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Agriculture Research and Extension Division
Department of Agriculture
Ministry of Agriculture and Forests
Thimphu: Bhutan

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Editorial
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FOREWORD

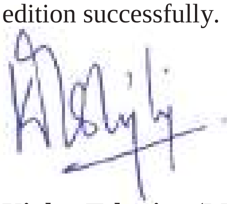
The Department of Agriculture is delighted to bring out the second volume of the Bhutanese Journal of Agriculture (BJA). BJA is a print open access English language journal on agriculture and publishes research articles annually with the primary purpose of providing an additional mechanism to disseminate appropriate technologies, and knowledge and information in the agriculture sector.

The BJA targets research findings that are innovative and relevant to sustainable agriculture development in Bhutan, and includes cross-cutting themes like postharvest, economics, agro-biodiversity, soil and water management, farming systems, pest and disease management and climate change.

We are continuously working at improving the quality of the journal and just this past year's single and maiden issue in itself has been a huge learning curve. Our editorial team, comprising members entirely from within the Department of Agriculture, is working hard to push a notch up with every issue. We are also looking at registering a DOI (digital object identifier) for the journal and launching its electronic version.

It is indeed rewarding to note that the BJA has, up to a significant extent, succeeded in motivating our researchers and field colleagues in maintaining a vibrant culture of research and scientific communication – key to success in research for agriculture development.

The department would like to thank the authors and the reviewers alike for their contribution. Above all, the BJA Editorial Board is commended for putting in extra effort in publishing this edition successfully.



Kinlay Tshering (Ms)
DIRECTOR

EDITORIAL

After a successful launch in February 2018, we are pleased to publish the second volume of the Bhutanese Journal of Agriculture (BJA). We are in the process of conscientiously improving the BJA in terms of its technical content, professional outlook and scientific relevance for our agricultural scientists. As intended, the BJA provides a platform for our researchers and rural development workers to showcase their scientific work and build a career path that is competitive both nationally and globally. We are encouraged by article contributions from the non-RNR sector such as the Sherubtse College in Kanglung and Samdrup Jongkhar Initiative (SJI). We promote and value such contributions.

In our second volume of the journal, we have a wide range of technical papers from crop production, economics, post harvest management, agro-biodiversity and morpho-agronomic analysis of new crop varieties. The paper on evaluation of chilli drying methods conclude that poly-tunnel drier is more efficient, economic and convenient compared to raised bamboo mat or bamboo mat with plastic roofing. The study on the analysis of SPAD value and chlorophyll content of organic vegetables under a protected system found a significant positive correlation between SPAD values, chlorophyll content and dry weight for spinach, fenugreek and amaranthus. Analysis of the status of on-farm diversity of field crops in two *geogs* of Samdrup Jongkhar revealed that on-farm agro-biodiversity plays a critical role in the food security and livelihood of farmers. Important insect pests, diseases and beneficial insects in fruits and vegetables in west central Bhutan were documented, which will form a scientific basis for appropriate crop protection interventions.

Three separate but similar studies on post harvest damage and loss in apple, mandarin and rice provide information on losses incurred during harvest and post harvest operations. The reaction of Bhutanese wheat varieties to rust diseases in mid and low altitudes showed a disease incidence of 2.5-16% and that the disease severity was positively correlated with temperature and humidity. A morpho-agronomic analysis of new rice germplasm was carried out at ARDC Bajo based on both qualitative and quantitative parameters. The paper on adaptation study of a new crop, Quinoa, under the Bhutanese farming system provides insight into its agronomic performance in different altitude regimes and crop sequences while the article on the efficacy of *Ageratina adenophora* confirmed that 10% aqueous extract can effectively control white rusts of crucifers. The study on growth and yield of oyster mushroom under different substrates indicated that Quinoa stubble was superior to banana leaf or lemon grass.

We wish you a happy reading of this rendition of the BJA!

Mahesh Ghimiray
EDITOR-IN-CHIEF

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Digital Soil Mapping of Soil Organic Carbon Stock in Bhutan

Tshering Dorji[a], Tsheten Dorji[b], Sangita Pradhan[b], Dawa Tashi[b], Karma Dema Dorji[b]

ABSTRACT

Soil organic carbon (SOC) plays an integral part in improving soil security, water security, food security, energy security, climate change abatement, biodiversity protection, and ecosystem services. It is important to understand its stock and spatial distribution for better management. However, not many countries have managed to map their national SOC stock and Bhutan is no exception. There is paucity of SOC information to clearly formulate plans and programs to increase Carbon (C) sequestration and enhance SOC storage in the country. A preliminary mapping of SOC stock of Bhutan for the top 30 cm depth was carried out to establish a baseline and contribute to global SOC mapping. A total of 993 data points was used for mapping SOC stock using regression kriging (RK). Regression tree model and ordinary kriging were used to perform the RK with elevation, land use land cover (LULC), slope, aspect, profile and plan curvatures, normalized difference vegetation index, SAGA wetness index, mean precipitation, mean temperature, geology, and terrain ruggedness index as environmental covariates. The model validation was done by repeated data splitting method. Preliminary results show that for the top 30 cm depth, Bhutan stores about 0.4 giga tonne carbon (GtC) with SOC density ranging from 0.5 to 315.3 ton ha^{-1}. Among the environmental covariates, LULC, topography, and climatic factors had significant influence on SOC stock and its spatial distribution. SOC stock was relatively low in the southern and eastern regions as opposed to the western and northern parts of the country. Under different LULC types, the SOC stock was lowest under agriculture land and highest under forest. These results are based on a small set of soil data and must be used with caution. However, for better SOC stock estimation and mapping, more and well distributed soil data will be necessary.

Keywords: *Soil Organic Carbon, Mapping, Land use land cover*

1. Introduction

Soil is essentially made up of minerals, organic carbon, water, and air. Among these four main components, soil organic carbon (SOC) forms the integral part of a functional soil. This is largely because SOC has the ability to improve the soil physical, chemical, and biological properties, which can enhance soil security. Enhanced soil security can improve food security, water security, energy security, climate stability, biodiversity, and ecosystem services (McBratney, Field & Koch, 2014). SOC also plays a key role in global carbon (C) cycle as it is

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the largest terrestrial C pool. Because of the important role it plays, SOC is often considered as a common indicator for soil security, water security, and ecosystem services. Further, SOC stock is one of the three indicators in assessing Land Degradation Neutrality (LDN) status by 2030. Bhutan being an LDN member country, information on SOC stock and its spatial distribution will be vital to assess its LDN status.

Globally, soil stores about 1500 GtC (1 GtC = 10^{15}gC) in the top one meter (Jobbágy & Jackson, 2000) which is approximately three times as much C found in the biosphere and twice as much C found in the atmosphere. For the top 30 cm depth, it stores about 680 GtC (FAO, 2017). Assuming that other components of global C cycle remain constant with current CO_2 concentration of 390 ppm, a change in global SOC storage by 1% may trigger a shift of about 8 ppm of CO_2 concentration in the atmosphere (Baldock, Wheeler, McKenzie & McBretney, 2012). This highlights the significance of C sequestration and storage in the soil to mitigate climate change. In order to enhance C sequestration and C storage, adequate information on SOC is necessary to formulate appropriate land management and C sequestration strategies. However, such information is limited in most of the countries, particularly in developing countries. This has posed a challenge to land managers in improving soil quality, increasing resilience to climate change, and enhancing ecosystem services through better SOC management.

The National Soil Services Centre (NSSC) under the Ministry of Agriculture and Forests made its first attempt to produce the SOC stock map of Bhutan for the top 30 cm depth with 993 observed data using digital soil mapping (DSM) techniques. The DSM of SOC stock of Bhutan was done with the objectives to set up a national baseline on SOC stock and contribute to global SOC stock mapping and formulate better C sequestration and SOC management strategies in the country.

2. Materials & Method

2.1. Study area

Bhutan is a landlocked country located in the Himalayas with China in the north and India in the east, west, and south. It has a geographical area of 38,394 km^2 with rugged terrain characterized by 'V' shaped valleys and high peaks. The valleys are characterized by narrow alluvial floors, fans, and terraces, with the lower slopes and alluvia often mantled with colluvia from upslope and aeolian deposits (Baillieet al., 2004; Caspariet al., 2006; Dorjiet al., 2009). Within less than 200 km (south-north), the altitudinal gradient increases from about 97 m to about 7570 m above sea level (masl). As such, there exist several agro-ecological zones with distinct climatic regimes in between. Monsoon dominates the climatic condition with annual precipitation varying from more than 2000 mm in the south to less than 1000 mm in the north and central parts of the country. The mean annual temperature ranges from approximately 14° to 26° C during summer and about -3° to 15° C in winter.

The Himalayan Mountains are young and still rising, leading to landscape dissection and natural soil erosion (Singh, Singh & Skutsch, 2010); the latter process is continually affecting soil development. There are four main soil zones grouped based on altitude i.e. i) moderately weathered and leached thin dark topsoil over bright subsoil up to about 3000 masl; ii) very bright orange-coloured non-volcanic andosolic soils and iii) acidic soils with thick surface litter that grade to weak podzols up to about 4000 m asl; and iv) alpine turf with deep dark and friable topsoil over yellowish subsoil mixed with raw glacial deposits above 4000 masl (Baillie et al., 2004).

