunguntla and Parchur) were air dried for 24 hours and sieved through a 2 mm sieve. Twenty mg of sieved soil was transferred to a Petri plate and spread uniformly on *Trichoderma* selective medium under aseptic conditions. The plates were incubated at 28 ˚C for three to five days and the colonies were allowed to sporulate. Thus, obtained *Trichoderma* cultures were sub-cultured on PDA after confirmation based on conidiophore morphology (Gams and Bissett, 2002). Pure cultures were obtained by hyphal tip method and then sub-cultured on PDA slants and incubated at 27±1 till the mycelium was fully grown over medium.

Cultural variability of *Trichoderma* isolates

Radial growth of 13 test *Trichoderma* isolates was observed to find their cultural variability.Three days old culture of each isolate was inoculated on PDA at 28 ± 1. The colony growth of the fungi on each plate was measured along the diagonals with a ruler, and the mean per plate was calculated (Kai *et al*., 2019). On the first day after incubation, T19002 isolate expressed maximum growth (4.2 cm), while isolate T19012 showed the least growth (2.2 cm), followed by T 19027, T 19028, T 19029 and T 19030 isolates with less than 3.0 cm growth, while the remaining seven isolates recorded growth between 3.00 to 4.00 cm. (Table 1). At

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Table 1. Cultural variability in the radial growth of *Trichoderma* isolates

S. No.	Isolates	Diameter of the colony (cm)*			Growth rate of the colony (mm h^{-1})			Average growth rate (mm h^{-1})
		1 DAI	3 DAI	5 DAI	1 DAI	3 DAI	5 DAI	
1	T 19001	3.0 (2.0)^{de}	5.3 (2.51)^{a}	7.9 (2.98)^{ab}	1.25	0.74	0.66	0.88
2	T 19002	4.2 (2.28)^{j}	8.5 (3.08)^{j}	9.0 (3.16)^{g}	1.75	1.18	0.75	1.23
3	T 19006	3.1 (2.02)^{ef}	7.5 (2.92)^{i}	9.0 (3.16)^{g}	1.29	1.04	0.75	1.03
4	T 19012	2.2 (1.79)^{a}	6.9 (2.81)^{f}	8.2 (3.03)^{d}	0.92	0.96	0.68	0.85
5	T 19015	3.4 (2.1)^{h}	6.8 (2.79)^{ef}	8.2 (3.03)^{d}	1.42	0.94	0.68	1.01
6	T 19020	3.6 (2.14)^{i}	9.0 (3.16)^{k}	9.0 (3.16)^{g}	1.50	1.25	0.75	1.17
7	T 19022	3.3 (2.07)^{gh}	7.5 (2.92)^{i}	9.0 (3.16)^{g}	1.38	1.04	0.75	1.06
8	T 19023	3.2 (2.05)^{fg}	7.2 (2.86)^{g}	8.6 (3.10)^{e}	1.33	1.00	0.72	1.02
9	T 19026	3.1 (2.02)^{ef}	7.4 (2.90)^{hi}	8.8 (3.13)^{f}	1.29	1.03	0.73	1.02
10	T 19027	2.9 (1.97)^{cd}	6.4 (2.72)^{d}	8.1 (3.02)^{cd}	1.21	0.89	0.68	0.93
11	T 19028	2.8 (1.95)^{bc}	6.2 (2.68)^{c}	8.8 (3.13)^{f}	1.17	0.86	0.73	0.92
12	T 19029	2.9 (1.97)^{cd}	6.4 (2.72)^{d}	9.0 (3.16)^{g}	1.21	0.89	0.75	0.95
13	T 19030	2.9 (1.97)^{cd}	5.7 (2.59)^{b}	8.0 (3.00)^{bc}	1.21	0.79	0.67	0.89
SEm(±)		0.03	0.04	0.04				
C.D. @ 5%		0.1	0.12	0.13				
CV (%)		3.05	2.69	2.53				

DAI- Days after inoculation; Figures with similar alphabets do not differ significantly; Figures in the parenthesis are square root transformed values

three days after incubation, 11 isolates attained >6.1 cm radial growth. The isolate, T19001 recorded the least growth of 5.3 cm followed by 5.7 cm in the isolate,T 19030. At 5 days after incubation, all the 13 isolates registered radial growth of>6.1 cm with five of the isolates *viz*., T 19020, T 19006, T 19002, T 19022, T 19029 completely occupied 9.0 cm in Petri plate and

seven isolates with radial growth of >8.0 cm *i.e.,* T19001, T 19012, T 19015, T 19023, T 19026, T 19027 and T 19028.The isolate, T 19030 recorded a radial growth of <8.0 cm (Table 1).

