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Preface

We would like to present, with great pleasure, the inaugural volume-6, Issue-6, June 2020, of a scholarly journal, *International Journal of Environmental & Agriculture Research*. This journal is part of the AD Publications series *in the field of Environmental & Agriculture Research Development*, and is devoted to the gamut of Environmental & Agriculture issues, from theoretical aspects to application-dependent studies and the validation of emerging technologies.

This journal was envisioned and founded to represent the growing needs of Environmental & Agriculture as an emerging and increasingly vital field, now widely recognized as an integral part of scientific and technical investigations. Its mission is to become a voice of the Environmental & Agriculture community, addressing researchers and practitioners in below areas

Environmental Research:

Environmental science and regulation, Ecotoxicology, Environmental health issues, Atmosphere and climate, Terrestric ecosystems, Aquatic ecosystems, Energy and environment, Marine research, Biodiversity, Pharmaceuticals in the environment, Genetically modified organisms, Biotechnology, Risk assessment, Environment society, Agricultural engineering, Animal science, Agronomy, including plant science, theoretical production ecology, horticulture, plant, breeding, plant fertilization, soil science and all field related to Environmental Research.

Agriculture Research:

Agriculture, Biological engineering, including genetic engineering, microbiology, Environmental impacts of agriculture, forestry, Food science, Husbandry, Irrigation and water management, Land use, Waste management and all fields related to Agriculture.

Each article in this issue provides an example of a concrete industrial application or a case study of the presented methodology to amplify the impact of the contribution. We are very thankful to everybody within that community who supported the idea of creating a new Research with *IJOEAR*. We are certain that this issue will be followed by many others, reporting new developments in the Environment and Agriculture Research Science field. This issue would not have been possible without the great support of the Reviewer, Editorial Board members and also with our Advisory Board Members, and we would like to express our sincere thanks to all of them. We would also like to express our gratitude to the editorial staff of AD Publications, who supported us at every stage of the project. It is our hope that this fine collection of articles will be a valuable resource for *IJOEAR* readers and will stimulate further research into the vibrant area of Environmental & Agriculture Research.

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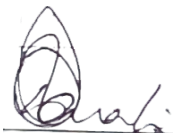

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Table of Contents

S.No	Title	Page No.
1	**Mapping of Milk Processing Units in Organized Sector: A Case Study for Haryana** **Authors:** Sukriti Sharma, Dr. D.K. Sharma **DOI:** https://dx.doi.org/10.5281/zenodo.3923263 **Digital Identification Number:** IJOEAR-JUN-2020-1	**01-06**
2	**Effect of Percent and Stage of Leaf Defoliation on the Quality of Sugarcane, at Arjo - Dedessa Sugar Factory, in Western Ethiopia** **Authors:** Kasahun Tariku **DOI:** https://dx.doi.org/10.5281/zenodo.3923267 **Digital Identification Number:** IJOEAR-JUN-2020-2	**07-13**
3	**Economic Analysis of Fluted Pumpkin (Telfaria Occidentalis) Production in Ibadan Metropolis, Oyo State, Nigeria** **Authors:** Akanni-John, R; Shaib-Rahim, H.O; Eniola O; Elesho R.O **DOI:** https://dx.doi.org/10.5281/zenodo.3931168 **Digital Identification Number:** IJOEAR-JUN-2020-3	**14-17**
4	**Isolation and Identification of Mycoplasma Species in Dogs** **Authors:** Maria Lucia Barreto, Mosar Lemos, Jenif Braga de Souza, Samara Gomes de Brito, Ana Beatriz Pinheiro Alves, Leandro dos Santos Machado, Virgínia Léo de Almeida Pereira, Nathalie Costa da Cunha, Elmiro Rosendo do Nascimento **DOI:** https://dx.doi.org/10.5281/zenodo.3931170 **Digital Identification Number:** IJOEAR-JUN-2020-4	**18-23**
5	**Effect of Genotype by Environment Interaction (GEI), Correlation, and GGE Biplot analysis for high concentration of grain Iron and Zinc biofortified lentils and their agronomic traits in multi-environment domains of Nepal** **Authors:** Rajendra Darai, Krishna Hari Dhakal, Ashutosh Sarker, Madhav Prasad Pandey, Shiv Kumar Agrawal, Surya Kant Ghimire, Dhruba Bahadur Thapa, Jang Bahadur Prasad, Rabendra Prasad Sah, Keshav Pokhrel **DOI:** https://dx.doi.org/10.5281/zenodo.3931172 **Digital Identification Number:** IJOEAR-JUN-2020-5	**24-40**

Mapping of Milk Processing Units in Organized Sector: A Case Study for Haryana

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Abstract— *The State Haryana is known for its major crops like wheat and rice and stands at the second largest contributor of food grains in India. Just like that Haryana ranks second in milk production. Dairy farming is also a form of agriculture in which milk is extracted from cow, buffalo, goat etc. then it sell by vendors from different rural and suburb regions to informal sector agents or to cooperative agents. This milk distributed further in different ways. Milk production is no more subsistence in nature and organized sector is a best example to prove it because cooperatives is an independent association of persons those fulfill their economic needs and distribution of milk and milk products is all a business as we can see it in "Haryana Dairy Development Cooperative Federation Ltd." This federation is famous by vita brand which was opened by the Haryana govt. on the pattern of Amul.*

Keywords— Dairy, Federations, Informal sector, Milk Production, Organized sector.

I. INTRODUCTION

Dairy is an agricultural industry in which milk alone valued more than combined value of wheat and rice. It covers about 1/3rd of gross income of rural households. Haryana, in spite of being a small state with only 1.3 % of total geographical area has a prominent space in the livestock map of the country. Haryana contributes 98.09 lakh tones milk per year which is more than 5.6% of the nation's total milk production. Before 1970s, the condition of dairy farming was not as appreciable as it is now because there is so much miscoordinance between rural milk producers and organized milk sectors. So this kind of poor connectivity creates problems like- poor rural milk producers didn't know the real price of their milk and on the other side, organized milk plants were deprived of from the valuable milk of rural area. But as we can say nothing is impossible, so on 13th January 1970, the "*White Revolution"* was started by Indian government with the objective to push the limits of dairy farming and to make it more valuable and economically more productive. This idea of "*Operation Flood*" was come through the success of "*Green Revolution*" in India. This revolution made dairy farming, a single self – sustaining industry in India. Over the span of three decades, India has transformed from a country of acute milk shortage to the world's leading milk producer. India is the largest producer of milk in the world since 1998. During 2016-17, the annual output was 165.40 million tons accounting for 20% of the world milk share. The per capita availability of milk during 2016-17 was 352 gm per day as against world average of 299 gm per day. After that, Haryana ranks second in country with availability of 877 grm. of milk per person today.

II. OBJECTIVES OF THE STUDY

1) To understand the problem of organized sector in dairy farming according to their demand and prize in Haryana State.
2) To mapping the formal sectors of milk production in Haryana.
3) To generalize the changes in milk production after white revolution.

III. DATA AND METHODOLOGY

The data has been collected for the study of organized sector of dairy farming and their problems in Haryana at local and state level. The required data was collected from secondary sources are- the websites looked into in order to gather the prior information and the related literature about the topic. This information is descriptive and analytical in nature.

IV. ROLE OF THE WHITE REVOLUTION AND HOW IT BECOMES BENEFICIAL FOR HARYANA'S MILK PRODUCTION

The father of White Revolution is Verghese kurrin who firstly introduced this concept and suggests ideas in development in the production of milk on its top level in India.

Haryana stands on second rank in the country as per capita per day availability of milk. Haryana is known for his commendable development in dairy industry and called as a milk pail of India. As we can see in the table given below that from the year 1966 to 2016, the production is increased chronically. As we discussed in the introduction that during 1970 when the revolution came in India, the production is hiked up tremendously in Haryana from 10.89 lakh tonnes to 17.27 lakh tones.

TABLE 1
YEAR-WISE MILK PRODUCTION IN HARYANA STATE (LAC TONNES)

Year	Milk Production
1966-67	10.89
1977-78	17.27
1981-82	22.75
1991-92	35.65
2000-01	48.49
2005-06	52.59
2006-07	53.67
2007-08	54.51
2008-09	57.45
2009-10	60.06
2010-11	62.67
2011-12	66.61
2012-13	70.40
2013-14	74.42
2014-15	79.01
2015-16	83.81

Source –Animal and dairying dept. of Haryana

There are 60.8 lakh buffaloes are in Haryana, in which "Murrah" buffalo is the most famous because of its buffer milk production. There are four types of cows in State like Exotic – (those are imported from foreign), Indigenous – (autochthonic, originating where it is found), Crossbreed – (Hybrid), Non- Descript – (ordinary) and two of buffalos (Indigenous and Non- Descript). Murrah is an autochthonic. In Table-2, trying to show the distribution of milk production through cows, buffalo and goats in different districts of Haryana with the help of survey which was done by Animal Husbandry and Dairying Department of Haryana in year 2017- 2018. Through this table I identified that Sirsa with 213.51('000' tonnes) cow's milk production, which is the highest from all other districts of the state. As given data of buffalo's milk production in which the state Bhiwani is leading with 668.36('000' tonnes) production and in goat's milk, Mahendragarh is the district which leads over other districts. But if talking about the total annual milk production in state then Bhiwani has won the race with 802.84 ('000' tonnes) production. The state's total milk production is about 9808.94('000' tonnes) which is second largest production after Uttar Pradesh in India. This is how it proved that how white revolution left a deep impression on state.

TABLE 2
DISTRICT WISE MILK PRODUCTION (IN '000' TONNES) IN HARYANA STATE 2017- 2018

Sr. no.	Districts	Total Cow's Milk Production	Total Buffalo's Milk Production	Total Goat's Milk Production	Annual Milk Production
1	Ambala	83.66	306.76	1.15	391.57
2	Bhiwani	127.66	668.36	6.81	802.84
3	Faridabad	45.74	189.84	1.38	236.96
4	Fatehabad	71.13	403.85	1.72	476.69
5	Gurugram	72.00	239.07	1.55	312.69
6	Hisar	94.61	634.12	2.45	731.18
7	Jhajjar	53.12	349.45	1.22	403.78
8	Jind	65.33	575.92	1.24	642.49
9	Kaithal	73.64	575.72	1.00	650.37
10	Karnal	188.34	445.19	1.39	634.93
11	Kurukshetra	119.44	282.04	0.54	402.02
12	Mahendragarh	60.04	350.72	7.82	418.58
13	Nuh	38.38	300.09	4.61	343.08
14	Palwal	43.67	387.24	1.18	432.09
15	Panchkula	22.18	105.91	1.45	129.54
16	Panipat	61.80	307.21	0,65	369.65
17	Rewari	62.13	295.24	3.77	361.13
18	Rohtak	45.29	347.56	0.80	393.65
19	Sirsa	213.51	423.55	5.48	642.53
20	Sonepat	102.71	476.70	1.25	580.65
21	Yamunanagar	176.10	275.19	1.32	452.60
22	Charkhi Dadri	-	-	-	-
State Total		1820.46	7939.71	48.77	9808.94

Source- Animal Husbandry and Dairying Department of Haryana (Sample Survey Report 2017 – 2018)

V. WHY HARYANA'S DAIRY SECTOR HAS FOR LONG BEEN UNORGANIZED

With liberalization of dairy industry, all kind of sectors got the opportunity to participate in different fields of dairy products. Most of the private sectors dominantly established their presence in milk distribution. One of the main reasons of their dominance is increasing population on a vast scale. Mostly the population of Haryana is rural and believed in old traditions, so people still are in favour of unpacked and unprocessed milk through their believe ones or local milk distributors "dudiya" because they believe it's good, fresh and more nutritious than packaged milk. More of the people who sell milk in rural areas would prefer to sell it in limited area of their periphery and those who want to sell it outside in urban areas too then they contact informal sector's agents who pander loose milk from dairy farmers and sell it in urban peripheries directly to consumers at their own fixed rates. It's a kind of procure with consumers and dairy farmers but this problem can't be solved unless the organized or formal sector didn't give their efforts to empower on informal sector and to standardized the equal rates everywhere.

TABLE 3
FLOW OF MILK THROUGH DIFFERENT CHANNELS

Share of marketable surplus	% of production	Total production (million tonnes)	Use
	100%	100	
	45%	45	Home consumption
	55%	55	Marketable surplus sold in urban and rural markets(informal and formal)
34.5%	19%	19	Sold in urban markets as loose unpackaged milk
40%	22%	22	Sold as processed products through informal markets
14.5%	8%	8	Sold as packaged milk through formal markets.
12.7%	7%	7	Sold as packaged milk products through formal markets

Source- India: Increasing demand challenges in the dairy sector by meena Punjabi

VI. THINGS THAT ORGANIZED SECTOR SHOULD PERFORM FOR BETTER RESULTS:

1) Quality or guarantee of freshness in products are the big issues in informal sector so if formal or organized sector wants to compete then they should take care of their quality of products for better results.
2) Organized milk producing agencies must enhance their interaction with small farmers and rural dairy vendors to earn their trust.
3) Area like Haryana where milk production is not even in all the districts so the formal sectors should engage their agents or managers to collect the ground reality data of dairy farms and to convey the milk sellers by giving them better packages more than the Informal's.
4) Raise packaged milk distribution in more areas.
5) Make policies that attract farmers to get a higher price for milk.
6) Make farmers more secure and give them strength in enhancing their production by animal insurances.
7) Prices should be same everywhere on the bases of amount of fat in milk.
8) Encourage commercial dairy farming and breed development.

VII. MAPPING OF ORGANIZED SECTOR OF MILK DAIRIES IN HARYANA

The most famous breed of buffalo named "Murrah" in Haryana mainly kept for milk production and it treated as a backbone of the state in dairy farming. Dairy farming in Haryana is now no longer just for illiterates and unemployed humans, many educated and private companies shown their interest and adopted it with the modern technology to maximize profit. After the establishment of Amul in Gujarat, Haryana's govt. also started a concept of organized milk plant in state, so on 1st April, 1977 "Haryana Dairy Development Cooperative Federation Ltd."was established by Haryana govt. for optimistic results and to encourage economic interests in producing milk. The products of this cooperative federation are recognized by the brand "vita". There are six plants of vita in Haryana districts named as- Jind, Ambala, Rohtak, Ballabgarh, Sirsa, and Kurukshetra. All of these plants are worked in three tiers.

Tier 1	Tier 2	Tier 3
Village level societies of milk production	District level cooperative unions	Milk federation at the state level as apex body

	Districts :	Products :
Vita milk plants	Jind	Liquid milk, ghee, paneer, mango drink etc.
	Ambala	Liquid milk, paneer, dahi, lassi, milk cake etc.
	Rohtak	Liquid milk, ghee, butter, paneer etc.
	Ballabgarh	Dahi, paneer, liquid milk etc.
	Sirsa	Paneer, kaju pinni, dahi, liquid milk etc.
	Kurukshetra	Liquid milk

There are six unions on district basis those carried out five important functions in dairy farming sector are – procurance, advertise or marketing of milk products involves (local and sample milk sale, dispatch of milk to milk union, payment etc.), processing and providing technical inputs (standardization of testing equipment and chemicals), institutional strengthening of milk cooperatives, enhancing women involvement in dairy cooperatives.