More than 58% of Bhutan’s population depends on agriculture, livestock and forestry for their livelihood. However, the cultivated agricultural land accounts only for about 3% of the total land area (LCMP, 2010) due to rugged terrain and extreme climatic conditions. As such, more than 70% of the total agriculture land is located on steep slopes with high incidence of soil erosion. On the other hand, about 71% of the country is under forest cover (LCMP, 2010) with very rich biodiversity. As expected, the spatial variation of different LULC types is greatly influenced by altitude, slope, and climatic regimes. As presented in Figure 1, broadleaf forest is predominant below 2500 masl with coniferous forest between 2500m and 3500 masl. However, shrubs and grassland occur all along the altitudinal gradient. As anticipated, snow and screes are largely confined to areas above 3500 masl. Conversely, agriculture land is mostly located on valley bottoms and mountain foot slopes.

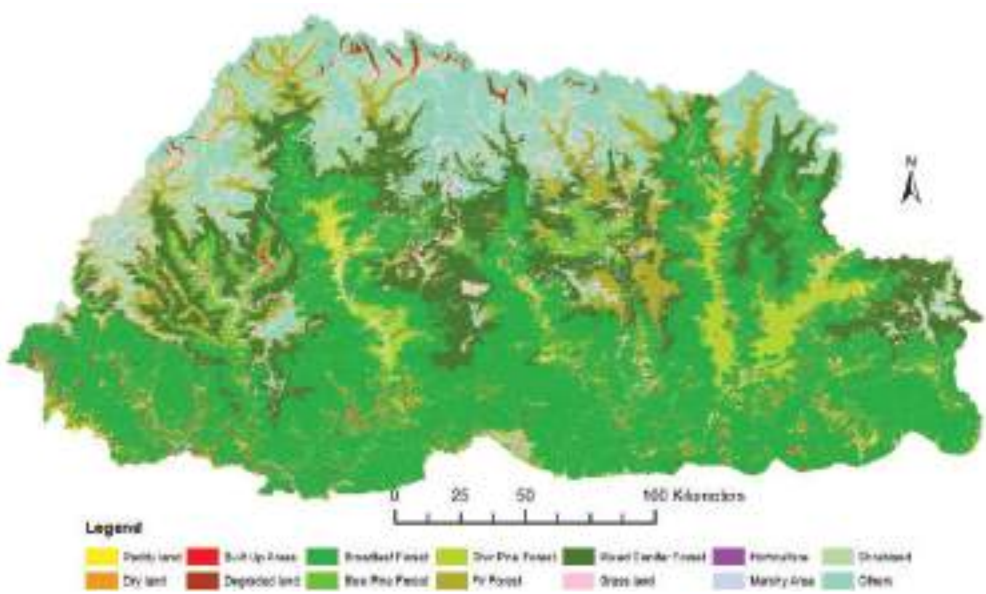

Figure 1.LULC map of Bhutan (LCMP, 2010)

2.2. Soil data

Soil information is limited in Bhutan as not many soil surveys have been done to date. As such, its soil resources are basically unexplored and not well documented. For this SOC mapping exercise, a total of 993 data points, from previous soil surveys (1997-2017), was used (Figure 1). About 80% of the total data points were from soil profile pits while the remaining data points were from auger bore holes. Soils were described and sampled based on genetic horizons. Samples were analysed for various soil parameters including carbon (C) concentration using (Walkley & Black, 1934) and bulk density using core ring method (Blake, Hartge & Klute, 1986).

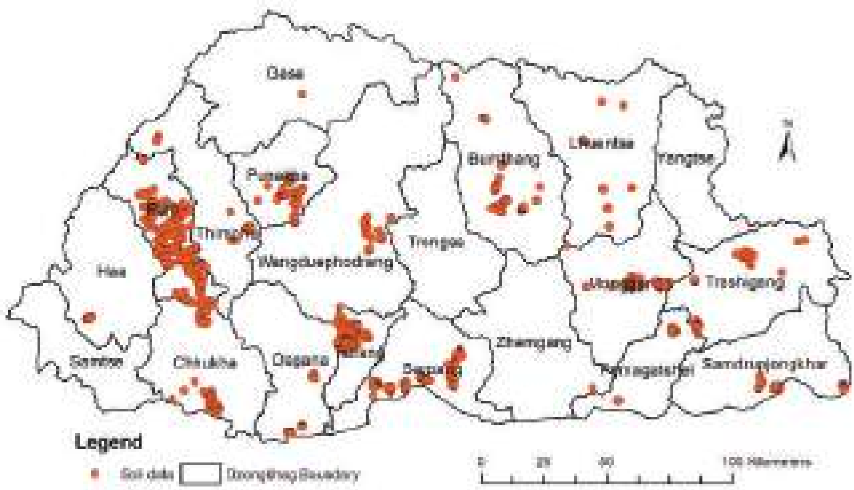

Figure 1.Distribution of soil observation sites

Since soil samples were collected based on genetic horizons, they had different soil depths and this posed a challenge to digitally map SOC stock for a particular soil depth. In this regard, an equal-area spline function was fitted to the profile values of the target soil variables using the CSIRO Spline Tool V2 (ASRIS, 2011) to convert the horizon-based values to the desired soil depth (0-30 cm). The equal-area spline function is based on the quadratic spline model of (Bishop, McBratney & Laslett, 1999).

2.3. Acquisition and derivation of environmental covariates

Digital Soil Mapping (DSM) of any soil property hinges on the use of easily discernible ancillary soil and/or environmental attributes. To generate the terrain attributes a 30 m digital elevation model (DEM) covering whole Bhutan was extracted from the Shuttle Radar Topography Mission (SRTM) elevation data portal (http://earthexplorer.usgs.gov) and was re-sampled to 1 km resolution. Slope gradient, aspect, slope curvatures (profile and plan), SAGA wetness index (SWI), and terrain ruggedness index (TRI) were derived from the DEM using the System for Automated Geo-scientific Analysis (SAGA) software (http://www.saga-gis.org/en/index.html) and Arc GIS software (version 10.3). In addition to the above covariates, the LULC data (LCMP, 2010), geological map (GEO) - Department of Geology and Mines), mean temperature and precipitation (www.worldclim.org), and normalized difference vegetation index ($NDVI$) were used as covariates after re-sampling to 1 km resolution.

2.4. Spatial modelling of SOC concentration and bulk density

Digital Soil Mapping (DSM) of any soil property is done with the assumption that a soil property of interest is closely associated with easily discernible ancillary environmental variables. This enables the target variable to be predicted by establishing relationships between it and the ancillary variables (McBratney et al., 2003). Based on this assumption, several methods have been used to digitally map the target variable. (Odeh, McBratney & Chittleborough, 1995) compared several methods of DSM: multi-linear regression, ordinary kriging, universal kriging, isotopic co-kriging, heterotopic co-kriging, and some variants of regression kriging (RK) models, and found that RK model to be most superior. A later study by Minasny and McBratney (2007) reported RK model to be more practical and robust than other prediction models. We used RK to digitally map the SOC stock.

RK has two main components i.e. regression and kriging (Figure 2). For the regression part, regression tree model (RTM) was used (Cubist 2.09 package) with elevation (DEM), LULC, slope, aspect, profile and plan curvatures, NDVI, SWI, mean precipitation, mean temperature, geology and TRI as covariates to predict the target variable. The RTM is found to be robust and appropriate for complex landscapes, such as in the Himalayas. The RTM is a non-parametric prediction model, which predicts the target variable based on linear regression models instead of discrete values predicted by the classical tree models (Minasny & McBratney, 2008).

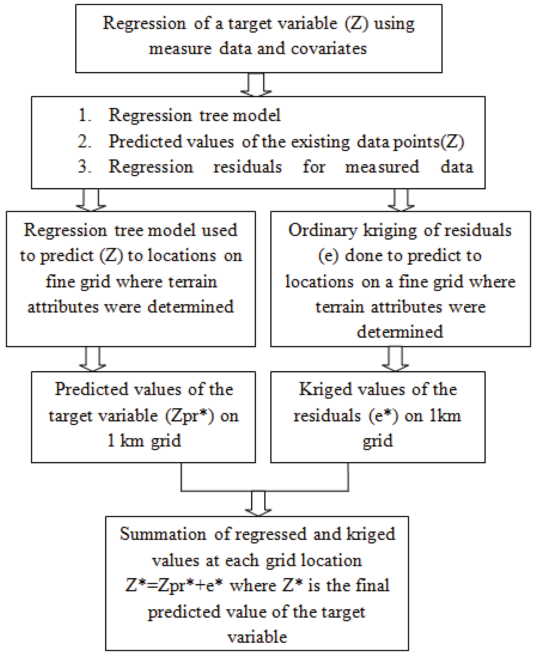

Figure 2.Flow chart showing the steps of RK for DSM (adapted from Odeh et al. (1995)).

At each node of the tree model, conventional linear least-squares regression is used to create the model associated with each of the terminal rules. Thus, the model generates a set of comprehensible rules, each of which has an associated multivariate linear model. When the rule conditions are met, the model predicts the target variable for each grid cell that has values for the appropriate predictor covariates (Minasny & McBratney, 2008). For the kriging part, the residuals, which are the difference between the measured and regressed values, were interpolated onto the entire 1 km grid, using a simple kriging, embedded in the package: Variogram Estimation and Spatial Prediction plus Error (VESPER) (Minasny, McBratney & Whelan, 2005). The final predicted value of the target variable at each 1 km grid cell was computed by summing up the regressed value from the RTM and the kriged residual (Figure 2).