Growth rate of 13 *Trichoderma* spp. was calculated at 1, 3 and 5 days after incubationby dividing the colony diameter by 24 h, 48 h and 78 h. The highest growth rate was observed in T19002 isolate (1.75 mm h^{-1}), followed by T19020 isolate (1.5 mm h^{-1}) and least growth rate was found in T19012 isolate (0.9 mm h^{-1}), after one day of incubation. At three days after incubation, high growth rate of >1.0 mm h^{-1} was found in the isolate, T19020 and the remaining 12 isolates recorded a radial growth of <1.0 mm h^{-1}. At 5 days after incubation,high radial growth rate was observed in T19001, T 19028 and T 19029 isolates and no growth was observed in case of T19020 isolate. Average growth rate was found highest in T19002 isolate with 0.91 mm h^{-1} radial growth followed by T 19020 (0.87 mm h^{-1}) and least growth rate (0.71 mm h^{-1}) was recorded by the isolate T 19012 (Table 1).

It was observed that all the isolates recorded a growth rate of 1.0-2.0 mm h^{-1} at 1 DAI. The isolate, T 19001 showed a growth rate of <0.5 mm h^{-1} and 11 isolates recorded 0.5-1.0 mm h^{-1} of growth rate and T 19020 expressed a growth rate of 1.0-2.0 mm h^{-1} at 3 DAI. At 5 days after inoculation all isolates, except two isolates (T 19028 and T 19029) recorded 0.5-1.0 mm h^{-1} of growth rate *i.e.*, <0.5 mm h^{-1} (Table 2). Among all the *Trichoderma* isolates, 11 isolates except T 19001 and T 19030 were found to be fast growing isolates.

Further isolates were grouped on the basis of radial growth and growth rate. Radial growth of *Trichoderma* isolates was recorded at 1, 3 and 5 DAI. All the 13 isolates were grouped into four categories as very fast (>6.1 cm), fast (4.0 - 6.0 cm), medium (2.1 - 4.0 cm) and slow growing (<2.0 cm) based on radial growth. It was observed that isolate T 19002 exhibited fast growth, while the remaining 12 isolates showed medium growth at day 1 after incubation. Eleven isolates showed >6.1 cm radial growth and were categorized as very fast which were considered as better proliferators, Two isolates, T 19001 and T 19030 were categorized as fast growing isolates at 3 days after inoculation. After five days of incubation, all isolates recorded very fast radial growth (Table 2).

Mohammad *et al*. (2015) observed that fourout of seven isolates reached 5.0-8.5 cm diameter in five days. Sharma and Singh (2014) reported that most of the isolates of *Trichoderma* showed fast growth of 5.03-5.06 cm within three days after incubation.

Variation among all the 13 *Trichoderma* isolates, in terms of growth habit, nature of mycelium, colony margin and conidiationwas presented (Table 3). Mycelium showed aerial, submerged and both types of growth habit; arachnoid, cottony and floccose to arachnoid type of mycelium with smooth or wavy margin; conidiation varied from 2-3 concentric rings with sparse or concentrated sporulation.Gams and Bisset (2002) reported that colonies of *Trichoderma* spp. strains showed rapid growth, concentric halos and floccose or compact surface that looked like tufts on the culture medium. The mycelium, initially of a white color, acquired green, yellow shades, or remained white, due to the abundant production of conidia which showed smooth or rough-appearance.

Based on the growth habit, *Trichoderma* isolates were categorized into three groups. Out of the 13 isolates, T 19001, T 19002 and T 19028 showed aerial growth habit; T 19006, T 19012, T 19026, T 19027 and T 19029 showed submerged; and remaining isolates *i.e.,* T 19015, T 19020, T 19022, T 19023 and T 19030 showed both aerial and submerged growth habit (Tables3 and 4).