TABLE 4
NAME OF UNIONS AND RESPECTIVE YEAR OF REGISTRATION

Name of the unions	Year of registration
The Ambala district cooperative milk producer union limited Ambala	1973
The Rohtak district cooperative milk producer union limited Rohtak	2003
The Hisar – Jind district cooperative milk producer union limited Jind	1991
The Kurukshetra – Karnal district cooperative milk producer union limited Kurukshetra	1991
The Sirsa district cooperative milk producer union limited Sirsa	1978
The Ballabgarh district cooperative milk producer union limited Ballabgarh	2003

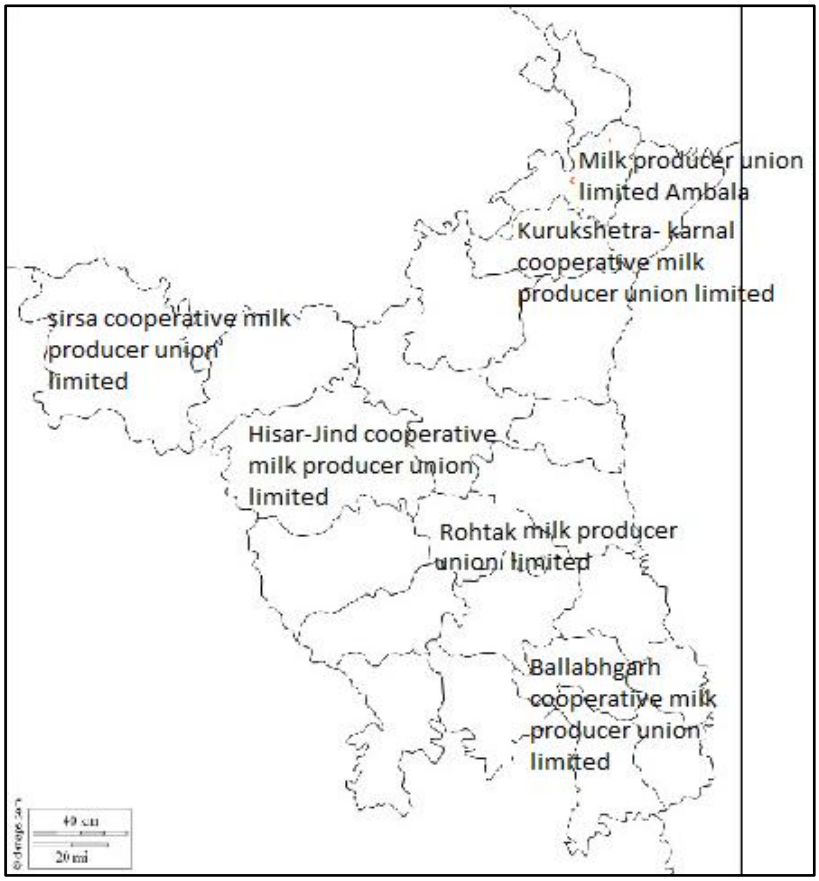

FIG. 1 - Milk unions of Haryana

VIII. CONCLUSION

Milk is a complete staple food in our life and dairy farming is a class of agriculture for long term production of milk, which is processed and sale as a dairy product. So this paper represents the dairy farming as an agriculture form in which milk extracted from animals like – cows, buffalo, goat etc. and then this milk processed into various products like – ghee, butter, cheese, curd and many more. So these processing are done in many ways because it's depend on our demand that which method we prefer. There are two kinds of milk processing units- organized and unorganized, the basic difference is that organized sector is of cooperatives and unorganized or informal sector's agents collect loose milk from rural vendors and sell them with quick access, and this is the main reason that's why our 70% of the population in state takes milk from informal sectors. There are six unions of milk federation in Haryana those are working but not as a team, so there is a highlight through this paper for formal sectors that they should improve their working skills and make efforts to attract public from loose milk to packaged milk with guarantee of freshness and nutrients. Vita federation was leased out the plants to the milk unions in six districts of Haryana those are not enough because demand is likely to grow in future years and government must unlock some schemes for dairy farmers to attract their interest by giving them appropriate price of their product.

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Effect of Percent and Stage of Leaf Defoliation on the Quality of Sugarcane, at Arjo - Dedessa Sugar Factory, in Western Ethiopia

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Abstract— *The research was conducted at Arjo-Didessa Sugar Factory which is located in East Wollega Zone of Oromia Regional State with the objective to determine the effect of leaf defoliation at different stages of sugarcane (Saccharum officinarum) on the quality. Sugarcane (Saccharum spp.) is unusual among field crops in that it is not the seed that have economic values, but rather the stalk. Sucrose is extracted from the large stalks that are produced by sugarcane plants. The effect of percent and stage of leaf defoliation on sucrose content as well as recovery percentage of sugar cane is still unknown. Effect of leaf defoliation at three different stages on quality of sugarcane juice was studied under field conditions. The methodology used include seven percent of leaf defoliation comprises of 10%, 20%, 30%, 40%, 50%, 60% and 0% as a check and three growth stages of defoliation at 9, 10 & 11 month of sugarcane was arranged in Randomized Complete Block Design. The results depicted that, significant variation among leaf removal and cane age was noted for quality parameters. Thus, significantly higher percent of sucrose percent (11.70%) at 30% of leaf removal and (11.50%) at20% of leaf removal were obtained from 11 and 10 month age of NCO-334 sugarcane variety respectively ,however, lower percent of sucrose (9.03%) at 11month age was recorded from undefoliated (check).In addition to these results the sugar cane plants that could be partially defoliated with changing sucrose production and retention of defoliated leave between furrow providing advantage, that increase soil moisture leads to water conservation especially for sugarcane grown under rainfall condition like Arjo Dedessa sugar factory, reduce weed growth, and prevent substantial losses of C and N due to sugarcane leaf burning at harvesting time. However, further future research is required to strengthen the investigation by confirming similar research on different location are necessary to recommend to all Ethiopian sugar factories*.

Keywords— sugarcane, defoliation, stage, quality.

I. INTRODUCTION

Sugarcane is one of the most important crops in the world (Dagar *et al*., 2002). Sugarcane belongs to the genus (*Saccharum* L.,) of the grass family (Poaceae) and originated in Papua New Guinea, as original habitat and from where it spread to south East Asia and India in the course of few thousand years (Bull, 2000).

The Office of Agricultural Economy, (2008) reported that the sugarcane burned in the field had many disadvantages such as weight reduction, microorganism destroyed easily, rapid decrease of sweetness, high production cost of plant, that organic material and structure in soil were destroyed and decreased sugar production. Sugarcane harvesting is a critical step that must be managed to maintain good quality and quantity of sugarcane production. Farmers harvesting sugarcane have a leaves-removing or leave defoliating step and cut the stem closing to the soil, then cut the top of sugarcane stem. Leaves and leaf sheaths of sugarcane caused delay of harvesting. Moreover, the sugarcane crop that has not been fully leaves-removed (leave defoliations) could carry some soil, sand and mud, thus damaging the downstream sugarcane process machine and reduced sugar yield (Yangyeun and Wongpicheth, 2008).

The contamination will be increased more when using the car to grip sugarcane to the truck. Sugarcane leaf-defoliating tools could help to speed up sugarcane harvest and reduce contamination. However, researchers in the past had focused on tools or equipments used to help harvest sugarcane crop; for example, sugarcane harvester, knife used for sugarcane crop on performance to sugarcane harvester. However, leaf-removal machinery can solve the problems of sugarcane burning and reduce contaminants. Retention of unburned residues can increase nutrient conservation, reduce weed growth, and conserve soil moisture on the other hand substantial losses of C and N due to sugarcane residue burning have been reported (Viator *et al*., 2006).

In general physiological and morphological responses of individual plants to defoliation was evaluated in chronological sequence beginning with plant function during "steady state" growth prior to defoliation, followed by the short-term effects of defoliation, and concluding with long-term processes contributing to the reestablishment of "steady-state" growth (Steingraeber *et al*., 1993). Particularly according to Gutierrez *et al* (2004), mechanical defoliation of sugar cane plants

(*Saccharum* spp.) will provide leaves that can be used as fodder but the effect of partial mechanical defoliation on sucrose content, enzyme activities and agronomic parameters of sugar cane is still unknown and also the concentration of sucrose in the stems of partial defoliated plants was significantly different from that found in intact plants. Similarly, Dendooven *et al* (2004) indicated that some agronomic parameters and enzyme activities were different in defoliated plants compared with intact plants except for the moisture content which was higher in defoliated plants than in intact ones. These makes sugar cane plants could be partially defoliated changing sucrose production and agronomic parameters while providing leaves that could be used as fodder.

The Ethiopian Government is building modern sugar factories and expanding the existing ones with the aim of maximizing the production volume to alleviate the scarcity of sugar in the country (EIA, 2008). This work was conducted in view of the limited information on the effect of leaf defoliation at different stages of sugarcane on biomass yield and quality but the hypotheses tested in these studies, the effect of leaf defoliation at different stages of sugarcane on biomass yield and quality were superior in defoliated than undefoliated sugarcane. This research was initiated with the objective of to evaluate the effect of defoliation at different stages on quality, and response of sugarcane crop to defoliation and its advantage to increase sugar recovery at Arjo-Didessa Sugar Factory which is located in East Wollega Zone of Oromia Regional State.

II. MATERIALS AND METHODS

2.1 Description of the Study Area

The experiment was conducted at Arjo-Didessa Sugar Factory located in East Wollega Zone of Oromia Regional State. Arjo Dedessa Sugar Factory is located at $09^{0}41^{m}48^{s}6^{ms}$ N latitude and $036^{0}26^{m}01^{s}9^{ms}$ E longitudes, with an altitude of 1053-1600 masl. The area has a mean maximum and minimum monthly temperature is 31.1°c and 19.1°c, respectively with annual rain fall of 358.4mm (Source: Arjo metrological station). Based on Arjo Dedessa feasibility study report (2005) the soil types of the experimental site are dominated by Vertisol and Luvisol.

2.2 Experimental Materials and Design

NCO -334 sugarcane varieties was used as an experimental material. Treatments comprising six levels of defoliation percent of 10 %, 20 %, 40 %, 50 %, 60 % and one control of 0 % of percent of defoliation at three different stages of sugarcane, that is, at 9 month growth stage (S_1), 10 month growth stage (S_2) and at 11 month growth stage (S_3) and each of which replicated three times. The percent of defoliation was made after counting total number of leaf from three randomly sampled and the leaf was defoliated according to percent of defoliation treatment. The two factors were combined factorially and arranged in randomized complete block design (RCBD). The actual experimental area was designed with PL = number of treatment x plot length + spacing between plot x number of block – 1i.e (7 X 5m + 2m x 3 -1= 40m) and PW = number of block x plot width + spacing between block + number of spacing (3 x 7.25m + 2m x2 =25.75m). The total area used was 40m x 25.75m ($1030m^2$). Plot width = 1.45 x 5 and plot length= 5m, the distance between block used were 2m, between plot were 1m and the sugarcane was spaced at 1.45m between rows.

2.3 Data Collection and Sampling

The data were collected on four quality parameters (%Brix, %Pol, % purity and cane recovery (sucrose %) attributes. The middle two rows out of the four rows in each plot were used for data collection, the number of plants per row was 1260 and the distance between rows were 1.45m. Brix % was measured in the cane juice analytical laboratory of Arjo Dedesa Sugar Factory with the help of bench refracto meter. The refracto meter was adjusted to zero with distilled water. A drop of juice was placed on the refracto meter then the brix was read (Blackburn, 1984)

The polarization of juice was measured by a Polari meter according to the method described by Horne's dry lead (South African Sugar Technologist's Association (1985). Approximately 150 ml of the sample was taken in a bottle provided with stopper. Sufficient lead sub acetate powder (1.5g) was added for clarification, shacked vigorously to disperse the lead sub-acetate completely and then allowed to stand and filtered through a fluted filter paper held in the funnel. Some of the filtrate was used for rinsing the Pol tube and filled completely. Then the polarization (Saccharimeter reading) was read. The Pol % obtained from Schmitz's table by using the Brix of the sample and Saccharimeter reading. Pol % is actually cane sugar present in the juice, expressed in percentage (Khedkar *et. al*. 2000).

There for Purity % was determined with the help of the following relationship following Islam et al., (2011).

$$Purity\ \% = \frac{(\text{Pol}\ \%)\ \text{x}\ 100}{\text{Brix}\ \%}$$

Sugar recovery was calculated with the help of the following formula following Islam et al., (2011).

Recoverable Sucrose (%) = [% Pol- (%brix-%Pol) 0.52] 0.75

Where: 0.52 = Non-sugar factor

0.75 = Cane factor

2.4 Data Analysis

The data collected were subjected to analysis of variance (ANOVA) using SAS software (SAS, 2004). Treatment means that exhibited significant differences were separated using the least significant difference at 5% level of significance (SAS, 2004).

III. RESULT AND DISCUSSION

The of analysis of variance result for different characters are presented in Table 1 design. The analysis of variance table for percent of defoliation showed a highly significance difference for all quality parameters while stage of defoliation showed significantly ($P< 0.05$) affect all the parameters except for sucrose %. However, their interaction effect showed that a significance difference for all characters except for purity percent (Table 1).

TABLE 1

MEAN SQUARE VALUES FOR THE PARAMETERS RECORDED AS AFFECTED BY PERCENT DEFOLIATION, STAGE OF DEFOLIATION, AND PERCENT BY STAGE OF DEFOLIATION INTERACTION OF SUGARCANE GROWN AT ARJO DEDESSA IN 2014/15 CROPPING SEASON.

Characters	Sources of Variation				
	PD	ST	PD X ST	MSE	REP
Internodes number	70.88**	3.19^{ns}	4.91*	3.41	5.28
Internodes length	11.83**	2.49^{ns}	3.45*	2.72	3.15
Stem diameter	292.86**	3.05^{ns}	12.89**	10.39	90.04
Internodes weight	113476.73**	2997.57^{ns}	12844**	7548.7	1.34
Sugarcane height	0.032**	0.0043^{ns}	0.061**	0.012	0.02
Leaf area index	61.14**	7.43**	3.75**	0.2	0.54
Biomass yield	14.97**	3.70**	1.08*	0.43	4.3
Number of leaf	3.75**	1.15*	1.17*	0.65	2.49
Brix %	16.82**	1.03*	2.03*	0.58	0.97
Polarity %	22.99*	4.58^{ns}	16.39*	14.42	17.63
Purity %	575.65**	533.36**	366.68^{ns}	378.58	553.1
Sucrose %.	3.78**	0.011^{ns}	0.25*	0.22	0.4

****, ** and ns indicate significance at the 0.05 and 0.01 probability levels and non-significance level, respectively. PD = Percent of defoliation, ST = Stage of growth, PD x ST = Interaction of percent of defoliation and stage of growth, MSE = Mean square error and REP = Replication***

3.1 Effect of Defoliation on Brix %

When 20 % of leaf removal at 11 month growth stage was applied significantly higher brix % was recorded as 22.70 % (Table 2). But the lowest brix % was recorded at undefoliated with nine month growth stage as18.42 % (Table 2). While defoliating from 60 % of leaf removal at nine month growth stage, 30 % of leaf removal at ten month growth stage and 50 % of leaf removal at11 month growth stage was not significantly different from 40 % of leaf removal at 11 month growth stage (Table 2). This shows that defoliation from 30 % – 60 % recorded indicates the positive in increasing the total soluble solid in the juice.

On average mean value of total soluble solid in juice (brix %) recorded for the three growths stage gave 20.80% (Table 2). From this experimental study brix the highest % was recorded as different stage and percent of defoliation significantly increases. In general in this study the total solids content present in the juice expressed in percentage were unaffected by defoliation but increases quality of sugar (Table 2).

TABLE 2
MEAN VALUES FOR BRIX %, POLARITY % AND SUCROSE % AS AFFECTED BY PERCENT DEFOLIATION AND STAGE OF DEFOLIATION INTERACTION OF SUGARCANE GROWN AT ARJO DEDESSA IN 2014/15 CROPPING SEASON

Parameter	Brix %			Polarity %		
DF %	Stage of defoliation			Stage of defoliation		
	9	10	11	9	10	11
0	18.42	21.9	19.02	21.15	19	21.83
10	19.34	19	19.28	20.5	20.5	21.44
20	19.27	18.9	22.7	21.47	20.9	20.3
30	21.6	22.2	21.59	21.36	30.6	20.43
40	21.93	20.7	22.13	19.77	19.1	19.35
50	21.24	21.9	22.34	19.72	19.8	19.64
60	22.01	21.9	19.6	19.92	19.6	20.44
Mean	20.8			20.8		
CV %	3.67			18.23		
LSD 0.05	0.47			0.29		

, ** and ns indicate significance at the 0.05 and 0.01 probability levels.

Studies on the quality parameters of cane stalk, juice and leaves in comparison to defoliation have been conducted in a number of cane growing regions. Results from Louisiana (Birkett, 1965), South Africa (Muir et al., 2009; Reid & Lionnet, 1989; Scott et al., 1978) and Australia (Ivin & Doyle, 1989) shows that the presence of reasonable amounts of brix and fiber in the defoliated cane juice than undefoliated.

The results suggest that the stage of defoliation induces significant changes in sugarcane juice brix % composition and its sensory attributes. The effect of late leaf removal was much more effective than early leaf removal in affecting final brix composition and quality. Brix % from the late defoliation treatment was rated the most preferred as of global value. It has also found that in grape the removal of some of the leaf material from the canopy whilst berry ripening occurs can increase Brix in the fruit (Holzapfel and Rogiers, 2002). It is suggested that it was due to the increased photosynthetic rate of the remaining un-defoliated leave. Conversely (Ezahounani and Williams, 2003) leave defoliation of basal has shown to have no effect on Brix.

3.2 Effect of Defoliation on Polarization (Pol %)

The mean value recorded for polarity percent in table 2, was 20.80 % at overall growth stage. The highest polarity percent was recorded from 30 % of leaf removal at ten month growth stage (Table 2). However, the lowest polarity percent was recorded from 0 % of leaf removal at10 month of growth stage (Table 2). This shows that defoliation at 30 % was more beneficial than at 0 % to increase the actual sugar in the juice. Although defoliation affected by 0 %, 20 % and 30 % of leaf removal at 9 month growth stage was not significantly different from 10 % of leaf removal at 11 month of growth stage (Table 2). This indicates defoliation percent was more beneficial than growth stage in this study.