2.5. Data validation

Any prediction model needs to be validated to assess its accuracy and reliability. It can be done either through external or internal validation. The former uses a new validation dataset from the same or similar population for validating previous models and is considered to be relatively better than internal validation methods. However, the difficulty in obtaining a new independent external dataset forces to go for internal validation. Repeated data splitting is a common internal validation method and we used this to validate our models. The whole data was partitioned into two portions, called the training and validation datasets. The training dataset constituted 70% of the total data points (698 points) and was selected through a simple random sampling procedure.The remaining 30% data was used as a validation dataset. Firstly, RTM was fitted onto the training dataset (using Cubist 2.09) and the model was used to predict the target variable for the validation dataset. Secondly, the residuals for the training dataset were calculated by subtracting the regressed values from the measured values of the target variable. Thirdly, the residuals of the training dataset were kriged to predict the residuals of the validation dataset using VESPER. The final RK predictions for the validation dataset was obtained by summing the regressed values from RTM and kriged values (Figure 2). The performance of the RK model was assessed by plotting the predicted values with measured values of the validation dataset. This whole process was repeated for 10 times to assess the stability of the prediction accuracy of the RK model. At each iteration, the statistical parameters including: (i) root mean square error (RMSE), (ii) coefficient of correlation (*R*), (iii) coefficient of determination (R^2), and (iv) mean error (ME) were determined and averaged at the end to provide the overall prediction accuracy of the model. The RMSE, which provides a measure of accuracy of the prediction method, is defined as:

$$\text{RMSE} = \sqrt{\frac{1}{n} \sum_{j=i}^{n} [z(s_j) - z^*(s_j)]^2} \quad (1)$$

and the ME (Odeh et al., 1995), which measures bias of prediction, is defined as:

$$\text{ME} = \frac{1}{n} \sum [z(s_j) - z^*(s_j \quad (2)$$

where $z(s_j)$ and $z^*(s_j)$ are the observed and predicted values, respectively (Eq. 1 and 2). For more accurate prediction, the RMSE should be as small as possible while the ME should be close to zero.

2.6. Computing SOC stock

Although SOC density and SOC stock are often used interchangeably in literature (Minasny *et al.*, 2006), they differ in scale and unit (Dorji *et al.*, 2014). SOC density is the SOC mass per unit area for a given depth, which can be calculated as:

$$SOC_d(kgm^{-2}) = SOC(kg/kg) * BD\ (kgm^{-3}) * D(m) \quad (3)$$

where SOC_d is SOC density (kg m^{-2}), SOC is SOC concentration (kg/kg), BD is bulk density (kg m^{-3}) and D is depth interval thickness (m). On the other hand, SOC stock is the actual SOC mass for a given soil depth and area. It was calculated by summing up the product of SOC density and area of the smallest mapping unit e.g. grid cell 1 × 1km^2.

$$SOC_{st}(t) = \sum_{i=1}^{n} (SOC_{di} * A_i)/10^3 \quad (4)$$

where SOC_{st} is SOC stock in metric tonne (t), n is number of 1 km grid cells, SOC_{di} is SOC density of grid cell for a particular depth interval (kg m^{-2}), A_i is an area of 1 km grid cell (1km^2) and 10^3 is the unit conversion factor.

3. Results and Discussion

3.1. Spatial modelling of SOC concentration and bulk density

As shown in Table 1, the RTM based on the whole dataset (993 data), used MT, GEO, NDVI, PLCUR, slope, ASP, MP, and ALT as conditions to perform the regression for SOC concentration. However, MP, MT, ALT, NDVI, TRI, slope, ASP, SWI, PLCUR, and PRCUR were used as covariates. Similarly, for bulk density, MP, ALT, SWI, MT, PRCUR, NDVI and ASP were used as conditions and MT, MP, ALT, NDVI, TRI, SWI, slope, PRCUR, PLCUR, and ASP as covariates. Among the environmental covariates, MP, MT, ALT, and NDVI showed more influence on both SOC concentration and bulk density, and their spatial distributions (Table 1).

Table 1.Usage (%) of covariates in the RTM for predicting SOC concentration and bulk density

Attribute usage	For SOC Concentration (0-30 cm depth)			
Conditions (Usage in %)	MT (99%)	GEO (72%)	NDVI (43%)	PLCUR (32%)
	Slope (25%)	ASP (24%)	MP (13%)	ALT (9%)
Environmental covariates used in regression tree model (Usage in %)	MP (89%)	MT (86%)	ALT (70%)	NDVI (60%)
	TRI (57%)	Slope (48%)	ASP (46%)	SWI (41%)
	PLCUR (14%)	PRCUR (11%)		
Attribute usage	For Bulk density (0-30cm depth)			
Conditions (Usage in %)	MP (75%)	ALT (49%)	SWI (45%)	MT (32%)
	PRCUR (12%)	NDVI (9%)	ASP (8%)	
Environmental covariates used in regression tree model (Usage in %)	MT (99%)	MP (95%)	ALT (91%)	NDVI (81%)
	TRI (74%)	SWI (67%)	Slope (62%)	PRCUR (31%)
	PLCUR (20%)	ASP (13%)		

TRI terrain ruggedness index, SWI SAGA wetness index, NDVI normalized difference vegetation index, MT mean temperature, MP mean precipitation, ASP aspect, PLCUR plain curvature, PRCUR profile curvature, ALT altitude, GEO geology.

Overall, the RTM performed well as indicated by low average error (AE) and ME for both SOC concentration and bulk density (Table 2). The AE was 0.89 g/100 g for SOC concentration and 0.05 g cm^{-3} for bulk density. The relative errors (RE) for both SOC concentration and bulk density were less than 1. The coefficient of determination (R^2) was moderately high for both SOC concentration (0.59) and bulk density (0.88). Looking at R^2 and ME values, the RTM was more robust and less bias in predicting bulk density than SOC concentration. This could be attributed to less spatial variation of bulk density compared to SOC concentration.

Table 2.Overall performance of RTM in predicting SOC concentration and bulk density

Depth (cm)	SOC (g/100g)					Depth (cm)	Bulk density (g cm^{-3})				
	AE	RE	ME	RMSE	R^2		AE	RE	ME	RMSE	R^2
0 - 30	0.89	0.61	0.005	1.34	0.59	0 - 30	0.05	0.23	0.0001	0.09	0.88

SOC soil organic carbon, AE average error, RE relative error, ME mean error, RMSE root mean square error, R^2 coefficient of determination

3.2. Validation of RTM and RK models

The overall performance of RTM and RK, in predicting SOC concentration and bulk density, was done using the repeated data splitting method. Based on the statistical parameters presented in Table 3 and 4, both RTM and RK performed better in predicting bulk density than SOC concentration. However, when compared between RTM and RK models, RK was supper in predicting both SOC concentration and bulk density with relatively low ME and RMSE and high coefficient of determination (R^2) (Table 3 & 4). Thus, RK was used to digitally map SOC stock.

Table 3.Performance of RTM in predicting SOC concentration and bulk density

Depth (cm)	SOC (g/100g)					Depth (cm)	Bulk density (g cm^{-3})				
	AE	RE	ME	RMSE	R^2		AE	RE	ME	RMSE	R^2
0 - 30	0.82	0.57	0.11	1.49	0.44	0 - 30	0.04	0.17	0.002	0.11	0.85

ME mean error, RMSE root mean square error, R^2 coefficient of determination

Table 4.Performance of RK in predicting SOC concentration and bulk density

Depth (cm)	SOC (g/100g)			Depth (cm)	Bulk density (g cm^{-3})		
	ME	RMSE	R^2		ME	RMSE	R^2
0 - 30	0.05	1.43	0.46	0 - 30	0.001	0.10	0.85

ME mean error, RMSE root mean square error, R^2 coefficient of determination

3.3. SOC density under different LULC types

Since SOC density provides better information for SOC storage than SOC concentration, SOC density (1×1 km^2 grid) was computed by multiplying RK predicted SOC concentration with bulk density (Eq. 1). Figure 3 shows relatively low SOC density in the valley bottoms where most of the agriculture fields are located. However, the upper slopes, which are mostly under forest, shrubland and grassland, have comparatively high SOC density. This indicates a strong influence of LULC and landform on the spatial distribution of SOC density. Under different LULC types, the mean SOC density for the upper 30 cm depth decreased in the order of mixed conifer forest> fir forest> others> grassland> shrubland> blue pine forest> marshy land> horticulture> dry land> paddy land> built-up areas> chirpine forest (Table 4). This is in line with the results reported by (Dorji, Odeh, Field & Baillie, 2014).