Based on the nature of mycelium, *Trichoderma* isolates were classified into three

Table 2. Grouping of *Trichoderma* isolates based on radial growth and growth rate

S. No.	Radial growth	*Trichoderma* isolates		
		1 DAI	3 DAI	5 DAI
1	Very fast (> 6.1 cm)	--	T 19002, T 19006, T 19012, T 19015, T 19020, T 19022, T 19023, T 19027, T 19026, T 19029, T 19028	All isolates
2	Fast (4.1 - 6.0 cm)	T 19002	T 19001, T 19030	--
3	Medium (2.1 - 4.0 cm)	T 19001, T 19006, T 19012, T 19015, T 19020, T 19022, T 19023, T 19027, T 19028, T 19026, T 19029, T 19030	--	--
4	Slow (< 2.0cm)	--	--	--
S. No.	**Growth rate (mm/h)**	***Trichoderma* isolates**		
		1 DAI	**3 DAI**	**5 DAI**
1	< 0.5	--	T 19001	T 19001, T 19002, T 19006, T 19012, T 19015, T 19020, T 19022, T 19023, T 19027, T 19026, T 19030
2	0.5 – 1.0	--	T19002, T 19006, T19012, T 19015, T 19022, T 19023, T 19027, T 19028, T 19026, T 19029, T 19030	T 19028, T 19029
3	1.0 – 2.0	All isolates	T 19020	--

groups. Eight isolates *viz*., T 19001, T 19006, T 19012, T 19015, T 19026, T 19029, T 19030 and T 19028 exhibited cottony mycelium and three isolates, T 19002, T19020 and T19023 had arachnoid; and floccose to arachnoid nature of mycelium was observed in two isolates (Tables 3 and 4).

Two types of colony margin were identified among the isolates of *Trichoderma*and grouped accordingly. Eleven isolates *viz.,* T 19001, T

Table 3. Variation in cultural characters among *Trichoderma* isolates on PDA medium

S. No.	Isolate	Growth Habit	Nature of Mycelium	Colony Margin	Conidiation
1.	T 19001	Aerial	Cottony	Smooth	Crusty spore mass sparsely scattered all over but more concentrated at centre
2.	T 19002	Aerial	Arachnoid	Smooth	3 Ring like zones with smooth dusty sporulation
3.	T 19006	Submerged	Cottony	Smooth	2 Concentric rings of granular sporulation
4.	T 19012	Submerged	Cottony	Smooth	3 Concentric zones of crusty granular spore mass
5.	T 19015	Aerial and submerged	Cottony	Smooth	2 Concentric rings of granular sporulation
6.	T 19020	Aerial and submerged	Arachnoid	Smooth	3 Concentric zones of dusty rough spore mass
7.	T 19022	Aerial and submerged	Floccose to arachnoid	Smooth	2 Concentric rings of dusty spore mass
8.	T 19023	Aerial and submerged	Arachnoid	Smooth	2 Concentric zones of crusty rough spore mass
9.	T 19026	Submerged	Cottony	Smooth	2 Concentric zones of granular spore mass
10.	T 19027	Submerged	Floccose to arachnoid	Wavy	Crusty spore mass scattered all over but more concentrated at the centre
11.	T 19028	Aerial	Cottony	Wavy	Single central zone of pale colored sporulation
12.	T 19029	Submerged	Cottony	Smooth	2 Concentric rings of dusty spore mass
13.	T 19030	Aerial and submerged	Cottony	Wavy	3 concentric zones with smooth dusty sporulation

Table 4. Grouping of *Trichoderma* isolates based on growth habit, type of mycelium and colony margin

S. No.	Character	Grouping	*Trichoderma* isolates
1	Growth habit	Aerial	T 19001, T 19002, T 19028
		Submerged	T 19006, T 19012, T 19026, T 19027 and T 19029
		Aerial and submerged	T 19015, T 19020, T 19022, T19023 and T 19030
2	Nature of mycelium	Cottony	T 19001, T 19006, T 19012, T 19015, T 19026, T 19029, T19030 and T 19028
		Arachnoid	T 19002, T19020 and T19023
		Floccose to arachnoid	T 19022 and T 19027
3	Colony margin	Smooth	T 19001, T 19002, T 19006, T19012, T 19015, T 19020, T 19022, T 19023, T 19026, T 19029 and T 19030
		Wavy	T 19027 and T 19028