From the result obtained even if differences exist among defoliation treatment after defoliation polarity percent was increased which have the advantage of having good quality of the actual cane sugar present in the juice. This is due to the increase in the photosynthetic potential of the remaining leaves and leads to enhanced Pol % resulted in biomass accumulation and sucrose yield. Khan and Ahsan (2000) working on *B. juncea* showed that eliminating the cost of maintaining senescing leaves by leaf removal may lead to increased plant yield. The Pol for the second stage of sugarcane sample meets maximum Pol 99.9oZ set for sugar yield (USC, 2008) and was significantly (p>0.05) higher than for the first and third stage of sugarcane development. Polarization for both defoliated and un-defoliated samples was measured by automatic Polari-meter calibrated in (ISS).

3.3 Effect of Defoliation on Purity %

On average the mean value of purity percent recorded was 91.1 % at all growth stage (Table 3). The highest purity percent (96.36 %) was recorded from 9 month growth stage but the lowest (86.31 %) purity percent was recorded at 11 month growth stage (Table 3); this shows that a higher purity indicates the presence of higher sucrose content out of the total solids. The purity of sugar cane process stream products (*e.g.*, cane juice, molasses, raw sugar *etc.*) is a measure of product quality and was determined by calculating the ratio of % Sucrose and % total Solids as a percentage which were measured by double polarization and dry substance measurements.

TABLE 3
MEAN VALUE ANALYSIS FOR PURITY % AS AFFECTED STAGE DEFOLIATION EFFECT OF SUGARCANE GROWN AT ARJO - DEDESSA IN 2014/ 2015 CROPPING SEASON

Stage of defoliation		% purity	% Defoliation	% purity
9 Month		96.36[a]	0	89.10[b]
10 Month		90.63[a]	10	91.72[b]
11 Month		86.31[b]	20	91.72[b]
Mean	91.1		30	94.96[a]
CV	21.35		40	86.30[b]
LSD	12.13		50	92.40[ab]
			60	91.60[b]
		Mean	91.1	
		CV	21.35	
		LSD	18.53	

Mean within columns followed by the same letters are not significantly different

3.4 Effect of Defoliation on Sucrose Percent

The analysis of variance for sucrose % showed that a highly significant effect for percent of defoliation and the interaction effect, however, stage of defoliation had no significant effect on the sugar recovery (Table 1). On average the different factor combinations gave 10.52 % of sucrose %. The highest sucrose percent (11.70 %) was recorded when 11 month growth stages were affected by 30 % of leaf removal and this followed by 20 % of leaf removal at 10 month growth stage. However, relatively the lower sugar recovery (9.03 %) was recorded from undefoliated control 0 % of leaf removal at 9 month growth stage (Figure 1). Hence the interaction effect of defoliation and stage more beneficiary than both of the main factor.

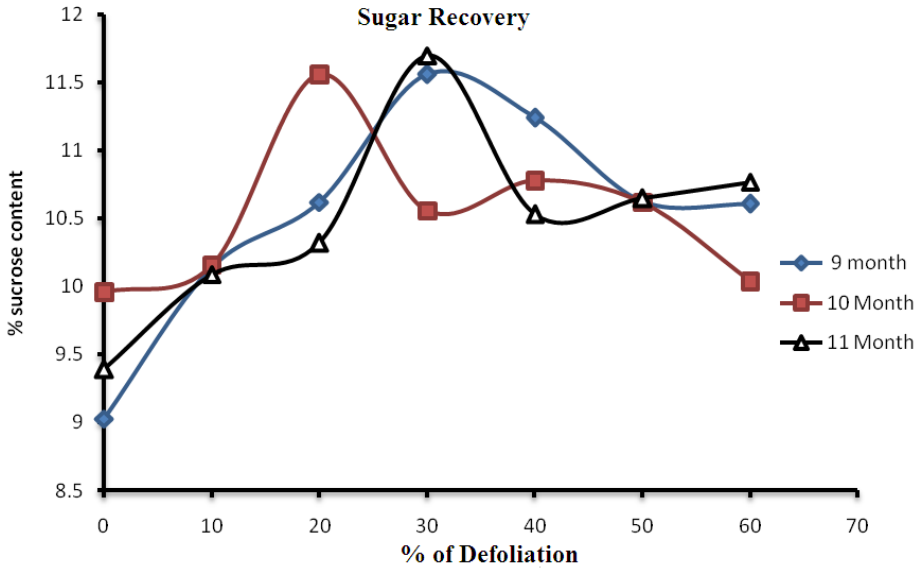

FIGURE 1: Sucrose concentration % in juice of sugarcane as affected by different stage of defoliation and percent leaf defoliation interaction grown at Arjo Dedessa in 2014/15 cropping season

Therefore results obtained after affecting the different growth stage by defoliation treatment significantly increases quality of sugar recovery within the juice used to increase the actual cane sugar. Results obtained indicated that the interaction effect was more beneficial than defoliations and stage for improving cane biomass yield and sugar recovery and also defoliated leaves could be used as animal fodder after 9month of crop age without affecting sugarcane yield.

The sucrose concentration in defoliated sugarcane was significantly different from the un-defoliated sugarcane and there were also significance differences between treatments of defoliations (Figure 1). This agreed with A study by Pammenter and Allison (2002) has shown that defoliation of alternate fully emerged lamina of sugarcane (*Saccharum officinarum* L.) at 155 d decreased the laminas area by 40% and also resulted in proportional partitioning of assimilate in leaves and stem with increased accumulation of sucrose.

Other studies also reported that Partial defoliation has resulted in increases of sucrose contents in Cherry trees (*Prunus Cereasus*) (Layne and Flore 1995), but (Neefs et al., 2002) reported that mechanical partial defoliation of witloof chicory (Cichorium intybus) affected plant growth. They found that the total fresh weight of defoliated plants stayed markedly lower compared with intact plants. Integrating production of sugar and using the leaves as fodder will be economically (Naseeven 1986, Namer 1991) and ecologically beneficial. Studies on *B. juncea* have shown that removal of shaded leaves (50% of lower leaves at 40 DAS) increases the supply of assimilate more than demand and thus improves growth and photosynthetic potential of the rest of the leaves (Khan *et al.,* 2002b). Anten and Ackerly (2001) reported that partial defoliation (50 and 66% of leaves removed) in palm (*Chamaedorea elegans* Mart.) significantly increased the light available to the remaining leaves and light-saturated photosynthesis per unit leaf area by 10–18%. Recently, (Li *et al*., 2010) reported that defoliation at flowering in chickpea (*Cicer arietinum* L.) when crop canopy is closed allows light penetration into deeper canopy and improves photosynthesis.

Partial defoliation has rejuvenating (make younger) effect on the remaining leaves, restoring the photosynthetic capacity to near the value of newly formed leaves (Wareing *et al.,* 1968, Khan *et al.,* 2008). The plants after defoliation require more assimilates for re growth which is balanced by the increased leaf assimilatory capacity and efficient N use (Lone and Khan 2007).

Ryle et al., (1985) reported that recovery of N2 fixation in *T. repens* after removal of half of the shoot tissue was related to the reestablishment and increased photosynthetic capacity after 5, 6, or 9 d of re growth. C4 plants accumulate greater quantities of carbohydrate than C3 plants (Downton 1971). This 'extra' photosynthetic could be used for re growth following defoliation. An advantage of the C4 system is that its net photosynthesis, compared to C3 plants, is greatest in young foliage (*Long et al., 2006*). Thus, the new foliage produced in response to defoliation would replenish the carbohydrate reserves (sucrose) more rapidly in C4 species than in C3 species.

IV. CONCLUSION

In light of the results obtained, the different levels and stages of defoliation have a significant effect on all the parameters studied .Partial defoliation in sugarcane (i.e. removal of half leaves) has been shown to not have a long-term negative effect on the quality parameters. Generally the results obtained in this study are based on data of superimpose experiment and, hence do not warrant the formulation of a clear-cut recommendation, However, suggestive enough to draw the following recommendations:

- When defoliation was applied on 30 % of leaf removal at 11 month growth stage in relative to other percent of defoliation and stage higher recovery percentage was recorded. On the other hand trash or leave without defoliating that is delivered with the stalks to the factory could also reduce the quality of sugarcane juice. However, further study is required to support some leaves defoliated in the field should be utilized as a soil fertilizer there is still plenty available for use as biomass; retention of unburned leave can increase nutrient conservation, reduce weed growth, conserve soil moisture and also defoliated leaves could be used as animal fodder after 9month of crop age without affecting sugarcane yield.
- Defoliation could also be used to renew, refresh and increase growth and photosynthetic rate in sugarcane plants under abiotic stress conditions. However, further research is required to strengthen the investigation and repeating similar research on different location are necessary to recommend to all Ethiopian sugar factories.

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Economic Analysis of Fluted Pumpkin (*Telfaria Occidentalis*) Production in Ibadan Metropolis, Oyo State, Nigeria

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Abstract— *The study was carried out to analyze the economics of fluted pumpkin production in Ibadan metropolis. A total of 80 fluted pumpkin farmers were selected using multistage sampling method. Data were collected using a set of questionnaire. Analysis of the data obtained from the questionnaire was carried out through the use of descriptive statistics such as frequency, percentage, profit function analysis, gross margin and multiple regression analysis. From the analysis, all the farmers interviewed were literate. From the gross margin analysis, fluted pumpkin production was found to be a profitable venture in the study area. The profit function analysis result of R^2 (0.8910) showed that 89.01 percent of the variability in profit in explained by the combined effect of the variable price items in the function. This is indicative of the price variable for output price had a positive significant effect on the profit level of farmers. The regression result showed that the coefficient for farming experience was positive and significant at 5 percent level. Recommendations from the study area include among others, government should provide inputs such as chemicals and planting seed at subsidized rate to farmers and also aim at solving the problems in vegetables production*.

Keywords— Gross margin, Profit function, Production, Fluted Pumpkin, Chi-squre.

I. INTRODUCTION

Agricultural production in Nigeria is dominated by small-scale farmers who produce the bulk of the food consumed in the country. One of the major crops produced are fluted pumpkin which represent an essential part of agricultural products. Their production remains entrenched in Nigerian agriculture and forms an important condiment in the national diet (Nwangwa *et al.*, 2007). Agriculture is considered the largest sector in Nigeria's economy. It employs 70 percent of the nation's labor force, contributes at least 40 percent of the gross domestic product and accounts for over three-quarters of the non-oil foreign exchange earnings (Ajekigbe, 2007). Fluted pumpkin is a very important vegetable that is popular in West Africa. It belongs to the family Telfaria Occidentalis Hook F. cucurbitaceae. It is a leafy vegetable that produces fruits. (Enabulele and Uavbarhe, 2001). Tindall, (1989) defined leafy vegetables as herbaceous plants used for culinary purposes. They are used to increase the dietary quality of soups. The fruit on full maturity has a weight of 10kg. and an appearance of 10 distinctive longitudinal ribs on the surface. It is popular in West Africa. The edible part of this vegetable are the large red seeds, leaves and young shoots used for traditional soup. Protein rich seed can be roasted or grounded for use in porridge. The flesh of the fruit has good oil content which can be used as cooking oil.

Amongst the different vegetable foods, production and consumption of fluted pumpkin is very important because of their contribution to good health by providing inexpensive sources of minerals and vitamins needed to supplement people's diet which are mainly carbohydrates (Yang *et al*., 2002) cited in Abu and Asember (2011). Fluted pumpkin is the most important and extensively cultivated food and income generating crops in many parts of Africa (Adebisi-Adelani *et al*., 2011).Fluted pumpkin is a very important vegetable that is popular in West Africa. It belongs to the family *Telfaria Occidentalis Hook F. cucurbitaceae* . It is a leafy vegetable that produces fruits. (Enabulele and Uavbarhe, 2001).

Fluted pumpkin consumption has improved over the years and it is an important component of the daily diets of Nigerians (Okoli and Mgbeogu, 2003). Due its hypolipidemic action, it lowers blood cholesterol and thus protects from a large range of associated complications like cardiac problems, hypertension and diabetes (Margret, 2011).

II. MATERIAL AND METHOD

2.1 Area of Study

Ibadan, the capital of Oyo State is the third largest city in Nigeria by population (after Lagos and Kano), and the largest in geographical area. At independence, Ibadan was the largest and the most populous city in Nigeria and the third in Africa after Cairo and Johannesburg. The city of Ibadan is located approximately on longitude 3°55East of the Greenwich Meridian and latitude 7°23North of the Equator at a distance some 145 kilometers Northeast of Lagos. Ibadan is located in southwestern Nigeria about 120 km east of the border with the Republic of Benin in the forest zone close to the boundary between the

forest and the savanna. There are eleven local governments in Ibadan metropolitan area consisting of five urban local governments in the city and six semi-urban local governments in the fewer cities. The five urban local governments are: North East, North Ibadan, Northwest Ibadan, Southeast Ibadan, and Southwest Ibadan. Urban cores (high-density) and hinterlands (low-density) characterized Ibadan metropolis. The population of Ibadan metropolis is 2, 550,593 according to 2006 census. However, its population at 2016 is estimated to be 3.16 million. The general land use pattern of the Ibadan metropolitan area shows a clear distinction purely residential use. According to Ayeni (1994) residential land use is the most predominant among all land uses in the built up part of Ibadan. The administrative and commercial importance of Ibadan has resulted in land being a key investment, an asset and a status symbol for the population.

2.2 Sampling Techniques

Multi-stage sampling procedure was use to sample the respondent for proper data collection during the field survey as stated below. 1st stage: identification of fluted pumpkin farmers use in the study area of many marketers in the market. 2nd stage: the farmers of fluted pumpkin production in the market was sample for proper data collection. 3rd stage: 80 copies well structure questionnaire was randomly distributed to the respondent and allow them to have equal chance when the survey is being carried out.

2.3 Method of Data Analysis

Statistical tools such as frequency distribution, Gross Margin, Chi-square and Profit function analysis. Multiple regression analysis was used to identify the determinant of peasant farmer production in the study area. Below is the model specification:

$$Y = b0 + X1 + X2 + X3 + X4 + X5 + X6 + X7 + X8 + \mu$$

Where Y = Output (kg), X1 = Age, X2 = Educational level , X3 = Farming experience , X4 = problem farmer facing , X5 = solution to famer's problem , X6 = Farm size, X7 = Capital, X8 = Labour used = Coefficient μ = error team.

III. RESULTS AND DISCUSSION

Table1 present the average costs and returns of pumpkin production in the study area. Total revenue of ₦367,150.6 was realized per hectare of pumpkin. The total cost of ₦138,737.60 was incurred. Of this, variable cost constituted about 83.03 percent (₦115,197.60) of total cost of pumpkin production. Further analysis of the variable cost component showed that labor accounted for 88percent, manure3.4%, pesticide 8.6percent and planting material l1.3percent of total variable cost of production. A gross margin and Net Income of ₦251, 953 and ₦228, 413 were realized per hectare. This indicated that pumpkin production is profitable in the study area. Comparing the net return with the current national minimum wage of ₦18, 000 revealed that pumpkin production is a profitable venture in the study area, hence can constitute a good source of employment for our young school leavers and the teeming population.

TABLE 1
INCOME AND EXPENDITURE BY PUMPKIN FAMERS PER HECTARE

Items	Units	Value ₦
Revenue Items		
Value of Output	Kg	367,150.60
Total Revenue		367,150.60
Cost Items		
Variable Cost		
Labour Cost	Naira	101,376.00
Cost of Manure	Kg	3,926.00
Pesticide	Litres	9,895.60
Planting Materials	Kg	1,500.00
Total Variable Cost		115,187.60
Fixed Cost		
Land	Hectare	10,120.00
Depreciation on Fixed Items		13,420.00
Total Fixed Cost		23,540.00
Total Cost		138,737.60
Gross Margin		251,953.00
Net Income		228,413.00

Source: Field survey, 2019

Table 2 presents the regression result for the factors affecting fluted pumpkin output in the study area. Of the four functional forms that were estimated, (linear, semilog, double log and exponential), the linear model was chosen as the lead equation because of the high R^2 value and the significant number of explanatory variables. The coefficient for farming experience was positive and significant at 5 percent level. Experienced farmers are perceived to better understand and processed new farming information from extension agents and other sources and hence, improves upon their efficiency and output. They are also known to be early adopters of new farming techniques. (Enete and Okon, 2010, Nwosu et al., 2012 Bassey and Okon, 2008) reported a significant difference between farming experience and water leaf, amaranth spp and cassava output in the study area respectively. Household size impacted positively on pumpkin production in the study area at 5 percent level. Since pumpkin production is labor intensive, large household sizes would imply available labor for pumpkin production. This is the case in the study area where the vegetative pattern and land tenure system does not favour mechanization. Other studies such as (Okoroji *et al*., 2012, Nwosu et al.,2012 Bassey and Okon, 2008) reported similar findings.