Table 4.Predicted SOC density under different LULC types (0-30 cm depth)

Sl #	LULC type	Mean SOC (t/ha)	Sl#	LULC type	Mean SOC (t/ha)	Sl#	LULC type	Mean SOC (t/ha)
1	Paddy land	62.16	6	Blue Pine Forest	84.79	11	Horticulture	71.58
2	Dry land	64.05	7	Chir Pine Forest	51.54	12	Marshy Area	74.1
3	Built Up Areas	60.52	8	Fir Forest	102.35	13	Shrubland	92.49
4	Degraded Land	81.75	9	Mixed Conifer Forest	105.21	14	Others	101.42
5	Broadleaf Forest	75.35	10	Grassland	98.26			

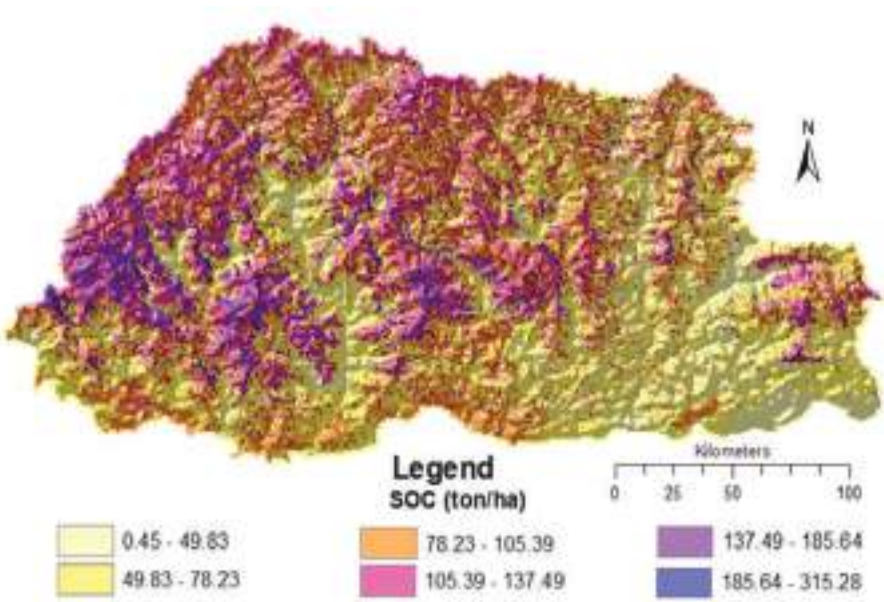

Figure 3.Predicted SOC density (1×1 km^2 grid) for the top 30 cm depth

The SOC stock for each grid was computed (Eq. 2) and added to estimate the overall SOC stock for the entire country. The preliminary results show that for the top 30 cm depth, Bhutan stores about 0.4 GtC with SOC density ranging from 0.45 to 315.28 ton ha^{-1}. The SOC stocks in the southern and eastern regions are relatively small as opposed to the western and northern parts of the country (Fig. 3). This is chiefly due to less forest cover and high rate of mineralization in the eastern and southern regions, respectively. The SOC stock under different LULC types was quite similar to what (Dorji et al., 2014) reported with SOC stock lowest under agriculture land and highest under forest.

4. Conclusion

The preliminary results show that Bhutan stores about 0.4 GtC in the top 30 cm depth. But the challenge now is how to maintain it against the backdrop of increased land degradation, unsustainable land management, and climate change. In this regard, land and land-based natural resources should be sustainably managed to reduce C emission and increase C sequestration. Furthermore, appropriate plans and policies need to be put in place to combat land degradation and increase SOC storage to mitigate climate change and enhance ecosystem services. This is the first attempt made to map SOC stock in Bhutan (0-30 cm depth) using DSM techniques. Since a small dataset was used for mapping SOC stock, the results presented here may not be very accurate and comprehensive. Hence, the information on SOC density and SOC stock should be used with caution. For more accurate and reliable SOC stock estimation and mapping, more and evenly distributed soil data is necessary. Furthermore, the capacity of the national staff on DSM needs to be developed.

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Morpho-agronomic Analysis of New Rice Germplasm at Agriculture Research and Development Centre, Bajo

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ABSTRACT

Extensive use of high yielding varieties has greatly reduced the genetic diversity in breeding germplasm of major food crops in the world. It is necessary to evaluate new rice germplasm to select superior lines of early maturing, high yielding, and resistance to biotic and abiotic stress breeding materials as strategy for rice improvement. This study was carried out to evaluate the morpho-agronomic characters of new rice germplasm and to compare the grain yield between 18 different rice germplasm, including the standard check BajoKaap 2. The experiment was conducted at the Agriculture Research and Development Centre, Bajo, Wangduephordang from April to October 2016. It followed a Randomized Complete Block Design with 18 treatments and three replications per treatment. The germplasm were evaluated based on 12 qualitative and 12 quantitative traits following the Descriptor for Rice Oryza sativa L and the standard evaluation systems during the different growth stages of the rice plant. Differences between the germplasm were observed in characters such as awning and flag leaf angle. Out of 18 treatments, eight had erect flag leaf angle while the rest exhibited intermediate type and only four had awns. Significant differences were observed between the treatments ($P<0.05$) in terms of plant height, leaf length, leaf width, number of tillers per hill, 50% flowering, panicle length, grain length, grain width and 1,000 grain weight. However, there was no significant difference in yield. Sinice these new rice germplasm are in the initial evaluation phase, further assessment may be required to ascertain their overall performance.

Keywords: *Descriptors for Rice, Germplasm, Quantitative trait, Qualitative trait, Rice, Yield*

1. Introduction

Rice is one of the most important food crops in the world. More than half of the world's population depends on rice for food and nutrition and in Bhutan, it is consumed three times a day and hence it is the staple crop. However, the domestic production of rice has not been able to meet the demand of the growing population due to low productivity. Today the self-sufficiency of rice is only 47% and the rest of the demand is met from imports. Bhutan imported 79,306 tons of rice in 2014 incurring a total cost of Nu 1787.60 million (NSB, 2016).

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Ghimiray (2012) pointed that low productivity and production in the country is due to limited wetland, use of low yielding traditional varieties, low input of inorganic fertilizers, lack of irrigation water and shortage of labour. He also recommended that one of the ways to increase rice production is to breed and develop high yielding varieties that can adapt to specific growing environments. Khush (2005) stated that to meet the challenges of producing more rice from the existing lands, varieties with higher yield potential and greater stability are needed and for that a rich genetic diversity is important in securing food and for sustainability as it allows the breeder to select appropriate genetic materials that can adapt to a specific location. Further, the wider the diversity the lesser the chance of pest and disease occurrence, so that loss to pest and disease can be minimized (NBC, 2008).

Bhutan has been introducing foreign germplasm since the 1960s and pedigree analysis of rice varieties indicated that 74% of the released varieties originated in other countries (Ghimiray & Vernooy, 2017). At the moment there are 23 improved rice varieties in the country. However, some of these varieties have lost resistance to the blast disease due to decades of cultivation. So, the need of the hour is to introduce as many rice lines or germplasm as possible to increase the gene pool, and to breed varieties that are resilience to biotic and abiotic stresses in the future. Screening and selection of new rice lines for identification is important to exploit the genetic diversity within rice germplasm. So the objectives of this study are to (1) To evaluate the morpho-agronomic parameters of new rice germplasm using descriptor for rice developed by IBPGR-IRRI Rice Advisory Committee and (2) To compare grain yield between 18 different breeding lines.

2. Materials and Methods

2.1 Experimental site

The study was conducted in the research field of the Agriculture Research and Development Centre (ARDC), Bajo, during the 2016 rice season. The site is located at 1,200 – 1,300 masl between 27^0 29’24.40’’N and 28^0 53’53.53’’E. The area falls under warm temperate-subtropical zone which is characterized by heavy rainfall during June to September and scanty during rest of the year.

2.2 Soil

The soil type of the experimental plot was fine sand to clay loam with neutral to slightly acidic pH, near total base saturations. It has low organic carbon, total N and available P contents. The low levels of organic matter are thought to be mostly due to long use of the soils for wetland rice.

2.3 Design and layout

The experiment was laid out in randomised complete block design (RCBD). The field was divided into three blocks, which were further subdivided into 54 plots where treatments or germplasm were randomly assigned. The plot size was 5m x 2m or 10 m^2. Row to row and plant to plant spacing were 20 cm x 20 cm.

2.4 Treatments

There were 18 treatments: IR 06M 144, IR 06M 150, IR 96 120, IR 11A 208, IR 10F 336, IR 09A 228, IR 09A 220, IR 10N 269, IR 05A 235, PK 3445-3-2, CB 08 514, IR 06N 170, IR 10A 134, IR 09 N 522, IR 08N 210, CT 16658-5-2-3SR-2-1-MMP, Salabhagi and Bajo Kaap 2 (BK 2) (Standard check) with three replications each.

2.5 Nursery, field preparation, transplantation, intercultural operation and aftercare

The nursery was raised on 23rd of April 2016. The field was ploughed and levelled thoroughly. The seeds were sown in line on the prepared beds and the beds were mulched and tagged. Seedbeds were irrigated as and when required to maintain the moisture.

Field preparation such as ploughing, harrowing, levelling and the application of basal dose of fertilizers were done a day prior to transplanting operation. The 45-day old seedlings were transplanted at 20 cm x 20 cm spacing and optimum water level of 4 - 6 cm was maintained.

All necessary intercultural operations were carried out during the cropping period for proper growth and development of the seedlings. A day after the transplanting, butachlor was applied at the rate 0.5 kg ai ha^{-1} to control grasses and sedges followed by hand weeding two weeks after planting. Hand weeding was targeted to uproot obnoxious rhizomes of Shochum/pondweed (*Potamogeton distinctus)* and other weeds from the field, and also to prepare the field for incorporation of urea fertilizer. Irrigation was done as and when required throughout the cropping season.