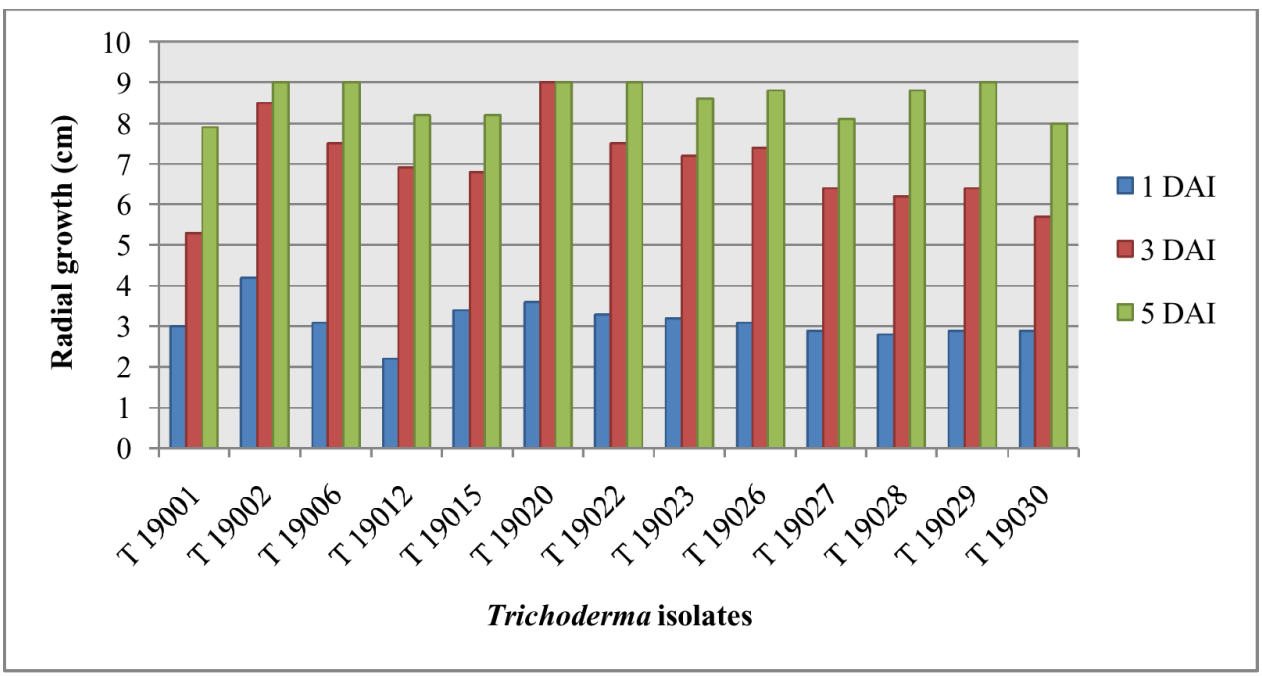

Figure 1. Variability in the radial growth of *Trichoderma* isolates

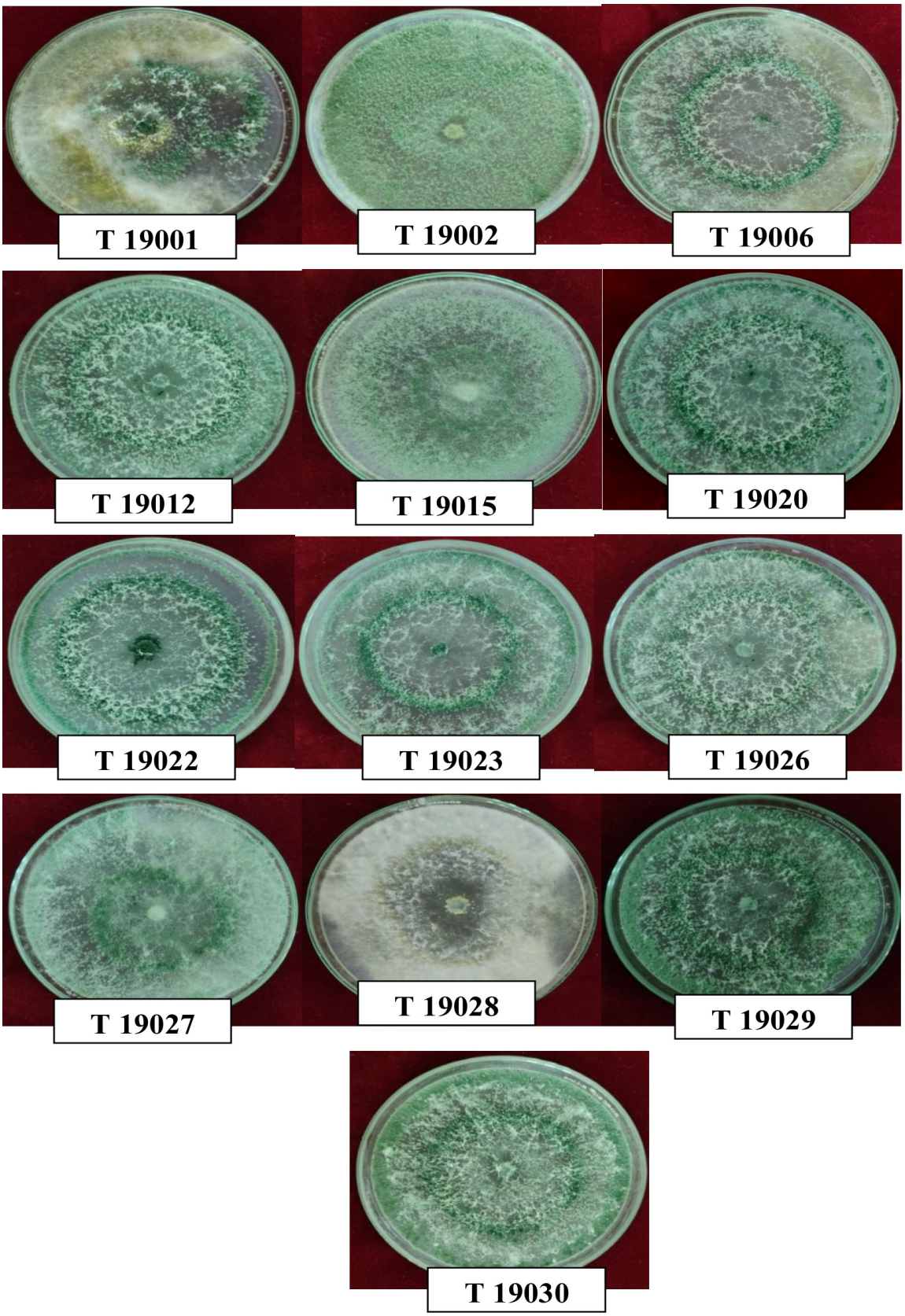

Plate 1. Pure cultures of *Trichoderma* isolates

19002, T 19006, T 19012, T 19015, T 19020, T 19022, T 19023, T 19026, T 19029 and T 19030 showed smooth margin and two isolates presented wavy margin (Tables 3 and 4).

Sangle *et al*. (2017) classified *Trichoderma* isolates based on mycelia growth, ring formation, sporulation and pigmentation. Muthu and Pratibha (2016) found floccose and arachnoid in twelve isolates of *Trichoderma* (Younesi *et al.,* 2021).

To be an effective (Harman *et al*., 2004) biological agent, faster proliferation and colonization of root surface *i.e.,* rhizosphere or rhizoplane before the entry and establishment of the pathogen at court of infection is a pre-requisite. Hence, the isolates exhibiting radial growth of 9.0 cm within three days were selected as a better isolates to test the bioefficacy against Fusarium wilt and dry root rot pathogens of chickpea.