TABLE 2
PROFIT FUNCTION ANALYSIS FOR FLUTED PUMPKIN

Parameter	Coefficient	Standard error	t-value	p-value
Intercept	3135.61	2265.01	1.4015	0.2152
Labour cost	3.8459	0.4361	8.6327	0.0000
Cost of manure	2.3260	1.7631	1.2326	0.3190
Output price	8.3716	0.9074	9.0282	0.0000
Capital cost	0.5317	0.5147	1.0341	0.2920
Land value	0.9436	0.0818	12.9328	0.0000
Cost of pest control	3.3150	0.1261	27.9237	0.0000
Cost of planting materials	0.3465	0.3253	1.4163	0.2289
R^2	0.8901			
Adjusted R^2	0.78873			

Source: Field survey, 2019

The result of analysis of constraints encountered by fluted pumpkin farmers in the study area ranked from most critical to the least in table 3. The table showed that lack of access to irrigable land (water) took the lead indicated by 22.5%. This was followed by the high cost of equipments (17.5%) and inadequate finance (12.5%). It is interesting to note that these three constraints identified as most important constraints sum up to over half (52.5%) of the problems of fluted pumpkin farmers in the study area. It may be concluded that if these three constraints are looked into, other impediments such as $4^{th,}$ 6^{th}, 7^{th}, 8^{th}, and 10^{th}, constraints may cease to exist or reduce to minimum in the study area.

TABLE 3
CONSTRAINT FACED BY FLUTED PUMPKIN FARMERS IN THE STUDY AREA

Constraints	*frequency	percentage(%)	
Inadequate irrigable land water	27	22.5	1^{st}
High cost of irrigated equipments	21	17.5	2^{nd}
Inadequate credit facility	15	12.5	3^{rd}
Pest and disease problem	14	11.7	4^{th}
Polluted water	12	10	5^{th}
Inadequate inputs	9	7.5	6^{th}
Transportation	7	5.8	7^{th}
Pilfering	6	5	8^{th}
Marketing problem	4	3.3	9^{th}
High cost of hired labor	3	2.5	10^{th}
Others	2	1.7	11^{th}
Total	120	100	12^{th}

****Multiple response***

IV. CONCLUSION AND RECOMMENDATIONS

The study revealed that there is more married male participating in fluted pumpkin production in the study area. It also revealed that most of the respondents were between the age of 31-35 years. It was found that majority of the respondents were married; few of them were single. It was also concluded that most of the respondents have 5-10 household size that could be of help in their family labour. From the gross margin analysis carried out in this study, fluted pumpkin production is said to be a profitable venture. Based on the findings of this study, the following policy recommendations are given in order to improve production efficiency of fluted pumpkin in Ibadan metropolis (IAR&T AND NIHORT), Oyo State, Nigeria.

i. Government should provide inputs such as chemicals (pesticides and herbicides) planting seeds etc at subsidized rates to farmers and also aim at solving major problem of vegetables production.

ii. It is advised that policies and opportunities that meets the needs of the ideal situation of farmers should be established, and not just those that favour large scale farmers only.

iii. Given the low level of cash income that farmers have at their disposal, promoting or making micro finance institutions accessible to small holder farmers could contribute immensely to the use of modern input.

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Isolation and Identification of Mycoplasma Species in Dogs

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***Abstract*—** *Mycoplasmas can be associated with several canine health issues, mainly when dogs do not respond to antimicrobial treatment usually aimed at bacterial infections. Different mycoplasma species can be found in both healthy and sick animals; however, the following subjects have yet to be fully understood: The role played by mycoplasmas in canine habitats and the various diseases caused by them. The aim of the present study is to assess the presence of mycoplasma in dog samples at NUDMIC/UFF, RJ, Brazil, over a timeframe of ten years. Out of all assessed dogs, 9.67% (15/155) had respiratory symptoms, whereas the rest of them were asymptomatic. Moreover, 29.96% of the cultured samples (77/257) were positive for mycoplasmas. Typical colonies of said samples were divided into 42.86% (33/77) of oropharynx samples, 51.95% (40/77) of urogenital samples and 5.19% (4/77) of samples from other sources. Species Mycoplasma canis, Mycoplasma edwardii and Mycoplasma cynos were identified by PCR and/or immunoperoxidase. The most common species was M. canis. M. cynos was found in a dog with signs of respiratory disease. Despite the recent improvement in early identification and the biomolecular knowledge surrounding canine mycoplasma, the etiopathogenesis of canine mycoplasmosis remains uncertain..*

Keywords*— *Diseases, dogs, isolation, Mycoplasma, PCR.

I. INTRODUCTION

It has been more than 80 years since Shoetensack first reported mycoplasma species in dogs. The initial studies were very slow and fruitless due to the challenge of growing mycoplasmas in samples contaminated by other bacteria. In addition, there were few techniques to isolate the mycoplasma mixtures available (Rosendal 1979). It is worth remembering that Watson and Crick (1953) published their studies on DNA structure in 1953, and that Mullis et al. (1986) only developed the Polymerase Chain Reaction (PCR) technique in the 1980s. Nevertheless, mycoplasmas in dogs were little studied until the 2000s, when several reports started to arise more often in many countries. However, many mycoplasma, acholeplasma and ureaplasma species had already been described, namely: *Mycoplasma cynos, M. canis, M. edwardii, M. bovigenitalium, M. gateae, M. spumans, M. feliminutum, Acholeplasma laidlawii* and *Ureaplasma* sp. The first three species were the most commonly reported in dogs. A new species, *Mycoplasma mucosicanis* SP. Nov., was isolated from both the mucosa and the urogenital tract of asymptomatic dogs (Spergser et al. 2011).

M. Canis and *M. edwardii* may often appear alone or combined with other mycoplasma species in the upper respiratory tract. However, these species do not seem to be associated with respiratory disease as a primary pathogen *(*Chalker et al. 2004; Johnson et al. 2013). On the other hand, *M. cynos* affects the lower respiratory tract, causing pneumonia alone or often combined with Canine Infectious Respiratory Disease Complex (CIRD) (Chalker et al. 2004; Johnson et al. 2013). The most common symptoms of this disease are dry cough, anorexia and apathy. Lesions caused by *M. cynos* similar to those caused by *M. pulmonis* infection and found in laboratory rodents (Barreto et al. 2003; Souza et al. 2016) — are pathognomonic for mycoplasma pneumonia (Hong and Kim 2012).

M. canis is the species most associated with infertility, mucopurulent discharges and cystitis in the urogenital system (L'Abee-Lund et al. 2003; Ulgen et al. 2006). However, other species, such as *M. edwardii* and *M. spumans*, may also be isolated in healthy animals (Maksimović et al. 2018).

Although the respiratory and urogenital tracts are affected by most isolated species in dogs, *M. edwardii* was isolated from a 12-year-old female dog that has presented acute polyarthritis followed by septicemia (Stenske et al. 2005). Moreover, a picture of purulent meningoencephalitis indicated a brain tissue condition in a six-week-old female dog (Ilha et al. 2010). *Mycoplasma* spp. was isolated from the ear canal of healthy dogs with external otitis for the first time in Paraíba State, Brazil (Santos et al. 2016).

Mycoplasma canis and *Mycoplasma spumans* were isolated in a cat (Walker et al. 1995) after a dog bite; likewise, *M. canis* was isolated in a 62-year-old woman (Klein, Klotz, and Eigenbrod 2018). These findings suggest the existence of a new mode of mycoplasma transmission.

Isolation had originally prevailed as the diagnostic method, however, PCR has been the method of choice for detecting and typifying mycoplasma in dogs since 2000 (Chalker 2005; Janowski et al. 2008; L'Abee-Lund et al. 2003). This method has contributed to raise the number of reports. The difficulty in obtaining specific antisera for mycoplasma in dogs is an obstacle to serological typing (Chalker 2005). Alternatively, methods such as immunoperoxidase and immunofluorescence are extremely useful in typing mycoplasma isolates, mainly the canine ones (Nascimento et al. 2010).

Despite the onset of enzymatic and biomolecular techniques, isolation remains the gold standard for the diagnosis of animal mycoplasmosis such as the canine one. Therefore, isolation is essential for the validation of PCR methods (Chalker 2005) and to enable the application of immunoenzymatic techniques such as immunofluorescence and immunoperoxidase (Santos et al. 2010; Zeugswetter et al. 2007).

Laboratory challenges in the diagnosis of canine mycoplasmosis lead to underreported cases and to poorly informed veterinarians and breeders, who often use antibiotics that do not work for mycoplasma (Chalker 2005). This scenario results from factors inherent to microorganisms and hosts (Berčič et al. 2012; Maksimović et al. 2018; Mannering et al. 2009) involved in sample preparation and cultivation methods (Chalker 2005; L'Abee-Lund et al. 2003).

In Brazil, Oliveira, Costa, and Silva (1998) assessed the vaginal microbiota diversity of healthy female dogs, but did not isolate mycoplasma. However, Costa et al. (2004) and Nascimento et al. (2010) isolated *Mycoplasma* spp. from the respiratory and urogenital tracts of asymptomatic dogs. These mycoplasma species were then identified as *M. canis* and *M. edwardii* through Indirect Immunoperoxidase, thus confirming the presence of mixed infections in dogs (Nascimento et al. 2010).

Due to the difficulty of diagnosis and of contributing to the knowledge regarding the occurrence of canine mycoplasma. The aim of the present study was to assess the presence of *Mycoplasma* spp. in dogs with and without mycoplasmosis over a timeframe of ten years.

II. MATERIALS AND METHODS

2.1 Study design

Samples were collected from dogs (n=155) in academic or private veterinary clinics in Niterói and Rio de Janeiro Cities. Some of the samples were collected from pet dogs belonging to veterinary students, under their request. The samples were processed at the Diagnostic Mycoplasma Laboratory (NUDMIC) of UFF Veterinary School.

Samples were subjected to cultivation and the isolates were identified by indirect immunoperoxidase. These samples were concurrently assessed by generic PCR, if positive; they were subjected to specific PCR identification.

2.2 Sample collection

Main samples were collected from the oropharynx and urogenital tracts. However, ear and conjunctival swab samples were eventually collected. All the samples were placed in sterile tubes filled with appropriate transport medium (modified Frey's agar and glycerol – 1:1).

2.3 Cultivation and isolation

Aliquots of 0.1 ml were removed from the transport medium. Then, the aliquots were inoculated into 0.9 ml of modified Frey's broth enriched with 50% horse serum and 50% swine serum, and into 0.1 ml of modified Frey's agar plate at 37 ºC under microaerophilic conditions, usually used in the NUDMIC. The cultures were followed up for 21 days by optical screening in a Novex RZ series stereomicroscope at 6.5-45x magnification range.

2.4 Immunoperoxidase

The indirect immunoperoxidase was performed according to Imada, Uchida, and Hashimoto (1987). The antisera were *M.canis, M. edwardii, M. gateae. M. molare* and *M. arginini*, in the 1/20 dilution. *Mycoplasma agalactiae* was used as negative control at the same dilution; as conjugated to goat anti-rabbit IgG conjugated with peroxidase at 1/80 dilution. The wash buffer was made up of TBBS, horse serum and Tween 20, and the developing solution was made with cold methanol, 4-chloro-1-naphtol, TBS and 30% hydrogen peroxide. Plates were screened for stained colonies in stereomicroscope.

2.5 PCR

DNA was extracted from the samples and/or isolates based on the phenol-chloroform method for the PCR assay according Sambrook (2001). A set of generic primers (GPO3 5'GGG AGC AAA CAG GAT TAG ATA CCC T 3' and MGSO 5'TGC ACC ATC TGT CAC TCT GTT AAC CTC 3") was used to amplify a 270-bp fragment of *Mycoplasma* spp (Van Kuppeveld et al. 1994). Subsequently, 5 µL of target DNA was used for a final 100-µL reaction mixture of 1x PCR buffer, 2 mM MgCl2, 0.5 mM dNTP mix, 2 µL (100 pmol) of both primers and 1.5U of Taq DNA polymerase.

Species-specific PCR was performed as described by Chalker et al. (2004): Clinical specimens were isolated and identified in the reference strains of *M. canis*, *M. edwardii*, *M. cynos*, *M. molare*, *M. gateae* and *M. felis*. Then, primers and processes were described (Table 1). PCR reactions (50 µl) included the following reagents: 5.0 µl of 10x magnesium-free buffer (0.1 M Tris-HCl, 0.5 M KCl, pH 8.3), 1.5 mM MgCl2, 0.5 mL (0.5 units) of Taq DNA polymerase, 0.2 mM of PCR nucleotide mix, 0.025 mg of forward primer (Myc1; 59-CACCGCCCGTCACACCA-39), 0.025 mg of reverse primer for each mycoplasma (Table 1); and 1 µg of DNA from either the sample, the positive control or the negative control (1 µl of water).

TABLE 1
PCR PRIMERS, CYCLES AND AMPLICON SIZE IN DOG SAMPLES

Species	Primer sequence	Cycle conditions (x30)	Product size (bp)
Forward primer Myc1	5 ’CACCGCCCGTCACACCA3’	according to reverse primer	according to reverse primer
M. canis	5’CTGTCGGGGTTATCTCGAC3’	95 °C 1min, 55 °C 30s, 72 °C 1min	247
M. cynos	5’GATACATAAACACAACATTATAATATTG3’	95 °C 45s, 55 °C 30s, 72 °C 20s	227
M. edwardii	5’CTGTCGGGTTATCATGCGAC3’	95 °C 45s, 55 °C 30s, 72 °C 20s	250
M. molare	5’AGCCTATTGTTTTTGATTTG3’	95 °C 1min, 55 °C 30s, 72 °C 1min	397
M. gateae	5’GTTGTATGACCTATTGTTGTC3’	95 °C 1min, 55 °C 30s, 72 °C 1min	312
M. felis	5’GGACTATTATCAAAAGCACATAAC3’	95°C 45 s, 51°C 30 s, 72°C 20 s	238

2.6 Data analysis

Data were compiled in Microsoft Excel® spreadsheets and were calculated the frequences of micoplasma canine. Pearson's chi-square test or Fisher's exact test was used, with a 95% confidence interval to compare the association between the techniques for diagnostic used and collection site. The analyzes were performed using the BioEstat® 5.0 software. (Ayres et al., 2007)

III. RESULTS

3.1 Sample collection

The assessment included 257 samples of 155 dogs from Niterói and Rio de Janeiro Cities. The samples included 55 males, 87 females and 13 dogs of non-specified sex. There was significant difference between the number of males and females ($p<0.05$). Ages ranged from 5 to 180 months (47 months, on average). The two main collection sites were the oropharynx and the urogenital tracts: 41.63% (107/257) and 40.07% (103/257), respectively. Samples from other body parts, such as the ear and the conjunctiva, totaled 18.30% (47/257).

3.2 Cultivation, isolation, immunoperoxidase and PCR

Among the cultures, 29.96% (77/257) were positive for mycoplasma species (Table 2), out of which, 42.86% (33/77) of typical isolated colonies were found in the oropharynx; 51.95% (40/77), in the urogenital tract; and 5.19% (4/77), in other sites (Table 3). These positive samples fermented glucose and were categorized as Mollicutes based on the Dienes staining method. Finally, 31.13% (80/257) of the samples subjected to PCR were positive for *Mycoplasma* spp (Table 2).

TABLE 2
MYCOPLASMA-POSITIVE DOG SAMPLES BASED ON THE DIAGNOSTIC METHOD

Results/Technique	Culture	PCR
Positive	77 (29.96%)[a]	80 (31.13%)[a]
Negative	180 (70.04%)	177 (68.88%)
Total	257 (100.00%)	257 (100.00%)

Same lowercase letters on the rows indicate no significant difference (p> 0.05).

TABLE 3
DOG SAMPLES SUBJECTED TO IMMUNOPEROXIDASE ASSAY ACCORDING TO COLLECTION SITE

Collection site	Oropharynx tract	Urogenital tract	Other sites	Total
Positive	16 (48.48%)[a]	19 (47.50%) [a]	2 (50.00%) [a]	37
Negative	17 (51.52%)	21 (52.50%)	2 (50.00%)	40
Total	33 (100.00%)	40 (100.00%)	4 (100.00%)	77

Same lowercase letters on the rows indicate no significant difference (p> 0.05).

There was no significant difference (p> 0.05) between the diagnostic methods of canine mycoplasma. There was no significant difference (p> 0.05) among the isolates of oropharynx and urogenital tract samples and of other sites.