2.6 Data collection

The 18 germplasm were evaluated during the particular stages of the rice plant following the descriptors published by International Rice Research Institute and International Board for Plant Genetic Resources (IRRI, 1980). A total of 12 qualitative and 12 quantitative morpho-agronomic traits were used. The qualitative traits were: leaf sheath colour, flag leaf angle, panicle exertion, panicle type, presence of awn, presence of apiculus, apiculus colour, panicle shattering, lemma and palea colour, grain category, threshability and aroma/scent. The quantitative traits were: effective tillers per hill, plant height, days to heading, days to flowering, days to maturity, panicle length, leaf length, leaf width, 1,000 grain weight, grain length, grain width and grain yield.

2.6.1 Grain yield

Grain yield (t/ha) was measured at 85% maturity or when 85% of the grains had matured. From each experimental plot of 10 m^2 crop cut was taken from an area of 8.6 m^2 (excluding the border rows) and was calculated using the following formula:

$$\text{Grain yield (t ha-1) at 14\% MC} = \frac{\frac{\text{yield}}{\text{plot}} \text{ x } 10{,}000 \text{ m}^2 \text{ x MC(adj)}}{\text{plot size}(8.64 \text{ m}^2) \text{ X } 1{,}000}$$

2.6.2 1,000 grain weight

The average weight was then multiplied with the adjusted moisture content. Moisture content was adjusted to 14% derived from the formula:

$$\text{MC (adj)} = \frac{\text{(100-MC)}}{\text{100-14}}$$

where MC = Moisture constant

2.7 Data analysis

Excel spread sheets of Microsoft office 2013 was used for data entry, segregation, descriptive statistics reporting and basic graphical presentations. All quantitative data have undergone Analysis of Variance (ANOVA) to verify the variation in the traits measured using the statistical software Statistix 8. A dendrogram was constructed from 1,000 grain weight, grain length and grain sizes in SPSS using ward linkage to cluster the germplasm.

3 Result and Discussion

3.1 Agronomical characterization

3.1.1 Days to 50% flowering

The study showed that days to 50% flowering for the entries ranged from 117 to 133 days (Table 1). Out of 18 entries, IR 11A 208 (133 days) took the longest period for flowering followed by PK 3445-3-2 with 128 days. Whilst IR 09A 220 took the minimum days for flowering at 117 days. The standard check BK 2 took 121 days. In rice plant, the days to heading starts three days before the flowering and the grains mature after 30 days from flowering. Environmental factors especially temperature affect the rice flowering dates and maturity (McKenzie, Rutger & Peterson, 1980).

3.1.2 Tillers per hill

The range of effective tillers was 9.23 to 12.87 as shown in Table 1. The highest number of effective tillers per hill was produced by IR 10A 134 (12.87) which was followed by IR 06M 150 (12.13). On the other hand germplasm IR 05A 235 (9.23) and Salabaghi (9.70) produced the minimum number of effective tillers. Effective number of tillers is one component that is attributed to increase in yield (Aslam, Akram & Ashraf, 1995). Out of 18, 11 germplasm had more effective no of tillers than BK 2.

3.1.3 Plant height (cm)

The plant height ranged from 95.2 to 108.13 cm (Table 1). Salabhagi was the tallest variety 108.13 cm followed by IR 05A 235 (106.93 cm) while CT 16658-5-2-3SR-2-1-MMP was the shortest (95.27 cm) followed by IR 96 120 (97.63 cm). Reduction in plant height may improve their resistance to lodging and reduce substantial yield losses associated with this trait (Aslam et al., 1995).

3.1.4 Panicle length (cm)

The range of panicle length was 22.84 to 28.28 cm (Table 4.1). The highest panicle length 28.28 cm was recorded in the germplasm IR 09A 228 which was closely followed by IR 09A 220 (27.90 cm). The lowest panicle length 22.84 cm was recorded in IR 05A 235 and closely followed by CT 16658-5-2-3SR-2-1-MMP with 22.97 cm. Although, panicle length contributes positively yet maximum panicle length is not the only factor responsible for higher grain yield (Aslam al., 1995).

3.1.5 1,000 grain weight (g)

The range of 1,000 grain weight was 16.77 to 32.59 g (Table 1). The highest grain weight 32.59 g was obtained from IR 06M 144 and was followed by IR 10N 269 with 32.15 g. The lowest weight 16.77 g was obtained from CB 08 514 and was followed by IR 05A 235 with 21.03 g. Yoshida (1981) stated that grain growth during ripening is characterized by the increase in size and weight of kernels as starch and sugars are translocated from culms and leaves.

3.1.6 Leaf length (cm) and leaf width (cm)

Leaf length ranged from 27.08 to 38.78 cm (Table 2). The highest length 38.78 cm was measured from IR 05A 235 followed by IR 09A 220 with 33.29 cm. The lowest length 27.08 cm was measured from IR 10F 336 and BK 2 with 27.16 cm. Leaf width ranged from 1.40 to 1.91 cm (Table 4.2). The highest length 1.91 cm was found in CT 16658-5-2-3SR-2-1-MMP and the lowest 1.40 cm in IR 06N 170. Thakur (1981) reported leaf angle as erect, horizontal or droopy and was largely influenced by leaf length. The wider the angle, the more the spread of leaves for light interception, especially in the lower leaves.

3.1.7 Grain length and grain width (mm)

Grain length ranged from 6.93 to 10.82 mm and grain width ranged from 2.31 to 2.76 mm (Table 2). The longest grain length was measured from IR 09A 220 (10.82 mm) followed by IR 10A 134 (10.39 mm) and the shortest from CB 08 514 (6.93 mm) and IR 05A 235 (7.84 mm). The widest grain width was measured from Salabhagi (2.76 mm) followed by IR 11A 208 (2.73). And the least wide was measured from CB 08514 (2.31 mm) and IR 09A 220 (2.34 mm). Grain length and width is the strongest determinant of grain size (Takeda, 1986). Increasing grain size has been proposed as one of the means to increase not only paddy yield but also the milling yield of rice (Venkateswarlu, Vergara, Parao & Visperas, 1986).

Table 1.Traits of days to 50% flowering, plant height, no. of tillers per hill, 1,000 grain weight and panicle length for 18 rice germplasm

Treatments	Days to 50% flowering	Plant height (cm)	No. of tillers per hill	1,000 Grain weight (g)	Panicle length (cm)
IR 06M 144	119^{ef}	104.87^{ab}	11.33^{abc}	32.59^{a}	27.68^{a}
IR 06M 150	120^{def}	106.83^{a}	12.13^{a}	27.69^{bcde}	25.31^{ab}
IR 96 120	120^{def}	97.63^{de}	11.40^{abc}	25.98^{cde}	24.23^{ab}
IR 11A 208	133^{a}	102.10^{bc}	9.90^{bcd}	27.69^{bcde}	25.66^{ab}
IR 10F 336	120^{def}	102.03^{bc}	11.63^{abc}	28.83^{abcde}	25.91^{ab}
IR 09A 228	120^{def}	99.23^{cde}	11.93^{ab}	25.31^{def}	28.28^{a}
IR 09A 220	117^{g}	102.13^{bc}	12.20^{a}	27.07^{cde}	27.90^{a}
IR 10N 269	121^{de}	99.20^{cde}	11.63^{abc}	32.15^{ab}	26.48^{ab}
IR 05A 235	123^{c}	106.93^{a}	9.23^{d}	21.03^{fg}	22.82^{b}
PK 3445-3-2	128^{b}	106.50^{a}	11.27^{abcd}	28.20^{abcde}	27.79^{a}
CB 08 514	120^{ef}	98.57^{cde}	10.80^{abcd}	16.77^{g}	24.12^{ab}
IR 06N 170	120^{def}	100.87^{bcd}	11.63^{abc}	26.78^{cde}	26.06^{ab}
IR 10A 134	121^{d}	100.80^{bcd}	12.87^{a}	28.19^{abcde}	27.86^{a}
IR 09N 522	121^{de}	99.33^{cde}	10.93^{abcd}	30.18^{abc}	25.84^{ab}
IR 08N 210	119^{ef}	101.30^{bcd}	11.40^{abc}	25.08^{ef}	26.18^{ab}
CT16658-5-2-3SR2-1MMP	127^{b}	95.27^{e}	9.77^{cd}	29.76^{abcd}	22.97^{b}
Salabhagi	119^{ef}	108.13^{a}	9.70^{cd}	26.22^{cde}	25.12^{ab}
BK 2(Standard check)	121^{d}	97.73^{de}	11.00^{abcd}	29.72^{abcd}	23.98^{ab}
$P<0.05$	**	**	**	**	**
C.V	0.51	4.57	20.64	5.38	10.05

Means followed by the same letter in the column are not significant at Tukey test ($P<0.05$)

Table 2.Traits of leaf length, leaf width, grain length and grain width for 18 rice germplasm