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CONSTRAINTS PERCEIVED BY THE FARMERS IN THE ADOPTION OF SUSTAINABLE CULTIVATION PRACTICES OF NAGA KING CHILLI

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Naga king chilli (*Capsicum chinense* Jacq) with GI tag (PIB, 2021) is best known worldwide for its intrinsic value and holds a distinct place in global trade due to its unique characteristics of high pungency, flavour and bioactive components. Thus, acknowledged as one of the most valued spice crops of Nagaland. Nagaland produces about 4000 MT of Naga king chilli annually. 70 percent of the production is marketed by traders in Kolkata and Guwahati (YES Bank Ltd. and IDH, 2018). The indigenous practice of Naga king chilli which is largely organic by default and agro-climatic conditions favouring sustainable cultivation, the prospects of sustainable Naga king chilli cultivation in Nagaland are massive. However, the ramifications of climate change, lack of access to improved technologies and postharvest losses have posed a significant threat to the sustainable Naga king chilli production in Nagaland, making the farmers' livelihood vulnerable. Hence, the study was conducted to assess the constraints perceived by the farmers in adopting sustainable cultivation practices of Naga king chilliand suggest strategies to overcome them.

The study was carried out during the year 2020-2023 applying an *ex post-facto* research design. Naga king chilli is a crop of economic importance and is grown in almost all the districts of the state of Nagaland. The three districts *viz.,* Peren, Dimapur and Mon districts of Nagaland werepurposively selected. Further more, two Rural Development (RD) blocks *viz.,* Tening and Jalukie block of Peren district, Niuland and Medziphema block of Dimapur district and Mon and Wakching block of Mon district and two villages from each of the selected RD blocks were included based on random sampling procedure.Thus, a total of 12 villages *viz.,* Old Tesen(30), Upper Sinjol(20), Dungki(22), Lamhai(18), S. Hetoyi(22), Ghokuto(18), Tsiepama(30), Tsiepama Model(14), Pongkong(24), Wangla(18), Wanching(18) and Tanhai(16) were selected. Finally, adhering to a proportionate random sampling procedure,30 percent of the Naga king chilli farmers from each of the selected villages were obtained for the study making a total of 250 respondents. Primary data was collected based on the personal interviews with the help of a structured interview schedule. The Henry Garrett Ranking Technique was employed for the study. Where the respondents were asked to rank the given problem according to the magnitude of the problem. The orders of merit given by the respondents were converted into ranks by using the following formula: Percent position= $100*(R_{ij} -0.5)/N_j$

Where, $R_{ij=}$ rank given for $_i^{th}$ constraints by j^{th} individual;

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N_i= number of constraints ranked by j^{th} individual

The percentage position of each rank thus obtained was converted into scores by referring to the table given by Garrett (1969). Then, for each factor, the scores of individual respondents were added together and divided by the total number of the respondents for whom the scores were added. These mean scores for all the factors were arranged in the order of their ranks and inferences were drawn.

Constraints perceived by the respondents in the adoption of sustainable cultivation practices of Naga king chilli in the region were studied under nine different aspects of the constraints. One of the major constraints recorded was biotic and abiotic constraints.

Where, the most severe constraints were epidemics of pests and diseases with a mean score of 70.34 followed by fluctuation of temperature (65.20), drought during crop period (60.76), occurrence of showers during harvest (60.76) and weed infestations (60.64). In the case of technical constraints, lack of knowledge about insect pest and disease management (66.85) was a major constraint among the respondents, followed by lack of mechanization of the farm (60.91), lack of knowledge about seeds/seedling treatment (57.28) and lack of knowledge about value addition of Naga king chilli (56.64). The highest extension constraint recorded was the lack of technical guidance from extension staff (63.30) followed by extension agents not being available for consultation (63.26), untimely visits of extension agents (58.87) and insufficient extension activities like training, demonstrations, kisan mela etc. by extension agencies (53.91). The top post-harvest constraints in the adoption of sustainable cultivation practices of Naga king chilli were the lack of proper storage facilities (61.46) followed by the lack of processing facilities at the local level (60.89) and increasing processing costs (59.08). The major input supply constraints were the high requirement of manure and fertilizer (62.26) followed by non-availability of fertilizers and bio-pesticides in time (60.61), lack of irrigation facilities (53.10) and non-availability of seeds and planting materials in time (52.64). The top marketing constraints perceived by the respondents were poor access to market information (66.15) followed by lack of proper market (64.73), distressed sales (59.62), high charges on transporting (52.40), exploitation by middlemen (48.94) and fluctuation in market price (47.61). In the case of land/soil-related constraints, steep and undulated land (62.18) was a serious limitation followed by soil erosion (61.39), poor land preparation (52.45) and soil fertility (46.43).Economic constraints experienced by the farmers were associated with the high cost of inputs (60.56) followed by the high cost of planting material (56.22), lack of credit facilities (52.00) and labour-intensive crop (50.66). Major social constraints were no institutional support for commercial Naga king chilli cultivation (60.86) followed by farmers having poor resource base (53.32), younger generation not interested in farming (52.96), poor educational status (52.36) and lack of motivation for Naga king chilli cultivation (51.68). The above findings were in line with the findings of Rais *et al.* (2021), Kumar *et al*. (2023) and Sahoo *et al.* (2023).