The specific PCR technique was used to identify 23.75% (19/80) of *M. canis* and 21.25% (17/80) of *M. edwardii*. There was a *M. cynos* sample among *M. spp*. The indirect immunoperoxidase technique was used to identify the isolates, out of which 67.57% (25/37) were *M. canis* and 43.24% (16/37) were *M. edwardii*. There were 4 positive samples for both species (table 4). There was no significant difference between *M. canis* and *M. edwardii* in PCR in comparison to the significant difference between *Mycoplasma.spp* and other species (p <0.05).

TABLE 4
DETECTION OF MYCOPLASMA SPECIES THROUGH PCR AND IMMUNOPEROXIDASE

Technique	PCR			Immunoperoxidase**	
Results	***M canis***	***M edwardii***	***M spp****	***M canis***	***M edwardii***
Positive	19 (23.75%)[A]	17 (21.25%)[A]	44 (55.00%)[B]	25 (67.57%)[a]	16 (43.24%)[b]
Negative	61 (76.25%)	63 (78.25%)	36 (45.00%)	12 (32.44%)	21 (56.76%)
otal	80 (100.00%)	80 (100.00%)	80 (100.00%)	37 (100.00%)	37 (100.00%)

Same lowercase and capital letters on the rows indicate no significant difference (p> 0.05).
**** One of the samples was typified as M. cynos.***
*****The total of 4 samples were positive for both species.***

Results in Table 4 suggest that both methods are efficient to typify mycoplasma species in dog samples, although the indirect immunoperoxidase technique showed better results than those obtained through the PCR technique.

IV. DISCUSSION

The present study investigated the presence of Mycoplasma spp in dogs for over a timeframe of ten years and identified three canine Mycoplasma species. *M. canis* and *M. edwardii* were the most prevalent species, whereas *M. cynos* was found in only one animal among the 15 dogs that have presented respiratory symptoms.

Mycoplasma canis and *M. edwardii* were found in respiratory and urogenital tract samples from male and female dogs by PCR and immunoperoxidase. These findings support the evidence that these species inhabit both the upper respiratory (Chalker 2005) and the urogenital tracts (Maksimović et al. 2018).

Findings regarding *M. cynos* were similar to those described by Hong and Kim (2012) and Canonne et al. (2018). Hong and Kim (2012) identified *M. cynos* by PCR in lung tissue samples from 5.0% (1/20) of Beagle dogs with respiratory disease. Canonne et al. (2018) studied dogs diagnosed with eosinophilic bronchopneumopathy and chronic bronchitis, as well as healthy dogs, by using the qPCR technique. They detected *M. cynos* in 6.67% (4/60) of sick dogs and in 3.33% (2/60) of healthy dogs.

The detection of *M. cynos* in only one case of respiratory disease suggests that the likelihood of *M. cynos* infection is low and facilitated by the following complications: Serious respiratory diseases such as CIRD, and association with other microorganisms (Chalker et al. 2004; Mannering et al. 2009); moreover, *M. cynos* is more easily detected through PCR (Mitchell et al. 2017). Such species has been defined as an emerging pathogen (Priestnall et al. 2014) associated with other CIRD microorganisms; nevertheless, its ability to cause disease on its own has not yet been proven, since it can be detected both in asymptomatic dogs (Lavan and Knesl 2015) and in dogs vaccinated against CIRD (Mitchell et al. 2017).

The urogenital tract is the common isolation site of several mycoplasma species. However, the association of the urogenital and respiratory tracts to mycoplasmosis remains uncertain due to the incidence of isolation in both healthy animals and in animals with urogenital disorders (L'Abee-Lund et al. 2003; Maksimović et al. 2018; Ulgen et al. 2006).

L'Abee-Lund et al. (2003) isolated *M. canis* in dogs with clinical signs of urogenital disease and suggested that this microorganism could be the causative agent of it. However, the present study showed that *M. canis* was more common in healthy dogs than in the sick ones, as reported by Janowski et al. (2008) and Maksimović et al. (2018). Janowski et al. (2008) suggested that *M. canis* was a part of the vaginal microbiota of healthy female dogs. Maksimović et al (2018) isolated *M. canis, M. spumans, M. edwardii* and *Mycoplasma* spp in vaginal samples from domestic and street female dogs who were either healthy, intact or subjected to total hysterectomy. The present study also evidenced *M. edwardii* and *Mycoplasma* spp in the urogenital tract of healthy animals, but *M. cynos* was not found in it, a fact to be unusual in this site; however, it has been reported in dogs with urogenital disease, along with *M. canis* and *M. spumans* (Jang et al. 1984).

The diagnostic results of the comparison between isolation and PCR have supported the findings by Costa et al. (2004), since there was no significant difference between the aforementioned techniques; thus, both of them can be recommended for diagnosis.

The detection of *M. canis* and *M. edwardii* alone and/or in mixed infections by indirect immunoperoxidase reaction in both the respiratory and urogenital tracts by the present study had already been previously reported (Nascimento et al. 2010). Based on this finding, these species can be part of the microbiota found in both the respiratory and urogenital tracts. Indirect immunoperoxidase can be used to identify mycoplasma species in dogs (Nascimento et al. 2010) and in other animals alike (Santos et al. 2010).

V. CONCLUSION

- Canine mycoplasmas have been found in sick animals, but they are more likely to be found in seemingly healthy animals.
- *M. canis* and *M. edwardii* were the most prevalent species in the assessed sites and through the diagnostic method.
- *M. cynos* was detected in a single animal who presented a respiratory disease.
- The highly consistent detection of mycoplasma species in dogs encourages instructive studies on their etiopathogenesis.

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Effect of Genotype by Environment Interaction (GEI), Correlation, and GGE Biplot analysis for high concentration of grain Iron and Zinc biofortified lentils and their agronomic traits in multi-environment domains of Nepal

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Abstract— *Lentil (Lens culinaris Medik. culinaris) is a cool season food legume contains the high quality of proteins and minerals. Selecting genotypes for high mean yield and yield stability has been a challenge for lentil breeders. The complexities of genotype × environment interaction (GEI) make selection difficult to identify the best performing and most stable genotypes. Therefore, this study was carried out to apply a GGE biplot and AMMI analysis model to evaluate the magnitude of the effect of GE interaction on grain yield of 25 lentil accessions at three environments during the year of 2016 and 2017 seasons in alpha-lattice design (5x5) with three locations and to evaluate relationships between test environments for identification of favorable genotypes for lentil production areas. Combined pooled mean analysis of variance for grain yield tested at three environments over the two subsequent years 2016 and 2017 showed that highly significant differences in genotypes, environment and G x E interaction effect indicating the possibility of selection for stable accessions. The stability of the assessed genotypes using some stability statistics derived from three types of statistical concepts (variance and regression analyses), AMMI (additive main effect and multiplicative interaction) analysis and GGE biplot (genotype main effects and genotype-by-environment interaction effects) models were applied to obtain good understanding of the interrelationship and overlapping among the used stability statistics. Research results showed that lentil accession WBL-77 (1451 kg ha-1) , RL-79(1446 kg ha-1) and PL-4(1429 kg ha-1) were the best performer and well adopted across the environments and over the years. AMMI analysis of variance for lentil grain yield (tha^{-1}) of lentil accessions tested at three environments over the years showed that 80.71% of the total sum of squares was attributed to environmental effects, only 8.38 % to genotypic effects and 10.90% to genotype × environment interaction effects. The partitioning of GGE sum of squares through the GGE biplot analysis showed that PC1and PC2 accounted 74.75%, and 25.24% of GGE sum of squares respectively over the years. Accessions ILL8006, RL-6, Shital, ILL3490 and simal were more close to the center point and indicated that stable across the environments. In another words, the genotypes which have low stability value (ASV) is said to be stable and the breeder chose the stable genotypes along with grain yield above the mean grand yield. In this experiment accessions RL-6(G-2) ranked 1st stability (ASV-0.53) followed by Simal (ASV-2.05), ILL-3490 (ASV-2.42) and Shital (ASV-2.72) and suitable for all environment.*

Keywords— Stability parameters, lentil, GGE biplot, AMMI-additive main effects and multiplicative interaction; ASV–AMMI stability value.

I. INTRODUCTION

Lentil (*Lens culinaris* Medik. culinaris) is a cool season food legume and "the house of essential nutrients", contains the high quality and quantity of proteins (up to 35%) and minerals calcium, phosphoros, potassium, folic acids, iron, zinc, selenium and vitamins. Lentil is the fourth most important crop grown after rice, maize, wheat & millet in terms of area (MoAD, 2016). In Nepal, it is mostly eaten as dal (Concentrated soup) with rice besides various food preparations. Rice or wheat bread and dal are the best combination in daily dish of low income people of Nepal who cannot afford the animal products. Virtually the major

proportion of rural people relies on lentil and other pulses for their nutritional security. It has diverse role in farming system which adds 42-75 kg biological nitrogen fixation. Lentil seed is rich in protein for human consumption, and lentil straw is a valued animal feed. It is also known as the exportable commodity, in 2016/17 lentil was exported in the value of USA $ 10 million from Nepal. It is grown as sole crop as well as mixed crop and intercropped with sugarcane, mustard, linseed, wheat, mango-orchard etc. In general, lentil is commonly grown as relay cropping system prior to rice harvest and in Nepal, still 0.24 million hectares of lands are rice fallows and has a great scope to vertical and horizontal expansion. In most lentils producing areas yield seems to be not more than one-half of potential yields while improved genotypes contribute to increase lentil production (Erskine, 2009). Lentil is adapted to low rainfall and is predominantly grown in the winter in regions (Sarker et al., 2003). Selecting genotypes for high mean yield and yield stability has been a challenge for lentil breeders. Yield is a quantitative trait while GxE interaction showed the yield stability and micronutrients heritability. It is also the interplay in the effect of genetic and non-genetic on development of any genotypes. Consequently Gx E interaction helps breeder to select the desirable varieties in the process of evaluation & increase efficiency of selection (Sabaghnia et al., 2008). It is reported the main environmental effects (E) and Genotype by Environment Interaction (GEI) as the most important sources of variation for the measured yield of crops. The yield is a combined result of the effects of the genotype (G), E and GE interaction. Environment is responsible more than 80% effects of the total yield variation, while Genotype and G x E interaction has small effect. Environmental factors include soil moisture, sowing time, fertility, temperature and day length which is strong influenced during the various stages of plant growth (Bull et al., 1992). Therefore GEI is an extremely important in the development and evaluation of plant varieties. Flores et al. (1998) compared 22 univariate and multivariate methods to analyze genotype by environment (GE) interactions. There are two possible strategies for interpreting GE interaction with univariate parametric methods including analysis of variance and simple linear regression analysis of cultivar yield. The requirement for stable genotypes that perform well over a wide range of environments becomes increasingly important as farmers need reliable production quantity (Gauch et al.,2008). Therefore, identifying most stable genotypes is an important objective in many plant breeding programs for all crops, including lentil. The performance of a genotype is determined by three factors: genotypic main effect (G), environmental main effect (E) and their interaction (Yan et al.,2007). Understanding genotype by environment (GE) interactions is necessary to accurately determine stability in lentil genotypes and help breeding programs by increasing efficiency of selection (Sabaghnia et al.,2008). The complexities of genotype × environment interaction (GEI) make selection difficult to identify the best performing and most stable genotypes (Yau, 1995). Thus, first we need to identify the stable genotypes for their yield and yield component traits. Stability of genotypes over wide range of environments is desirable and depends upon GEI (Ali and Sawar, 2008). AMMI analysis has been shown to improve both the post-dictive and predictive success of yield trials by altering the noise (random variation) from the data pattern, thereby improving predictive accuracy (Gauch and Zobel, 1988). Understanding the structure and nature of GEI is of utmost significance in crop improvement programs because the significant GEI can seriously impair efforts in selecting the superior genotypes (Danyaliet al., 2012). The objectives of this investigation were: to apply a GGE biplot and AMMI analysis model to evaluate the magnitude of the effect of GEI on grain yield of 25 high grain Fe and Zn lentil accessions tested across the three locations over the years and to evaluate relationships between test environments for identification of favorable genotypes for lentil production areas in terai agro-domains.

II. MATERIALS AND METHODS

2.1 Description of the Study Sites

G x E interaction trials were conducted at Grain Legumes Research Program (GLRP), Khajura, Banke at 81^0 37" East longitudes and 28^0 06" North latitude and an altitude of 181 meters above mean sea level, NMRP/NCRP, Rampur, Chitwan at 27^o 40′ N latitude, 84^o19′ E longitude at an altitude of 228m above mean sea level and RARS, Parwanipur at the latitude 27^0 4'40.9''N and longitude $84^0$56'9.85"E as well altitude 75m above sea level for two consecutive cropping seasons (October 2016 to March 2017) in Nepal.

2.2 Plant Materials

For this study, 25 high grain concentrations of iron (Fe) and zinc (Zn) lentil accessions including local landraces was planted for phenotypic evaluation. Sources of these accessions were originated from SAARC countries (14 accessions: Nepal-7, India-6, Bangladesh-1) and ICARDA (11 accessions). Released lentil varieties Shital and Simal was used as a check.

2.3 Experimental Layout and Design

Present experiment was carried out in Alpha-lattice design (5 x 5) with three replications. A unit plot comprise 2-meter length row with a plot size of (3 m^2). The accessions were planted in the third week of October to 2^{nd} week of November. Seeds of each

accession were distributed thoroughly to seed packets, representing number of rows of each plot size and then randomized plot numbers were assigned to each plot seed packets and arranged according to planned field- layout. Recommended agronomic practices were strictly followed for raising a good crop at all the testing sites. The crop was supplied as recommended dose of fertilizers @20:40:20 NPK during the final land preparation.

2.4 Data Collection

Quantitative traits were recorded on 10 randomly selected plants followed by IBPGR Descriptors (Anonymous, 1985). Data was recorded on plant basis for plant height (Plht cm), number of pods/plant (P/P), number of seeds/pod (S/P), number of seeds/plant (S/P), and seed weight/plant (SWPP) whereas Morphological parameters of quantitative data was recorded days to 50% flowering (DF), days to maturity (DM), above ground biomass (BY), 100-seed weight in gram (HSWT), seed yield/plant (SYPP), and *stemphylium* blight severity scored on a 1–9 scale (1–9 rating scale where 1 = highly resistant and 9 = highly susceptible) before flowering and after flowering was recorded on plot basis according to the Chen, 2007 that can be exploited for developing future breeding material in lentil breeding improvement program. Data for crop phenology, growth, yield and yield components were collected based on either from 10 randomly taken sample plants or from plants in net plot. Mean values of these samples were utilized to estimate the performance of each genotype for the traits under consideration.

2.5 Statistical Data Analysis

Plot means values was calculated for all traits and used for the analysis of variance (ANOVA). The estimation of genetic parameters was analyzed using R-stat version and GEA-R. Phenotypic and genotypic variances for the Alpha Lattice design (5 x 5) was computed for all traits based on the methods of Federer, 1961.

2.5.1 The GGE biplot

GEI is commonly observed by crop producers and breeders as the differential ranking of cultivar yields among locations or years (Samonte et al. 2005). Plant breeders conduct multi-location trial primarily to identify the superior accession for a target region and secondarily to determine whether the target region can be subdivided into different mega-environments (Yan et al. 2000).

Analysis of variance for genotype x environment interaction

Analysis of variance (ANOVA) was computed using Additive Main Effects and Multiplicative Interaction (AMMI) (Zobel et al., 1988; Guach, 1988) and regression models (Eberhart and Russell, 1966) for grain yield that exhibited significant mean squares for genotype and genotype by environment interaction. The GEI analysis of variance using Eberhart and Russell (1966) model was computed by GEA-R (Genotype by Environment Analysis with R) statistical software, while META-R (Multi-Environment Trial Analysis with R) and R-Stat version 3.5.3 statistical software was used to calculate ANOVA for AMMI (Zobel et al., 1988; Guach, 1988). The analysis of variance of each location (Annex i) and combined analysis of variance over locations (Annex ii) were done as per Gomez and Gomez (1984).

Specifically, for the data matrix Y= (yij); with response variables Yij, the ANOVA model is

$$Yij = \mu + \alpha i + \beta j + \phi ij + \varepsilon ij$$

where Yij is the yield of the genotype i in the environment j, μ is the overall mean, αi is the genotype (row) main effect, βj is the environment (column) main effect, ϕij is the specific genotype i (row) by the environment j (column) interaction, and εij is the error term of the model, where εij~iid N(0,δ2)

ANNEX 1
OUTLINE OF ANALYSIS OF VARIANCE FOR A SINGLE LOCATION

Source of variation	Degree of freedom	Sum of square	mean square	F value Expected mean Square
Block(R)	(r-1)	$(r-1)^2$	MSr	-
Genotypes	(g-1)	$(g-1)^2$	MSg	$\sigma^2_e + r\,\sigma^2 g$
Error	(r-1) (g-1)	$(r-1)^2\,(g-1)^2$	MSe	σ^2_e

Where: r= no. of blocks; g = number of genotypes; e = error; MSr= replication mean square; MSg= genotype mean square; MSe= error mean square

The combined analysis was done using mixed linear model as outlined in Annex ii to examine the additive and interaction effects of genotypes and environments.