Varieties	Yield (t ha^{-1})	Leaf length (cm)	Leaf width (cm)	Grain length (mm)	Grain width (mm)
IR 06M 144	6.12^{a}	29.07^{b}	1.62^{ab}	9.93^{cd}	2.69^{abc}
IR 06M 150	5.55^{a}	30.32^{b}	1.53^{ab}	9.22^{efg}	2.55^{cde}
IR 96 120	6.34^{a}	28.22^{b}	1.48^{ab}	9.63^{de}	2.71^{abc}
IR 11A 208	6.67^{a}	30.01^{b}	1.67^{ab}	9.19^{fg}	2.73^{ab}
IR 10F 336	5.98^{a}	27.08^{b}	1.48^{ab}	9.61^{def}	2.65^{abc}
IR 09A 228	5.40^{a}	30.72^{ab}	1.51^{ab}	9.87^{cd}	2.59^{bcd}
IR 09A 220	6.28^{a}	33.29^{ab}	1.46^{b}	10.82^{a}	2.34^{f}
IR 10N 269	6.16^{a}	32.31^{ab}	1.68^{ab}	9.69^{d}	2.63^{abc}
IR 05A 235	6.18^{a}	38.78^{a}	1.80^{ab}	7.84^{h}	2.66^{abc}
PK 3445-3-2	6.04^{a}	28.17^{b}	1.67^{ab}	8.99^{g}	2.44^{def}
CB 08 514	5.55^{a}	31.37^{ab}	1.79^{ab}	6.93^{i}	2.31^{def}
IR 06N 170	6.40^{a}	30.46^{ab}	1.40^{b}	9.78^{d}	2.59^{bcd}
IR 10A 134	5.76^{a}	32.18^{ab}	1.69^{ab}	10.39^{ab}	2.39^{ef}
IR 09N 522	6.40^{a}	30.20^{b}	1.50^{ab}	9.24^{efg}	2.65^{abc}
IR 08N 210	5.78^{a}	27.23^{b}	1.50^{ab}	10.26^{bc}	2.43^{def}
CT 16658-5-2-3SR-2-1-MMP	5.46^{a}	28.53^{b}	1.91^{a}	9.74^{d}	2.68^{abc}
Salabhagi	6.18^{a}	30.62^{ab}	1.54^{ab}	8.87^{g}	2.76^{a}
BK 2 (Standard check)	6.00^{a}	27.16^{b}	1.59^{ab}	9.12^{g}	2.67^{abc}
P<0.05	Ns	**	**	**	**
C.V	9.19	16.76	16.65	5.34	7.35

Means followed by the same letter in the column are not significant at Tukey test (*P*<0 .05)

3.2 Grain yield

There was no significant difference (*P*<0.05) between 18 germplasm in terms of grain yield (t ha^{-1}). However, the grain yield in absolute values ranged between 5.40 t ha^{-1}and 6.67 t ha^{-1} (Table 2 and Figure 1). Insignificant difference in grain yield was expected as seven test entries were the elite selections from the two years of vigorous on-station evaluation trial (ARDC-Bajo, 2015; ARDC-Bajo, 2016).

In 2013-2014, ARDC- Bajo has received a total of 80 advanced lines as part of IRRI nursery and has undergone introductory and observation nurseries in the past two years. Thus, the test entries were the best selections from the previous two years of varietal evaluations at the research station, and their insignificant difference in grain yield is normal since there are many other parameters considered for evaluation and screening (IRRI, 1996).

Among the germplasm tested in the current experiment, IR 11A 208, IR 06N 170 and IR 09N 522 were the top three performers with grain yield record at 6.67 t ha^{-1}, 6.40 t ha^{-1}, and 6.40 t ha^{-1} respectively. Lowest grain yield was recorded in IR 09A-228 (5.40 t ha^{-1}) and CT 16658-5-2-3SR-2-1-MMP (5.46 t ha^{-1}), and their values were also below that of the standard check (BK 2) which produced 6 t/ha^{-1}. IR 06M-150, IR 9L-120, IR 09A-220, IR 10N-269, IR 05A-235, and Salabhagi yielded slightly over 6 t h^{-1}. The grain yield of these entries agrees with the reports of Brennan and Malabayabas (2011) who reported yield range of 5.5 - 7.0 t ha^{-1} for the modern varieties in the recent years in South East Asia.

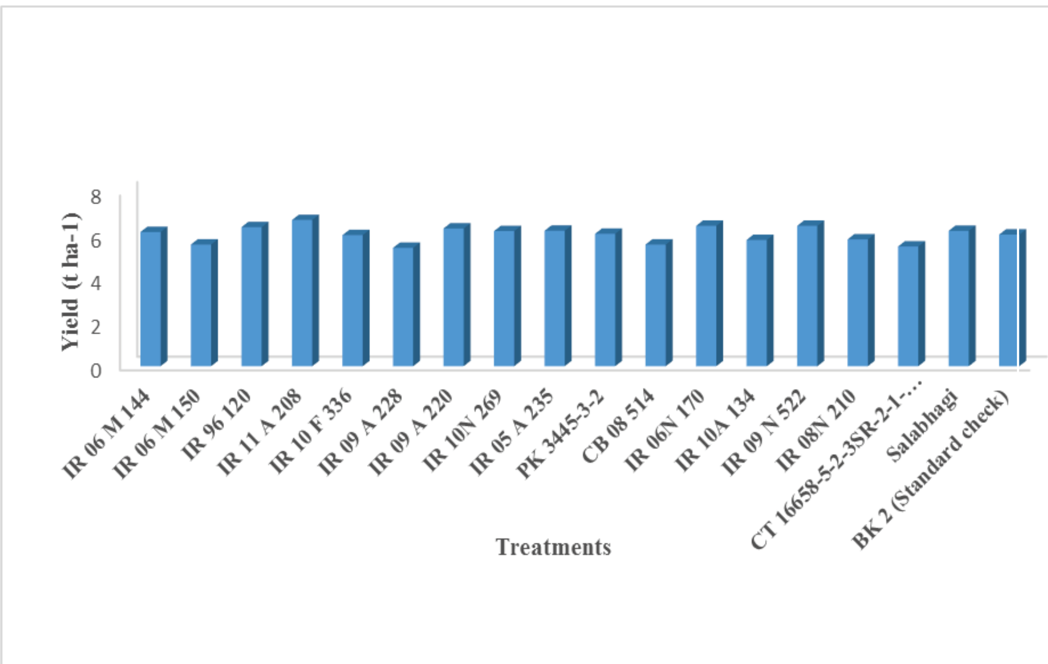

Figure 1.Grain yield of the germplasm materials

3.3 Clustering of the genotypes based on 1,000 grain weight, grain length and grain width

The dendrogram given in Figure 2 was constructed from 1,000 grain weight, grain length and grain width in SPSS using wards linkage. The dendogram showed two clusters. In cluster I there are ten germplasm (IR 09N 522, BK 2, IR 10F 336, CT 16658-5-2-3SR-2-1-MMP, IR 06M 114, IR 10N 269, IR 11A 208, Salabhagi, IR 96 120, IR 05A 235) and in cluster II there are eight germplasm (IR 09A 220, IR 10A 134, IR 08N 210, IR 09A 228, IR 06N 170, IR 06M 150, PK 3445-3-2, CB O8 514). The two main clusters are further subdivided into five clusters. The clustering of the varieties represents the similarities in grains: shape, size and weight. IR 09N 522 and the standard check BK 2 are clustered together which means the grains from these two varieties are similar meaning IR 09N 522 can be categorized as a slender grain type since BK 2 has a white slender grains. However the grains from the germplasm CB 08 514 showed a contrast to BK 2 and has small, round and bold shape and this could also mean that the grains which falls in cluster II and the II-subdivisions range from medium to round shape.

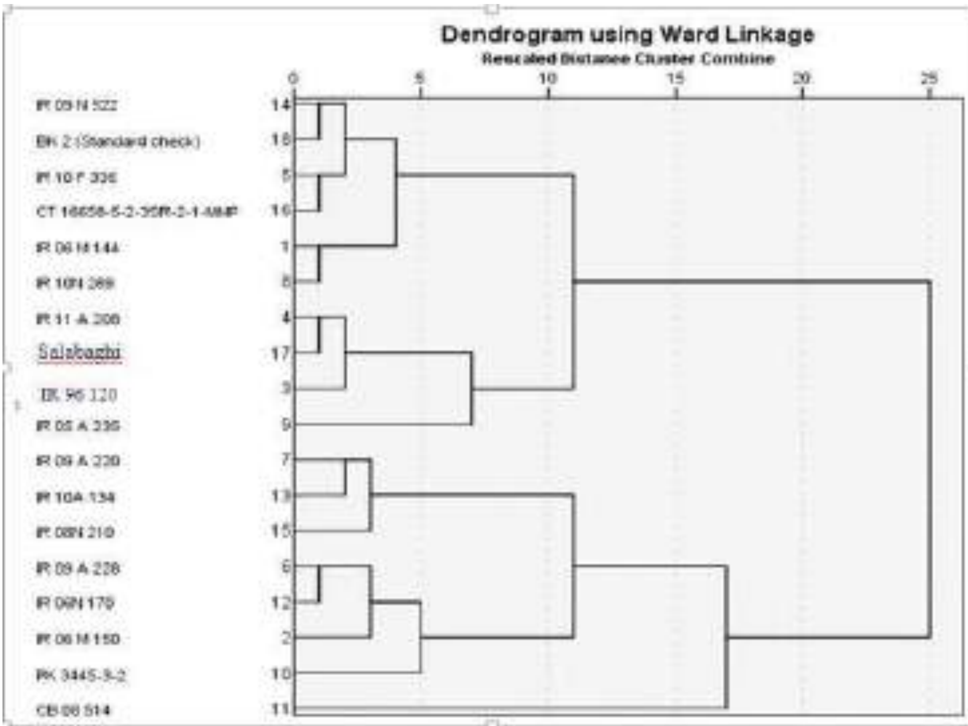

Figure 2.Cluster dendrogram showing similarities and dissimilarities among 18 germplasm in terms of 1,000 grain weight, grain length and grain width.