The repercussions of climate change have posed a significant threat to the traditional cultivation practices of Naga king chilli as farmers are fully dependent on natural resources for their livelihood.Naga king chilli is generally cultivated in jhum hills under rain-fed conditions using traditional cultivation practices. These traditional practices become inefficient during extreme shifts in temperature, erratic rainfall and high incidence of insect

pests and diseases.Therefore, water management strategies, technical assistance in core areas of Naga king chilli cultivation, location-specific production and management strategies might be effective in minimizing the problems faced by the farmers in sustainable Naga king chilli cultivation practices. Introduction of soil conservation-oriented farming systems like, Sloping Agricultural Land Technology (SALT) and Soil and Water Conservation (SWC) approaches with longer fallow periods (up to 15 years) to naturally build up soil fertility and integrated farming systems with fast-growing and nitrogen-fixing trees and shrubs on contours boundaries and hilltop may help in reducing soil erosion, enrich the soil, provide fodder, fuel-wood and biomass (FOCUS, 2018).These approaches may solve the problem of biotic and abiotic constraints, and land/soil-related constraints faced by the respondents.

Constraints related to extension and market could be curtailed through modern technology upskilling on the use of online platformsfor an efficient exchange of farm information and research knowledge while accessing market information and staying connected with buyers around the world. The farmers may also be motivated to form Farmers Producers Organization (FPO) among Naga king chilli farmers to increase sales and profits, eliminate exploitation by middlemen and acquire better price realization. Besides the sale of fresh and dried chillies, there is a huge scope for value addition of Naga king chilli.However, unfortunately, irrespective of much biological and commercial strength of the crop, the post-harvest handling and processing is still in the infancy stage. Therefore, training on post-harvest management activities like grading, processing, packaging and storage of Naga king chilli is essential. This will protect the farmers from post-harvest losses ultimately add value to their produce and improve economic stability among the farmers.

With no insurance or security against financial losses during crop failures, the farmers usually settle for sporadic intercrop of Naga king chilli in the jhum fields. These constraints may be averted through a comprehensive approach and community involvement by educating and engaging farmers, youths and village leaders on the available institutional credits and crop insurance schemes.These initiativesmay come as an essential tool in developing confidence among the farmers to take up sustainable cultivation of Naga king chilli on a commercial scale whilst increasing the production and economic status of the farmers.

In conclusion, epidemics of pests and diseases (70.34), lack of knowledge about insect pest and disease management (66.85), lack of technical guidance from extension staff (63.30), lack of proper storage facilities (61.46), high requirement of manure and fertilizer (62.26), poor access to market information (66.15), high cost of inputs (60.56), no institutional support for commercial Naga king chilli cultivation (60.86), steep and undulated land (62.18)were the most serious constraints perceived by the farmers in adopting sustainable Naga king chilli cultivation practices in the study area. Addressing the varied challenges of Naga king chilli farmers requires a holistic and collaborative approach. The threats of global climate change causing outbreaks of pests and diseases could be minimized to some extent by adopting Integrated Pest Management (IPM) strategies along with the use of biopesticides.Equipping farmers on livestock waste management and composting with available organic matter from the field may resolve the problem of manures and fertilizers. Moreover, educating farmers to take advantage of Information and

Communication Technology (ICT) to access vital production and market-related information. In addition, the provision of cold storage facilities at the village level might encourage the farmers to adopt sustainable Naga king chilli cultivation practices for increased production and productivity. Thus, a collaborative effort of policymakers, research organizations and other stakeholders could be instrumental in formulating and implementing adaptation strategies that are production and conservation-oriented.

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