ANNEX 2

THE OUTLINE OF THE COMBINED ANALYSIS OF VARIANCE OVER LOCATIONS

Source of variation	Degree of freedom	mean square	F value Expected mean Square	F-ratio
Environment (E)	e-1	MSE	$\sigma^2e + r\sigma^2g*e + gr\ \sigma^2e-$	MSl/MSr
Blocks in Loc[R]	1(r -1)	MSr	$\sigma^2e + g\sigma^2R(L)$	MSr/MSe
Genotype (G)	g-1	MSg	$\sigma^2_e + r\ \sigma^2g*e + r\Sigma\alpha i^2/g-1$	MSg/MSe
G * E	(g-1)(e-1)	MS ge	$\sigma^2_e + r\sigma^2ge$	MSge/MSe
Pooled error (e)	1(g-1)(r-1)	MSe	σ^2_e	
Total	1rg-1			

Where: E= number of locations; G = number of genotypes; r= number of blocks; MSE=environment mean square; MSr= block mean square; MSg= genotype mean square; MSGxE= GxE mean square; MSe=error mean square; σ²=Variance

2.5.2 AMMI model

The model AMMI uses the biplot constructed through the principal components generated by the interaction environment-genotype. If there is such interaction, the percentage of the two principal components would explain more than the 50% of the total variation; in such case, the biplot would be a good alternative to study the interaction environment-genotype, Crossa (1990).

The AMMI model is

$$Y_{ij} = \square + \alpha_i + \beta_j + \sum_{k=1}^{t} \lambda_\kappa \xi_{ik} \acute{\eta}_{ik} + \varepsilon_{ij}$$

where t is the number of SVD axes retained in the model, λk is the singular value for the SVD axis k, ξ_{ik} is the singular value of the genotype i for the SVD axis k, $\acute{\eta}_{jk}$ is the singular value of the environment j for the SVD axis k, and ε_{ij} is the error term of the models, where $\varepsilon_{ij} \sim^{iid} N(0,\delta^2)$.

We used GGE biplot to indicate any possible specific adaptations of accessions to these environments instead of evaluating the slopes. The basic model for a GGE biplot is:

$$Y_{ij} - \square - \beta_j = \sum_{k=1}^{K} \lambda_\kappa \xi_{ik} \acute{\eta}_{ik} + \varepsilon_{ij}$$

Where Yij = the mean yield of genotype i (= 1,2,...,g) in environment j (= 1,2,...e), μ = the grand mean, β_j = the main effect of environment j, $(\mu+\beta_j)$=mean yield of environment j, λk =the singular value (SV) of kth principal component (PC), ξ_{ik} = the eigen-vector of genotype i for PCk, η_{jk} = the eigen-vector of environment j for PCk, K is the number of PC axes retained in the model ($K \leq \min(g,e)$ and K = 2 for a 2-dimensional biplot) and ε_{ij} =the residual associated with genotype iin environment j.

2.5.3 Stability Analysis

The stability parameters are useful in characterizing genotype by showing their relative performance in various environments. This parameters we can calculate as follows.

A linear regression model with interaction genotype by environment is like:

$$Y_{ij} = \mu + d_i + (1 + \beta_i)e_j + \delta_{ij} + \varepsilon_{ij}$$

Where, Yij is the average phenotypic value of the i-th genotype in the j-th environment, μ is the general mean, di is the effect of the i-th genotype (i=1,...,t), ej is the effect of the j-th environment (j=1,...,s), 1 + βi is the regression of Y_{ij} in e_j, δ_{ij} is the deviation of the regression for the i-th genotype in the j-th environment, εij is the error. GEA-R (Genotype x Environment Analysis whith R for Windows) Version 2.0 was used to construct GGE biplot graph.

2.5.4 Pearson correlation coefficient

Principal component analyses (PCA) based on the correlation matrix was performed to obtain an understanding of the relationship among stability parameters. To correlate the relationship in between lentil lines and quantitative traits like days

to 50% flowering, plant height, 100 seed weight and grain yield were evaluated using Pearson correlation coefficient using BLUPs (Best Linear Unbiased Predictors) of single environment as well as across the environments.

III. RESULTS AND DISCUSSION

3.1 Weather patterns

Development of lentil plant is exceptionally touchy to climate conditions, particularly precipitation, high temperature and early frost. Precipitation in the wake of blossoming favors vegetative development. Unnecessary wet conditions at the time of planting delay it's planting, which results in the late advancement of lentil plant, leaving the crop defenseless against summer heat. High temperature stresses flowers, resulting in no podding or potentially excessive flowers and pod shedding. Late planting may likewise bring about insufficient root advancement and stemphyliun disease incidences and the vulnerability of crop to early frost in the fall.

Average monthly mean temperature during the growth period varied among the three environments. Khajura in 2017 had a hotter trimming season than Rampur and Parwanipur. . Anyway the mean temperature in the two years over the situations played out similar patterns (16-28.8 ^{0}C). During the two years of study, rainfall distribution varied among the environments. In 2016, Parwanipur got 260 mm of precipitation when contrasted with 202 mm in Rampur and 48.1 mm in Khajura. The major rainfall distribution difference was in the months of October received about 4 times higher rainfall as compared to Khajura). In 2017, Parwanipur, Khajura and Rampur received rainfalls of 204.2, 99.2 and 87.5 mm, respectively. Overall Parwanipur received much higher rainfall especially in both years as compared to other locations (Annex i). These weather conditions have an impact on the results obtained which are covered during discussion.

3.2 Pooled mean yield analysis of variance of twenty five high Fe and Zn grain biofortified lentil accessions tested at three environments

Pooled mean analysis of variance (ANOVA) for grain yield tested at three environments over the two subsequent years 2016 and 2017 showed that highly significant differences in genotypes, environment and G x E interaction effect (Table 1). From the study , it was concluded that accession WBL-77 (1451 kg ha^{-1}) , RL-79(1446 kgha^{-1}) and PL-4(1429 kgha^{-1}) were the best performer and well adopted across the environments while accessions ILL-7723 (970 kg ha^{-1}) and ILL-4605 (1076 kg ha^{-1}) were the poor performer and adopted in location specific. In Box and Whisker plot graph clearly also indicated that there was large variation of grain yield performances of lentil accessions tested in three environments over the years. Lentil accessions WBL-77 showed the highest yield in the graph followed by RL-79 and PL-4 than the check sagun (Fig. 1)

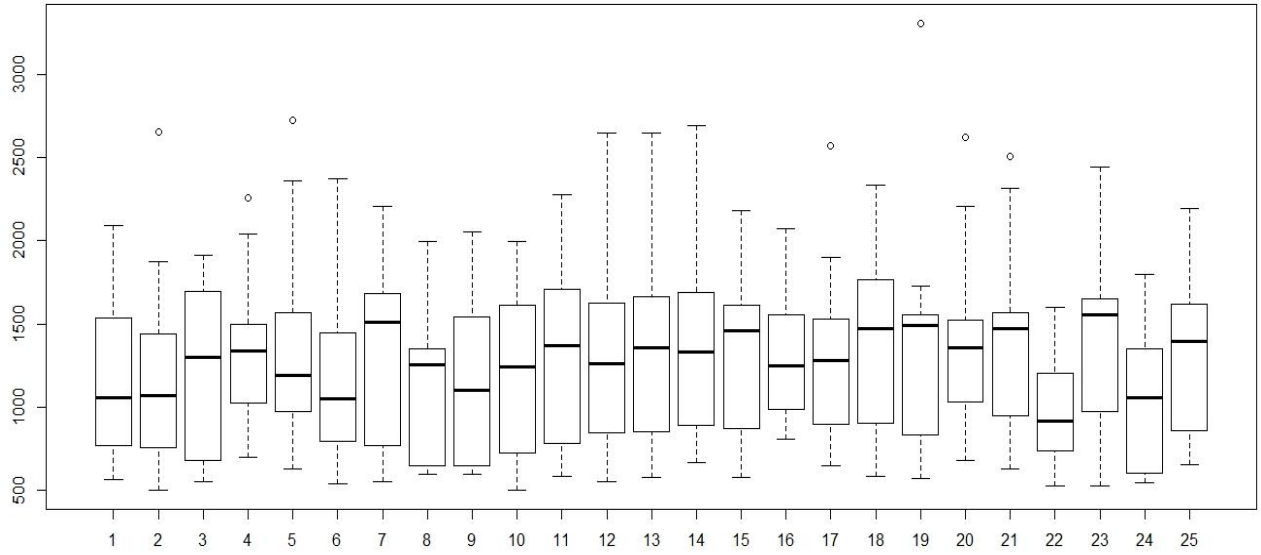

FIGURE 1: Combined mean yield analysis in Box and Whisker plot tested at three environments over the years 2016-2017

TABLE 1
COMBINED ANALYSIS OF MEAN PERFORMANCES OF LENTIL ACCESSIONS IN G X E BIO-TRIAL ACROSS THE LOCATIONS (KHAJURA, PARWANIPUR AND RAMPUR) AND OVER THE YEARS (2016-2017)

Gen	Names	DF	DM	Plht(cm)	PP	SP	HSWT	GY
1	ILL-8006	60	128	40	62	1.89	1.78	1188
2	RL-6	58	127	39	67	1.89	1.67	1206
3	RL-12	58	128	39	57	1.94	1.72	1270
4	ILL-7715	59	129	40	61	2.00	1.78	1301
5	ILL-7164	62	130	40	57	1.94	1.78	1316
6	ILL-3490	58	127	39	70	1.94	1.44	1136
7	Khajura-2	59	127	39	62	2.00	1.72	1326
8	Simal	58	129	41	62	1.94	1.78	1144
9	Shital	56	125	37	70	2.00	1.67	1134
10	Sagun	58	127	39	71	1.94	1.56	1209
11	HUL-57	58	129	42	55	2.00	1.78	1299
12	LG-12	58	128	40	59	2.00	1.72	1337
13	**PL-4**	58	129	40	67	2.00	2.22	1429
14	RL-11	58	129	39	65	2.00	1.61	1364
15	RL-4	59	130	40	64	2.00	1.83	1276
16	ILL-2712	60	129	38	59	2.00	1.61	1305
17	Black Masuro	67	134	40	66	2.00	1.61	1338
18	**RL-79**	56	124	40	62	2.00	2.11	1446
19	ILL-6467	60	131	45	63	2.00	1.67	1380
20	ILL-7979	56	130	37	68	1.94	1.78	1393
21	**ILL-6819**	59	130	40	59	2.00	1.72	1383
22	ILL-7723	60	136	39	52	1.94	1.94	970
23	**WBL-77**	57	129	42	56	2.00	1.89	1451
24	ILL-4605	58	127	38	65	1.94	2.89	1076
25	RL-49	54	122	39	64	1.83	2.61	1356
	Mean	59	129	40	62	1.97	1.84	1281
	P-Value							
	Genotypes	<0.001	<0.001	0.021**	0.35	0.29	<0.001	<0.001
	Environment	<0.001	<0.001	<0.001	<0.001	0.038*	<0.001	<0.001
	GxE	<0.001	0.95	0.066*	0.29	0.038*	0.037*	0.003**
	CV%	12.74	4.70	14.49	33.24	9.50	24.03	26.32
	LSD	5.09	4.08	2.84	13.79	0.11	0.277	225.61

3.3 Additive main effects and multiple interactions (AMMI) analysis

Results of AMMI analysis of variance for lentil grain yield (tha^{-1}) of twenty five lentil accessions tested at three environments over the years showed that 80.71% of the total sum of squares was attributed to environmental effects, only 8.38 % to genotypic effects and 10.90% to genotype × environment interaction effects (**Table 2**). The partitioning of GGE sum of squares through the GGE biplot analysis showed that PC1and PC2 accounted 74.75%, and 25.24% of GGE sum of squares respectively over the years. The two principal components explained a total of 99.99% variation. The average grain yield of each environment and accessions over the years 2016-2017 are given in **Table 3**. Environment grain yield ranged from 769 kg ha^{-1} (Parwanipur) to 1626 kg ha^{-1} (Khajura), while genotype grain yield ranged from 970 kg ha^{-1} (ILL-7723) to 1451 kg ha^{-1} (WBL-77). Also, results of AMMI analysis indicated that both AMMI PC1 and AMMI (PC2) were found non-significant. In the biplot, a total fifteen lentil accessions namely PL-4, RL-11, ILL-6819, ILL-7979, WBL-77, HUL-57, ILL-

7715, ILL-6467, ILL-7164, LG-12, ILL-2712, Black Masuro, Khajura-2, RL-79 and RL-49 and two environments (Khajura and Rampur) located on the right side of the black vertical line (Figure 2).

TABLE 2
AMMI ANALYSIS OF VARIANCE FOR GRAIN YIELD OF 25 LENTIL ACCESSIONS TESTED IN THREE ENVIRONMENTS OVER THE YEARS 2016- 2017

	SS	PORCENT	PORCENAC	DF	MS	F	PROBF
ENV	61407926	80.7139	80.7139	2	30703963	302.3473	0
GEN	6376975	8.38183	89.09573	24	265707.3	2.61647	0.00007
ENV*GEN	8296077	10.90427	100	48	172834.9	1.70194	0.00372
PC1	3100841	74.7544	74.7544	25	124033.7	1.21197	0.22479
PC2	1047197	25.2456	100	23	45530.3	0.44489	0.98857
PC3	0	0	100	21	0	0	1
Residuals	38081993	0	0	375	101552	NA	NA

TABLE 3
AMMI ANALYSIS OF VARIANCE FOR POOLED MEAN GRAIN YIELD OF 25 LENTIL ACCESSIONS TESTED AT THREE ENVIRONMENTS AND OVER THE YEARS 2016-2017

	TYPE	NAME	YLD	DIM1	DIM2	DIM3
1	GEN	1	1187.5	-0.17471	-0.0186	-4.2E-05
2	GEN	10	1209.222	-0.23513	0.223462	-5.9E-05
3	GEN	11	1299.167	0.231725	0.170429	-5.4E-05
4	GEN	12	1336.889	0.155857	0.314179	-6.4E-05
5	GEN	13	1429.222	0.674001	0.539839	2.94E-05
6	GEN	14	1364.222	0.542952	-0.15662	7.7E-05
7	GEN	15	1276.389	0.577529	0.000629	-4.2E-05
8	GEN	16	1305.389	-0.20384	-0.57267	-4.2E-06
9	GEN	17	1337.944	-0.42274	0.096082	5.68E-05
10	GEN	18	1446.222	-0.91191	0.581784	2.2E-05
11	GEN	19	1379.722	0.160574	0.37275	3.94E-05
12	GEN	2	1205.944	-0.02759	-0.01218	6.54E-05
13	GEN	20	1393.167	0.501999	-0.23486	-2.5E-05
14	GEN	21	1383.056	0.318877	-0.24163	-2.6E-05
15	GEN	22	969.7778	-0.55258	-0.7471	6.81E-06
16	GEN	23	1451	0.370912	0.274479	4.68E-05
17	GEN	24	1076	-0.54732	-0.23913	7.96E-05
18	GEN	25	1356.222	-1	0.165491	-5.7E-05
19	GEN	3	1269.778	-0.04115	0.278227	-6.2E-05
20	GEN	4	1301.167	0.203554	-0.38733	-1.6E-05
21	GEN	5	1316.278	0.216272	-0.34748	-1.9E-05
22	GEN	6	1135.889	0.107905	-0.14515	-3.3E-05
23	GEN	7	1326.333	-0.0568	0.313729	4.29E-05
24	GEN	8	1144.278	0.013274	-0.14325	-3.3E-05
25	GEN	9	1134.444	0.098347	-0.08508	7.08E-05
26	ENV	Khajura	1626.253	1	0.343942	-8.7E-05
27	ENV	Parwanipur	769.1267	-0.10927	-0.83216	-8.7E-05
28	ENV	Rampur	1448.847	-0.89073	0.488217	-8.7E-05