3.4 Morphological characterization

3.4.1 Basal leaf sheath colour

In all the 18 germplasm, the sheath colour was found green (Table 3). In rice, purple leaf sheath as well as purple apiculus and stigma is common in wild species and landraces; however, green leaf sheath (GSH) is prevalent in modern cultivars (Chin et al., 2016).

3.4.2 Flag leaf angle

Out of 18, eight entries about 44% showed erect leaf angle while the rest of the germplasm exhibited intermediate angle (Table 3). Flag leaf is very important for photosynthesis. Erect leaf angle is a desirable trait for high-yielding varieties. A plant community with vertically oriented leaves gives better light penetration and higher carbon assimilation per unit of leaf area (Tanaka, 1976).

3.4.3 Awn

Out of 18 entries, only four (22%) of the germplasm had awns present on the grains (Table 3) while the rest were without awns. The presence of awns is considered important trait in rice domestication (Hussain et al., 2014). Grains of wild rice have long awns that protect the grains from animal pilfering. The varieties with long awn or strongly awned are more resistant to bird attack than the varieties with no awns. On the other hand, cultivated rice varieties have short awns allowing for easier harvesting than varieties with long awns (Hussain et al., 2014).

3.4.4 Panicle type, panicle exertion, panicle shattering, apiculus, lemma and palea colour

All the entries were of intermediate panicle type, showed well exerted panicles and low panicle shattering. Out of 18 germplasm 14 (78%) had straw colour, three (17%) had brown and only one (6%) had red apiculus colour. Sixteen (89%) out of 18 germplasm had straw coloured lemma and palea except two (11%), which exhibited brown furrows on straw (Table 3).

Table 3.Qualitative traits showing pre-dominate state observed

Treatments	A	B	C	D	E	F	G	H	I	J	K	L
IR 06M 144	1	2	1	1	2	2	Absent	Present	2	1	1	2
IR 06M 150	1	1	1	1	2	2	Absent	Present	2	1	1	2
IR 96 120	1	2	1	1	2	2	Absent	Present	2	1	1	2
IR 11A 208	1	2	1	1	2	2	Absent	Present	2	1	1	2
IR 10F 336	1	1	1	1	2	2	Absent	Present	2	1	1	2
IR 09A 228	1	2	1	1	2	2	Absent	Present	2	1	1	2
IR 09A 220	1	2	1	1	2	2	Present	Present	2	1	1	2
IR 10N 269	1	1	1	1	2	2	Absent	Present	3	1	1	2
IR 05A 235	1	2	1	1	2	2	Absent	Present	3	1	1	2
PK 3445-3-2	1	2	1	1	2	2	Absent	Present	2	4	1	2
CB 08 514	1	2	1	1	2	2	Absent	Present	4	1	4	2
IR 06N 170	1	1	1	1	2	2	Present	Present	2	1	1	2
IR 10A 134	1	1	1	1	2	2	Present	Present	2	1	1	2
IR 09 N 522	1	2	1	1	2	2	Absent	Present	2	1	1	2
IR 08N 210	1	1	1	1	2	2	Present	Present	2	1	1	2
CT 16658-5-2-3SR-2-1-MMP	1	2	1	1	2	2	Absent	Present	2	1	1	2
Salabhagi	1	1	1	1	2	2	Absent	Present	3	1	1	2
BK 2 (Standard check)	1	1	1	1	2	2	Absent	Present	2	4	1	2

A. **Presence of awn:** Present, absent
B. **Basal leaf sheath colour:** Green (1), Purple lines (2), light purple (3), purple (4)
C. **Flag leaf angle:** Erect (1), Intermediate (2), Horizontal (3), Descending (4)
D. **Panicle Exertion:** Well exerted (1), moderately exerted (2), just exerted (3), Partly exerted (4), enclosed (5)
E. **Panicle shattering**: Low (1), Moderate (2), Moderately high (3), High (4)
F. **Panicle type**: Compact (1), Intermediate (2), Open (3)
G. **Threshability:** Difficult (1), Intermediate (2), Easy (3)
H. **Presence of apiculus:** Present, absent
I. **Apiculus colour:** White (1), Straw (2), Brown (twany) (3), Red (4), Purple (5)
J. **Lemma and palea colour:** Straw (1), glod furrows on straw background (2), Brown spots on straw (3), Brown furrows on straw (4), Brown (twany) (5), Reddish to light purple (6)
K. **Grain category:** Slender(1), Medium(2), Bold (3) , Round (4)
L. **Scent:** yes (1), No (2)

4 Conclusions

Morpho-agronomic characterization is an important prerequisite to evaluate phenotypic diversity within germplasm collection. It creates the basis to ensure effective utilization of the crop germplasm by both farmers and breeders amongst other users.

Evaluation of 18 rice germplasm revealed genetic variability in some of the traits. Differences among the germplasm were observed in characters such as: awning and flag leaf angle. Out of 18, eight (44%) treatments exhibited erect flag leaf angle while the remaining had intermediate leaf angle. Only four (22%) had awns while the rest showed awnlessness. All 18 germplasm showed well exerted panicles, low panicle shattering and intermediate panicle types respectively. These phenotypic traits could be explored for rice improvement in the future.

There was no significant difference in yield although other yield components were significant. The grain yield in absolute values ranged from 5.40 to 6.67 t ha^{-1}. Since the treatments included were the improved advance lines selected from the IRRI nursery and the introductions from India, higher yield was expected even under multi-environmental conditions and this could be the reason for performing well in terms of yield. This study is still in its initial phase, so all the treatments need to be selected and promoted to the next level advance evaluation trial for proper evaluation.

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Efficacy of *Ageratina adenophora* against White rust (*Albugo candida*): A laboratory and field study

Jigme[e], Ram Chandra Bajgai[e]

ABSTRACT

White Rust of Crucifers is caused by Albugo candida, which causes damage to quality and quantity of the produce. Conventionally, fungicides are applied to reduce disease incidences; however, improper and excessive use of chemical fungicides can be detrimental to the environmental health. In this study, different concentrations (2.5%, 5%, 7.5% and 10%) of freshly prepared aqueous extract of Ageratina adenophora were tested for antifungal activity in vitro and in vivo against A. candida using poison-food technique and Colony Forming Unit (CFU/ml). In vitro results revealed that 10% aqueous extract of A. adenophora was most effective against mycelial growth and biomass formation of A. candida.10% aqueous extract inhibited 92.7% of A. candida mycelial and total biomass formation followed by 78% at 7.5% concentration. The lowest inhibition was observed in 2.5% concentration with 2.4%. The in vivo antifungal activity of aqueous extract of A. adenophora was tested in the potted plants under normal conditions. The result revealed that the preventive control is most effective than the curative control. Preventive control of disease incidence using 10% aqueous extract led to 54.1% reduction, whereas the curative control reduced by mere 3.6%. Thus, 10% aqueous extract of A. adenophora demonstrates the potential for the control and management of white rust of crucifers.

Keywords: *Albugo candida, Ageratina adenophora, Aqueous extract, Antifungal activity, Curative control, Preventive control*

1. Introduction

Agriculture is the key source of sustaining livelihood and enhancing human life (Pino, Sánchez& Rojas, 2013). It supplies food and other essential commodities for human consumption. Apart from fulfilling food requirement of a growing population, agriculture plays pivotal role in improving the economy of a nation (Dutta, 2015). However, the damage and destruction inflicted on the crops by various pest and pathogens such as insects, microbes (bacteria, fungi, viruses and mycoplasmas), nematodes, weeds, animals and birds have posed serious challenges to farmers in terms of sustaining food productivity (Yadav, Kewal & Choudhary, 2015; Koul, 2011).

White Rusts (also called as white blister) are caused by several species of *Albugo*, belonging to the class Oomycetes. The disease attacks aerial parts of cruciferous plant including flower,

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leaves and stem. It does not attack the root of a large number of cultivated and wild crucifer plants (Sharma 2017). Cultivated plants which are susceptible to infection by white rust include cauliflower, cabbage, radish turnip, broccoli, and mustard. White rust caused by *Albugo candida* (Pers. ex. Lev.) Kunze (Saharan, Verma, Borhan & Singh, 2014) is an important and widespread disease in the world. According to (Lahiri & Bhowmik 1993; Sharma, 2017), the pathogen produces two types of infection i.e., local and systemic infection. Local infection is characterized by the formation of raised creamy white sporangial pustules on the under surface of leaves and on tender shoots whereas systemic infection is usually seen in young inflorescence and terminal leaves. Conventionally, fungicides are applied to reduce the disease incidence of white rust; however, improper and excessive use of chemically derived fungicides causes residual toxicity in the non-targeted organisms and leads to environmental degradation. Sustainable crop production needs eco-friendly methods of pest and diseases control. Therefore, the development and synthesis of bio-pesticides could be one of the options in conventional crop disease control system.