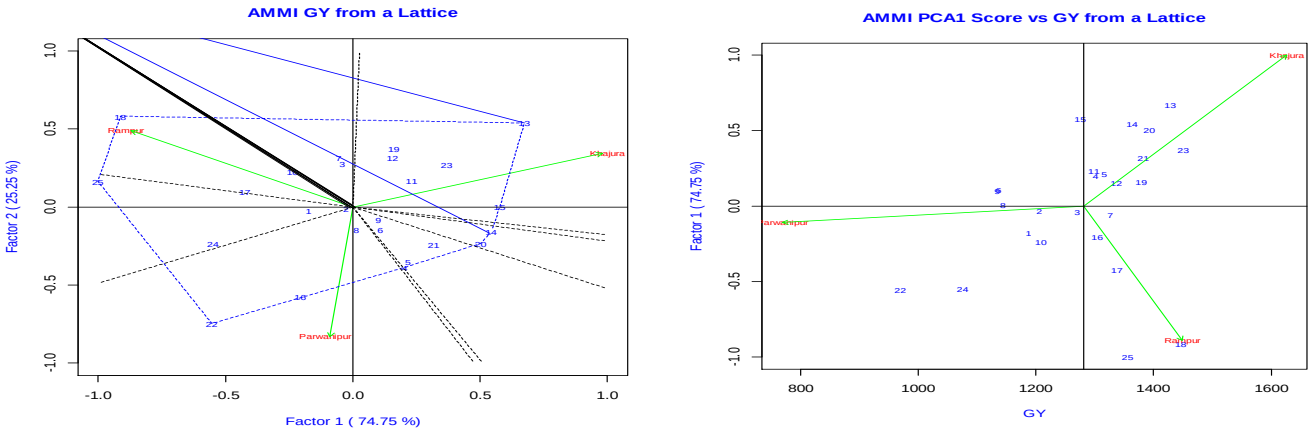

FIGURE 2: AMMI PCA 1 score vs GY from lattice tested at three environments over the years 2016-2017

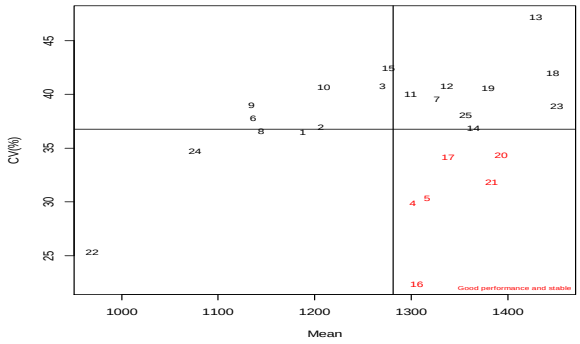

FIGURE 3: Stability and high yield accessions based on Plot CV over the years 2016-2017

Accordingly, the AMMI1 graph shows that accessions Khajura-2 and RL-12 stood out with the lowest PCA1 scores (Figure 12). This indicates that these were least involved with the interaction, and are therefore the most stable. However, only the yield of lentil accession Khajura-2 had high than the RL-12. On the other hand, the accessions WBL-77 and PL-4 were found the most unstable, however WBL-77 had with the highest average yield. Rampur environment stood out with a small contribution to the interaction and with a high contribution (Khajura) (Figure 3). Environments Rampur and Khajura averages were recorded above the overall averages (1281 kg ha^{-1}), indicating that these were favorable environment to obtain high means. The most ideal accessions should combine high yield and stable performance across a range of production environments. Among the high yielding accessions ILL-7979, ILL-6819, ILL-7715, ILL-2712, ILL-7164 and Black Masuro can be best evaluated based on stability and good performance of grain yield with combined low absolute PC1 score and high yield (Figure 3).

3.4 Stability coefficient analysis of different parameters in AMMI model

In the study over the years, lentil accessions Shital, ILL-7979, RL_6, RL-11 and Sagun (bi=0.9752-1.07) were found to be biologically stable which can be adapted to all the environments. Based on the value of Eberhart and Russel mean square deviation (S_2di) resulted from stability coefficient -35689.79 to -6012.4454) consequently lentil accessions RL-6, Simal, ILL-2712, ILL-6467, LG-12, RL-12, Shital, Khajura-2, ILL-3490, ILL-8006, HUL-57, Sagun, ILL-7715, ILL-7164 , WBL-77 , ILL-6819 and ILL-7723 were found to be stable and adapted to all environments. Based on the value of determination of the coefficient (R_2), lentil accessions RL-49, RL-79, ILL-7723 and ILL-4605 were found to be agronomic stable across the environments over the years. Based on the smaller values of variation coefficient (CV), lentil accessions ILL-2712, ILL-7723, ILL-7715 and ILL-7164 had small values in the ranges (22.38-30.38%) and therefore these accessions were found to be biologically stable across the environments over the years 2016-2017. Based on the small values (-1147.50) of Shukla's variance (ri^2), lentil accessions RL-6 was found to be agronomic stable across the environments over the years. Perkin and Jink's (1968) regression coefficient is similar to the FW method but the observations are adjusted for site effects before the regression is invoked. Based on the smallest values (127.56 -454.56) of Perkin's and Jinks(DJ_i) lentil accessions RL-6 and Simal were found to be stable across the environments over the years. Wricke's (1962) ecovalance (W2) stability parameter

gives the relative contribution of each genotype in a test of total GE interaction. Based on the lowest values (193.05) of Wricke's Ecovalence (W), lentil accessions RL-6 was found to be stable and well adapted across the environments over the years. Based on the Superiority Measure (Pi) increased the stability if it has small values (39457.39-51215.09), lentil accessions WBL-77 and ILL-6467 were found to be stable and well adapted across the environments over the years 2016-2017 (Table 4).

TABLE 4
STABILITY COEFFICIENT ANALYSES FOR GRAIN YIELD (KGHA-1) TESTED AT THREE ENVIRONMENTS OVER THE YEARS 2016-2017

		*	*	Francis	Eberhart &Russell	*	*	Shukla	Perkins &Jinks	*	Wricke's Ecovalence	Superiority Measure
GEN	Names	Mean	Sd	CV(%)	bi	S2di	R2	ri2	Bi	DJi	Wi	Pi
1	ILL - 8006	1187.5	434.0855	36.5546	0.9517	-29769.9	0.984	2552.755	-0.0483	6047.424	7001.536	134890.3
10	Sagun	1209.222	492.9771	40.7681	1.0702	-18620.6	0.9646	9189.179	0.0702	17196.74	19212.55	118494.6
11	HUL - 57	1299.167	520.9608	40.0996	1.1433	-28140.6	0.9859	7488.601	0.1433	7676.723	16083.49	84366.71
12	LG-12	1336.889	545.787	40.8252	1.2049	-34398.2	0.9976	8860.657	0.2049	1419.131	18608.07	65997.43
13	PL- 4	1429.222	675.4352	47.2589	1.4408	26754.72	0.9314	75986.58	0.4408	62572.08	142119.8	55675.34
14	RL - 11	1364.222	503.5754	36.913	1.0334	34155.08	0.862	37024.52	0.0334	69972.44	70429.59	82821.34
15	RL - 4	1276.389	542.6601	42.5153	1.1265	33643.07	0.8821	40057.43	0.1265	69460.43	76010.14	111156.3
16	ILL - 2712	1305.389	292.2599	22.3887	0.644	-34791.2	0.994	27497.54	-0.356	1026.134	52899.95	107702.7
17	Black Masuro	1337.944	457.4478	34.1903	0.9599	5474.282	0.9013	21546.14	-0.0401	41291.64	41949.37	71931.38
18	RL - 79	1446.222	608.152	42.0511	1.1172	192892.7	0.6908	126103.7	0.1172	228710.1	234335.2	53828.46
19	ILL - 6467	1379.722	560.5175	40.6254	1.2378	-34689.4	0.9982	11941.37	0.2378	1127.936	24276.58	51215.1
2	RL - 6	1205.944	446.7794	37.0481	0.9874	-35689.8	0.9997	-1147.51	-0.0126	127.5666	193.0533	125653.4
20	ILL - 7979	1393.167	479.227	34.3984	0.9819	28782.44	0.8594	33928.88	-0.0181	64599.8	64733.61	75123.05
21	ILL - 6819	1383.056	441.4315	31.9171	0.9382	-6474.46	0.9247	15543.44	-0.0618	29342.9	30904.39	71332.53
22	ILL - 7723	969.7778	246.3688	25.4047	0.473	-6012.45	0.7545	76739.32	-0.527	29804.91	143504.8	309070
23	WBL - 77	1451	565.3628	38.9637	1.2303	-16198.9	0.9693	21209.24	0.2303	19618.41	41329.46	39457.4
24	ILL- 4605	1076	374.7414	34.8273	0.7504	14517.34	0.8208	39964.34	-0.2496	50334.7	75838.85	211985.2
25	RL - 49	1356.222	517.5693	38.1626	0.8716	188947.6	0.5805	124571.7	-0.1284	224765	231516.4	90959.99
3	RL - 12	1269.778	517.955	40.791	1.1423	-33472.3	0.9956	4528.918	0.1423	2345.101	10637.68	89883.49
4	ILL - 7715	1301.167	388.9898	29.8955	0.8338	-17813.1	0.9405	14677.49	-0.1662	18004.29	29311.05	102708.4
5	ILL - 7164	1316.278	399.9915	30.3881	0.8583	-17392.3	0.9424	13230.8	-0.1417	18425.04	26649.14	95460
6	ILL - 3490	1135.889	429.8212	37.8401	0.9446	-31628.8	0.9887	1706.143	-0.0554	4188.546	5443.768	167579.1
7	Khajura - 2	1326.333	525.6886	39.6347	1.1582	-32294.6	0.9936	6231.401	0.1582	3522.722	13770.24	67354.29
8	Simal	1144.278	418.7663	36.5966	0.925	-35362.8	0.9987	246.3736	-0.075	454.5692	2757.793	161807.1
9	Shital	1134.444	442.8602	39.0376	0.9752	-32906.1	0.9926	466.5451	-0.0248	2911.289	3162.909	166078.4

3.5 Mean yield performances and stability analysis of the lentil accessions

GGE biplot based on environment-focused scaling for pooled mean performances and stability of the accessions over the years 2016-2017 and across the three environments indicated that lentil accessions PL-4, WBL-77, RL-4, RL-11 and ILL-7979 had the highest mean yield (Fig. 4) while accessions RL-79, RL-49, ILL-7723 and ILL-4605 were highly unstable and below average yield, whereas Shital, RL-6, ILL-3490 and Simal highly stable, were followed by HUL-57, ILL-8006 and RL-4 with above average yield over the years 2016-2017 across the three environments.

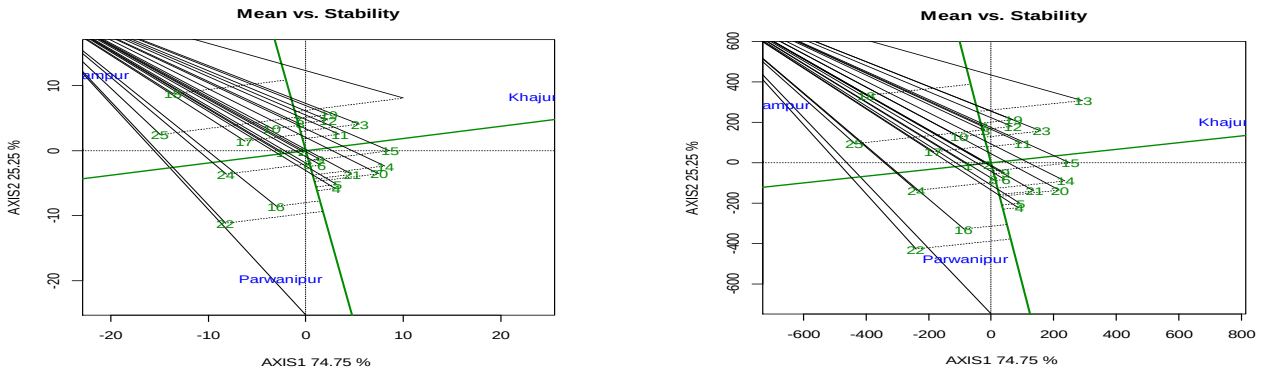

FIGURE 4, 5: GGE biplot based on environment-focused scaling for mean performance and stability of the genotypes over the years 2016-2017

3.6 Discriminating ability and representativeness of the test environment

GGE biplot discriminating ability and representativeness is an important measure of the testing environments. The concentric circles on the biplot as shown in Figure 5 help to visualize the length of the environment vectors, which is proportional to the standard deviation within the respective environments and is a measure of the discriminatory ability of the environments. Based on ranking accessions based on the discriminativeness against representativeness of test environments for biofortified lentil data over the years indicated that all environments looked diverse and they are discriminating based on the location specific across the three environments over the years (Fig 6). However, Parwanipur are looked close to the centers and might be discriminative (informative) whereas Khajura and Rampur least representative over the years (Fig vi). All three test environments (locations) that are discriminating and representative are good test environment for selecting wider adaptable accessions. Discriminating but non-representative test environments like Khajura and Rampur are useful for selecting specifically adaptable accessions if the target environments can be divided into mega-environments or they are useful for culling unstable accessions if the target environment is a single mega-environment.

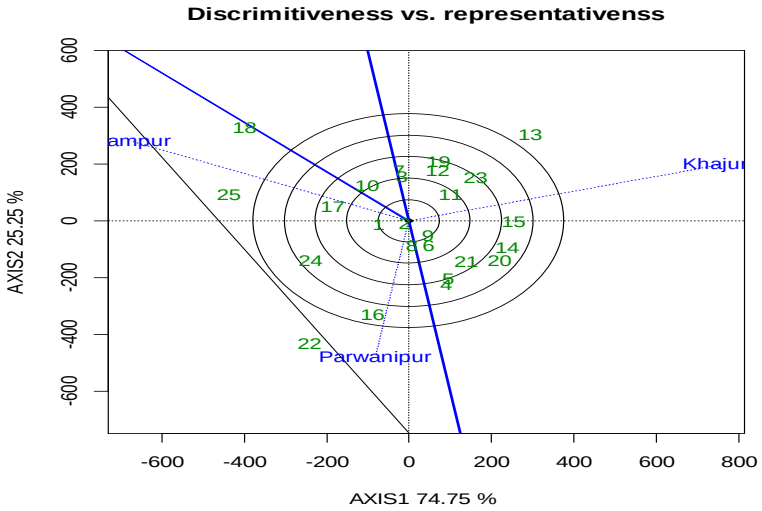

FIGURE 6: Ranking accessions based on the discriminativeness against representativeness of test environments for biofortified lentil data over the years 2016-2017

3.7 Ranking accessions relative to the ideal genotype

An ideal genotype should have the highest mean performance and be absolutely stable (that is, performs the best in all environments). Such an ideal genotype is defined by having the greatest vector length of the high yielding accessions and with zero GEI, as represented by an arrow pointing to it (Figure 7). A genotype is more desirable if it is located closer to the ideal genotype. Thus, using the ideal genotype as the centre, concentric circles were drawn to help visualize the distance between each genotype and the ideal genotype. Because the units of both PC1 and PC2 for the accessions are the original unit of yield in the genotype focused scaling (Figure 7), the units of the AEC abscissa (mean yield) and ordinate (stability) should also be in the original unit of yield.

3.8 Ranking of environments relative to the ideal environments

Based on this, Rampur located in the first concentric circle and has been the most ideal environment (Figure 8). Thus, Rampur environment was close to the ideal environment and this environment has been identified as desirable environments while Khajura environment was close to the second concentric circle and has been good for grain yield performances but Parwanipur environment was far distance than the centre therefore it might be useful to identify the location specific accessions. This difference between environments can be related to soil fertility, climate changes and other environmental variations from year to year.

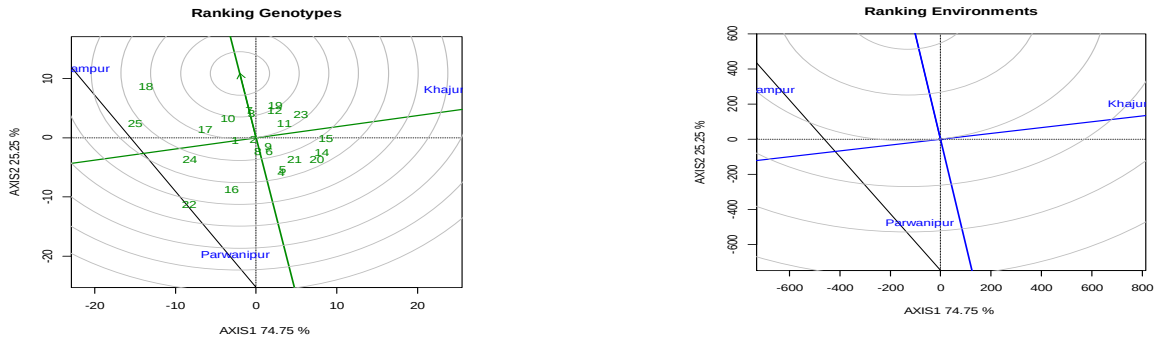

FIGURE 7, 8: GGE biplot based on genotype-focused scaling for comparison of the genotype with ideal genotype over the years 2016-2017 (Left) and Fig viii GGE biplot based on environment-focused Scaled by no scaling model centered by tester-centered G+GE for comparison of the environment with ideal environment over the years 2016-2017(Right)

3.9 Which genotype won where and mega environments with GGE bi-plot

One of the most attractive features of a GGE biplot is its ability to show the which-won-where pattern of a genotype by environment data set (Figure 9). Figure 9 indicated that accessions PL-4, RL-79, RL-49, ILL-7723 and ILL-7979 were the vertex accessions which showed the highest yield in specific environments. Here an example, the equality line among PL-4 and RL-79 indicates that PL-4 was better in Khajura while RL-79 was better in Rampur environment. This pattern suggests that the target environment may consist of two different mega-environments and that different accessions should be selected and deployed for each.

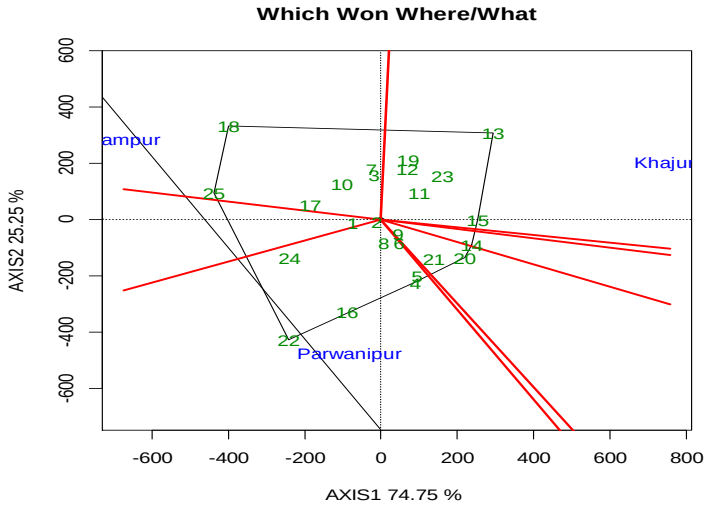

FIGURE 9: The which-won-where view of the GGE biplot to show which genotypes performed best in which environment in no scaling models centered by double centered GE over the years 2016-2017

3.10 AMMI 1 biplot analysis

Based on the stability analysis of AMMI Biplot Model Type 1 across the environments over the years in the graph showed that the genotypes which are in the right side of perpendicular i.e. accessions PL-4, RL-4, RL-11 and ILL-7979 are less affected by G x E inter action. Accessions ILL8006, RL-6, Shital, ILL3490 and simal were more close to the center point and indicated that stable across the environments (Figure 10).

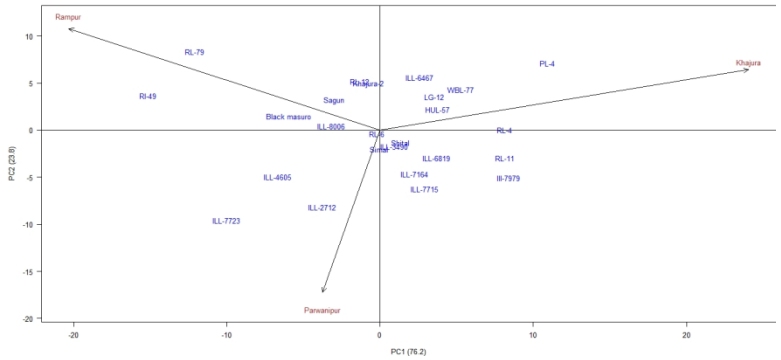

FIGURE 10: Stability analysis of AMMI Biplot Model Type 1 across the environments over the years (2016-2017)

TABLE 5

AMMI STABILITY VALUE AND YIELD STABILITY VALUE BASED ON THE # CROPS WITH IMPROVED STABILITY, 2016-2017

GEN	ASV	YSI	rASV	rYSI	means
2	0.53	19	1	18	1202
8	2.05	23	2	21	1146
6	2.42	25	3	22	1145
9	2.72	27	4	23	1136
7	5.08	16	5	11	1326
1	5.67	26	6	20	1196
3	5.71	24	7	17	1283
5	6.16	18	8	10	1329
10	6.18	28	9	19	1201
11	7.1	25	10	15	1295
21	7.24	15	11	4	1399
19	7.26	17	12	5	1379
12	7.31	25	13	12	1319
4	8.14	28	14	14	1299
23	10.41	16	15	1	1458
16	10.59	29	16	13	1311
17	10.79	26	17	9	1332
24	12.9	42	18	24	1047
15	14.59	35	19	16	1285
14	14.86	27	20	7	1363
20	15.87	27	21	6	1377
22	20.3	47	22	25	991
13	20.8	26	23	3	1420
18	23.2	26	24	2	1424
25	27.36	33	25	8	1359

The AMMI Stability Value (ASV) and AMMI stable index are calculated as suggested by Zobel et al, 1998 and Purchase et al.1997 and their ranks are presented in Table 5. The highest mean grain yield of genotypes averaged across the environments over the years 2016-2017 were produced by WBL-77(G-23) (1458 kg ha^{-1}) followed by RL-79(G-18) (1424 kg ha-1), PL-4(G-13) (1420 kgha-1) and ILL-6819(G-21) (1399 $kgha^{-1}$) while lowest by ILL-7723(G-22) (991 kg ha^{-1}). The genotypes which has low stability value (ASV) is said to be stable and the breeder chose the stable genotypes, having grain yield above the mean grand yield. In this experiment accessions RL-6(G-2) ranked 1st stability(ASV-0.53) followed by Simal(G-8) (ASV-2.05) , ILL-3490(G-6)(ASV-2.42) and Shital(G-9) (ASV-2.72) and suitable for all environment but out of test accessions; WBL-77(G-23), RL-79, PL-4 and ILL-6819(G-21), ILL-6467(G-19), ILL-7979(G-20), RL-11(G-14), RL-

49(G-25), Black Masuro(G-17),ILL-7164(G-5), Khajura-2(G-7), LG-12(G-12) and ILL-2712(G-16) produced the mean yield above grand mean (Table 2).

3.11 Pearson Correlation Coefficient Analysis

TABLE 6
PEARSON'S PRODUCT-MOMENT CORRELATION BETWEEN THE GRAIN YIELD AND YIELD CONTRIBUTING TRAITS OF LENTIL

Traits	DF	DM	PLHT	PP	SP	HSWT	GY
DF	1	0.576**	0.011	0.048	0.278**	0.057	0.306**
DM		1	0.103*	-0.036	0.216**	0.165**	0.497**
PLHT			1	0.324**	0.324**	0.383**	0.564**
PP				1	0.217**	0.278**	0.246**
SP					1	0.017	0.474**
HSWT						1	0.330**
GY							1

Note: ** and * indicates significant at 1% and 5% level of significance

Correlation between the grain yield and yield attributing traits was done as in Table 6. Yield is one of the most important trait during the selection criteria and influenced by different yield attributes i.e days to flowering, days to maturity, plant height, number of pods and seeds per plant, hundred seed weight. The correlation Table showed that there was a positive and highly significantly correlation with yield and days to 50% flowering (r=0.306, P≤.001). Similarly, based on the correlation, there was highly significant and positive correlation between the yields with the yield attributing traits like days to maturity, plant height, pods per plant, seeds per pod and hundred seed weight at 1% level of significance. Days to 50% flowering was positively and highly significantly associated with days to maturity (r=0.576, P≤.001) and number of seeds per pod (r=0.278, P≤.001). There was also positive but no significant correlation with 100 seed weight, pods per plant and plant .Similarly, there was positive and significant correlation between days to maturity and plant height (r=0.103, P≤.005), seeds per pod(r=0.216, P<0.001), hundred seed weight(r=0.165, P<0.001) and grain yield (r=0.497, P≤0.001) but negative correlation with pods per plant(r=-0.036) . Likewise, plant height had significantly positive correlation with number of pods per plant (r=0.324, P≤.001), seeds per pod(r=0.324, P≤.001), hundred seed weight(r=0.383, P≤.001) and grain yield (r=0.564, P≤.001) .There was a positive and significant correlation with number of pods per plant with seeds per pod(r=0.217, P≤0.001), and similarly positive association was observed with 100 seed weight(r=0.278, P≤0.001) and also with seed yield(r=0.246, P≤0.001). Similarly positive correlation was observed between the number of seeds per pod with the hudred of seed weight ((r=0.017) but the highly significant correlation with the grain yield of (r=0.474, P≤.001) and likely highly significantly and positive correlation with grain yield was also recorded between the 100 seed weight and the yield (r=0.330, P≤.001) .

IV. DISCUSSION

GGE biplot based on environment-focused scaling for pooled mean performances and stability of the accessions across the three environments over the years indicated that lentil accessions PL-4, WBL-77, RL-4, RL-11 and ILL-7979 had the highest mean yield whereas Shital, RL-6, ILL-3490 and Simal were found highly stable. Based on ranking accessions based on the discriminativeness against representativeness of test environments for biofortified lentil data over the years indicated that all environments looked diverse and they are discriminating based on the location specific across the three environments over the years. An ideal genotype is defined as one of the highest yielding across the test environments and is definitely stable in performance (Yan and Kang, 2003). In the genotype-focused the GGE biplot analyses, concentric circles are drawn to help visualize the distance between each genotype and the ideal genotype (Naroui Rad et al., 2013). Lentil accessions Khajura-2. RL-12, Sagun, LG-12 and ILL-6467 which fell into the centre of concentric circles, was the ideal genotype in terms of stability, compared with the rest of the accessions. In addition, RL-79, PL-4, WBL-77, HUL-57 and Black Masuro located on the next consecutive concentric circle, may be regarded as desirable accessions in terms of higher yielding ability. Based on this, Rampur located in the first concentric circle and has been the most ideal environment. The yield performances was found poor at Parwanipur because of high rainfall (204-260 mm) occurred during the cropping season and looked more prevalent of stemphylium diseases consequence reduced yield substantially than other locations. Many researchers find this use of a biplot intriguing, as it graphically addresses important concepts such as crossover GE, mega environment differentiation, specific adaptation etc. as discussed in Yan and Tinker (2006). Lentil accessions PL-4, RL-79, RL-49, ILL-7723 and ILL-7979 were the vertex accessions which showed the highest yield in specific environments. It has been proposed that GGE biplot analysis was a useful multi-location trial for the analysis of GE interactions (Butron et al., 2004;

Fan et al., 2007; Laffont et al., 2007; Yan and Kang, 2003; Samonte et al., 2005) and had been exploited in the variety evaluation of wheat (Yan and Hunt 2001; Yan et al., 2000), Maize (Fan et al., 2007) and soybean (Yan and Rajcan, 2002). Based on the stability analysis of AMMI Biplot Model Type 1 across the environments over the years in the graph showed that the genotypes which are in the right side of perpendicular i.e. accessions PL-4, RL-4, RL-11 and ILL-7979 are less affected by G x E interaction. Accessions ILL8006, RL-6, Shital, ILL3490 and simal were more close to the center point and indicated that stable across the environments. In another words, the genotypes which have low stability value (ASV) is said to be stable and the breeder chose the stable genotypes along with grain yield above the mean grand yield. In this experiment accessions RL-6(G-2) ranked 1st stability (ASV-0.53) followed by Simal (ASV-2.05), ILL-3490 (ASV-2.42) and Shital (ASV-2.72) and suitable for all environment. Based on the value of Eberhart and Russel mean square deviation (S_2di) resulted from stability coefficient (-35689.79 to -6012.4454) consequently lentil accessions RL-6, Simal, ILL-2712, ILL-6467, LG-12, RL-12, Shital, Khajura-2, ILL-3490, ILL-8006, HUL-57, Sagun, ILL-7715, ILL-7164 , WBL-77, ILL-6819 and ILL-7723 were found to be stable and adapted to all environments. Based on the lowest values (193.05) of Wricke's Ecovalence (W), lentil accessions RL-6 was found to be stable and well adapted across the environments over the years. Based on the small values (-1147.50) of Shukla's variance (ri^2), lentil accessions RL-6 was found to be agronomic stable across the environment. These stability coefficient parameters showed the diverse results of stable accessions however accession RL-6 was found the most stable accession because all the stability parameters provided the same results.

Plant breeders consistently face GE interactions when testing genotypes across the environments. Combined pooled mean analysis of variance (ANOVA) for grain yield tested at three environments over the two subsequent years showed that highly significant differences in genotypes, environment and G x E interaction effect. From the study, it was concluded that accession WBL-77 (1451 kg ha^{-1}), RL-79(1446 kgha^{-1}) and PL-4(1429 kgha^{-1}) were the best performer and well adopted across the environments. The different performance of genotypes across environments could also be indicative of wide variation in climatic conditions and soil types in the different growing environments. Consequently, comparisons can only be made in each environment separately (Breese, 1969). Crossa et al. (1991) had noted that the use of AMMI in G x E interaction analysis would lead to the selection of superior genotypes even in the field experiment. Here, AMMI analysis of variance for lentil grain yield (tha^{-1}) of lentil accessions tested at three environments over the years showed that 80.71% of the total sum of squares was attributed to environmental effects, only 8.38 % to genotypic effects and 10.90% to genotype × environment interaction effects. The partitioning of GGE sum of squares through the GGE biplot analysis showed that PC1and PC2 accounted 74.75%, and 25.24% of GGE sum of squares respectively over the years. In the biplot, a total of fifteen lentil accessions namely PL-4, RL-11, ILL-6819, ILL-7979, WBL-77, HUL-57, ILL-7715, ILL-6467, ILL-7164, LG-12, ILL-2712, Black Masuro, Khajura-2, RL-79 and RL-49 and two environments (Khajura and Rampur) located on the right side of the black vertical line showed the highest yield and more stable accessions. It seems that other statistical models such as regression procedures are more useful for understanding and describing G x E interactions. The GxE interaction is an important source of variation in any crop. Geographic differentiation of landraces of lentil emphasizes the specific adaptation of this crop (Erskine, 1997). According to Freeman (1972), one of the main reasons for growing genotypes over a wide range of environments is to estimate their stability and adaptability. Biological stability is not acceptable to most plant breeders, who prefer an agronomic concept of stability. In this concept of stability, it is not necessary for the genotypic response to environmental conditions to be equal for all genotypes.

V. CONCLUSION

Pooled mean analysis of variance for grain yield tested at three environments over the two subsequent years showed that highly significant differences in genotypes, environment and G x E interaction effect indicating the possibility of selection for stable accessions. Research results showed that lentil accession WBL-77 (1451 kg ha-1) , RL-79(1446 kg ha-1) and PL-4(1429 kg ha-1) were the best performer and well adopted across the environments and over the years. AMMI analysis of variance for lentil grain yield (tha^{-1}) of lentil accessions tested at three environments over the years showed that 80.71% of the total sum of squares was attributed to environmental effects, only 8.38 % to genotypic effects and 10.90% to genotype × environment interaction effects. Based on the value of Eberhart and Russel mean square deviation (S2di) resulted from stability coefficient (-35689.79 to -6012.4454) consequently lentil accessions RL-6, Simal, ILL-2712, ILL-6467, LG-12, RL-12, Shital, Khajura-2, ILL-3490, ILL-8006, HUL-57, Sagun, ILL-7715, ILL-7164 , WBL-77, ILL-6819 and ILL-7723 were found to be stable and adapted to all environments. Accessions ILL8006, RL-6, Shital, ILL3490 and simal were more close to the center point and indicated that stable across the environments. In another words, the genotypes which have low stability value (ASV) is said to be stable and the breeder chose the stable genotypes along with grain yield above the mean

grand yield. In this experiment accessions RL-6(G-2) ranked 1st stability (ASV-0.53) followed by Simal (ASV-2.05), ILL-3490 (ASV-2.42) and Shital (ASV-2.72) and suitable for all environment.

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ANNEX 3

MEAN AGRO-METEOROLOGICAL DATA FOR THE FY 2016-2017 AND GEOGRAPHIC INFORMATION

Environment		Mean Yield (Kg/ha)	Latitude	Longitude	Altitude (m)	Temp (0c)		Rainfall (mm)
Location	Year					Min	Max	
Khajura	2016/17	1351	28^0 06" N	81^0 37" E	181	14.81	25.26	48.1
	2017/18	1865				19.58	29.24	99.2
Parwanipur	2016/17	796	27^0 4' 40.9" N	$84^0$56' 9.85"	75	16.01	28.19	260
	2017/18	779				16.22	28.35	204.2
Rampur	2016/17	1387	27^o 40′N	84^o19′ E	228	14.82	28.18	202
	2017/18	1438				16.00	28.8	87.5

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