By nature, all plants synthesize and discharge numerous secondary metabolites, which enable them to defend against pathogens, pests, animal attacks and harsh environmental conditions (Cavoski, Caboni & Teodoro, 2011). According to Kumar, Singh, Sharma and Kishore (2017), *Ageratina adenophora* produces numerous secondary metabolites, which have antimicrobial (antibacterial and antifungal), antiseptic, analgesic, molluscicides and insecticidal potential. Although *A. adenophora* has meritorious chemical contents of diverse medicinal and antimicrobial properties, it is one of the most invasive weeds, where invasion of this weed has replaced larger part of the vegetation coverage and thus considered as a major threat to native biodiversity (Tripathi, Kushwaha & Yadav, 2006). Its allelopathic property makes it a noxious weed and dominates over other species (Subba & Kandel, 2013).In spite of potentially helpful biochemical characteristics and harmful biodynamic characteristic, the potential of *A. adenophora* for controlling fungal diseases in crops has not been evaluated (Sobrinho, de Morais, de Souza & dos santos Fontenelle, 2017). Thus, this study intended to evaluate the efficacy of fresh aqueous extracts of *A. adenophora* against White Rust of Crucifers.

2. Materials and Methods

2.1. Plant material collection, plant extracts preparation and media (PDA) preparation

Fully developed aerial parts of *A. adenophora* were collected from Sherubtse College campus, Kanglung, Trashigang, Bhutan. Aqueous extract of *A. adenophora* was prepared by grinding 20g of fresh leaf materials in an electric blender by adding sterile distilled water at the rate of 10 ml/g (Nashwa & Abo-Elyousr, 2012). The homogenates were filtered with Whatman No.1 filter paper. Then the filtrates were centrifuged at 5000 rpm for 10-15 minutes at room temperature and the supernatant were collected. The extracts were further diluted by adding sterile distilled water to have ranges of concentration (2.5%, 5.0%, 7.5% and 10%) and stored in refrigerator at 4°C. Potato Dextrose Agar medium (PDA) was prepared by dissolving 100g of potato infusion, 2.5g of dextrose and 10g of agar in 500ml of distilled water (pH 5.6±0.2). The dissolved medium was autoclaved at 15lbs at 121°C for 15 minutes.

2.2. Isolation of *Albugo candida*, antifungal activity, colony forming unit (CFU) and biomass evaluation

The infected plants were collected from local farmer in Kanglung gewog. The infected plant parts were cut, packed in the polythene bag and brought to the lab. They were thoroughly washed (leaf with shiny whitish pustules underside of *Brassica juncea*) in clean water followed by sterile water (distilled water), and with the help of sharp sterile razor blade, the infected tissues along with adjacent small unaffected tissue were cut into small pieces (25 mm squares). The cut pieces were transferred into sterile petri dishes containing 1% of sodium hypochlorite for surface sterilization for 30seconds. After surface sterilization, the sterilized pieces were transferred to petri dishes containing PDA and incubated at 25°C for 72 hours. A portion of mycelium from fungal colony was transferred to fresh potato dextrose broth for the pure culture.

The antifungal activity of aqueous extract of *A. adenophora* was assayed by poison-food technique and further confirmed by calculating Colony Forming Unit (CFU). The plant extract was incorporated into the molten PDB broth at a desired concentration at the ratio of4:1 (PDB and Plant extract) and then mixed thoroughly with Vortex Shaker. Then the medium was poured into 50 ml conical flask. The conical flasks were inoculated with 0.1 ml of fungal suspension and incubated in the incubator at 25°C for 48 hours. After 48 hours, 0.1 ml of fungal suspension serially diluted up to $10^4$was transferred and cultured on the petri plates containing PDA using spread plate method. The inoculated plates were incubated at 25°C for 48 hours. After 48 hours, the colonies on the petri dishes were counted under digital colony counter. The inhibitory activity of the extract was determined and evaluated using the following equation (1) modified from John, Ragi, Sujana & Kumar (2014):

$$IAG = \frac{NFC - NFT}{NFC} \times 100 \qquad (1)$$

Where, IAG = Inhibitory Activity of Growth, NFC = Number of Fungal colony in Control plate, NFT = Number of Fungal colony in Treated plates

To extract fungal biomass, 5 ml of different concentration (2.5%, 5%, 7.5% & 10%) of leaf extracts was incorporated into 20 ml potato dextrose agar broth in 50 ml conical flasks. The flasks were inoculated with 0.1ml of fungal inoculums. The cultures were incubated for 8 days at 25°C and the fungal biomass was harvested through centrifugation at 5000 rpm for five minutes. The fungal biomass pallets were collected and dried overnight in the oven at 35°C. The inhibitory activity of extract against fungal biomass was calculated using the following equation (2):

$$IAB = \frac{DWC - DWT}{DWC} \times 100 \quad (2)$$

Where, IAB = Inhibitory Activity on the Biomass, DWC = Dry Weight of biomass in Control, DWT = Dry Weight of biomass with extract Treatment

2.3. In vivo evaluation of extracts against *Albugo candida*

In vivo antifungal potential of *A. adenophora* aqueous extract was studied on potted plants under normal conditions. The potted plants were divided into Control (C), Curative Control (CC) and Preventive Control (PC). The plants in (C) and (CC) were infested with *A. candida* inoculum by spraying, when the symptoms appeared, the plants in CC were treated with the extract. For the preventive control test, the plants in PC were first treated with the extract by spraying and were infested with *A. candida* inoculum after 24 hours as per John et al., (2014).

2.4. Disease assessment

Table 1.Scoring method for evaluating the efficacy of *A. adenophora* extracts (Modified from Goss, Mafongoya, Gubba & Sam (2017))

Scale	Disease severity
0	No symptoms
1	Very few symptoms, 1-3 small lesions on one or two leaves
2	Small lesions on 3-5 leaves
3	Enlarged lesions on 3 or more leaves
4	Coalescing lesions forming wilted
5	Mildly chlorotic and appearance of green island as the leaf ages
6	Plants completely defoliated and dying

The disease severity index (DSI) was calculated by following equation (3) adopted from Alemu, Lemessa, Wakjira & Berecha (2014):

$$DSI = \sum \left[\frac{d \times n}{N \times m}\right] \times 100 \quad (3)$$

Where DSI = Disease Severity Index, d = disease rating on each plant, n = number of plants in each score, N = total number of plants examined and m = maximum disease rating possible.

The reduction of DSI on each plant was calculated using following equation (4):

$$PR = \left[\frac{PVC - PVT}{PVC}\right] \times 100 \quad (4)$$

Where, PR = Percent Reduction, PVC = Percentage Value of the Control and PVT = Percentage Value of the Treatment group.

3. **Result and Discussion**

3.1. Antifungal activity- inhibition percentage (IAF %) and Biomass of A. candida

Results revealed that all the extract concentrations (2.5%, 5%, 7.5% and 10%) showed positive results in suppressing the growth of *A. candida* with variable potency. The growth inhibition increased with increase in the extract concentration. The number of *A. candida* colony and biomass formation was found to be inversely proportional to the extract concentrations. The highest concentration (10%) of *A. adenophora* aqueous extract was found to be the most effective inhibiting *A. candida* growth in the lab. Therefore, 10% aqueous extract was chosen for experiments during the field test with the potted plants. Figure (1) and (2) depict the trends of inhibitory activity and biomass formation of *A. candida* in aqueous extracts of *A. adenophora*.

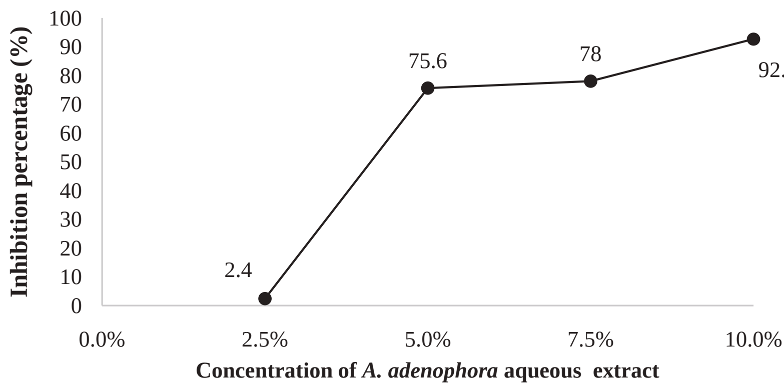

Figure 1.Inhibitory activity of aqueous extract of *A. adenophora* against *A. candida*

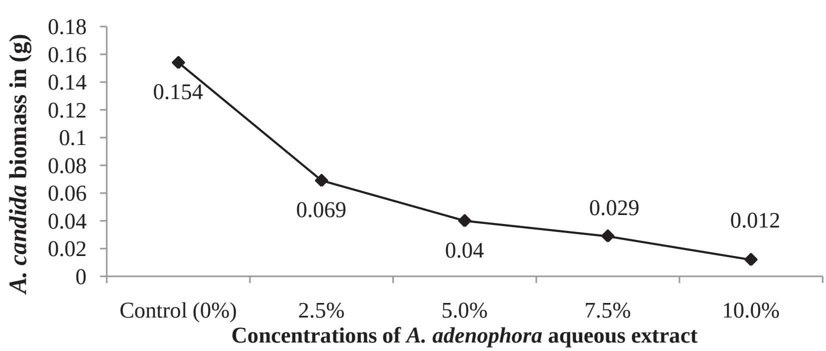

Figure 2.*A. candida* biomass formation in the presence of different concentrations of aqueous extracts *A. adenophora*.

The highest inhibitory activity of aqueous extract of *A. adenophora* against *A. candida* was observed in 10% concentration with 92.7% inhibition followed by 7.5% with 78% inhibition. The lowest inhibitory activity was observed in the lowest concentration (2.5%) with 2.4 percent inhibition followed by 5% extract with 75.6% inhibition (Figure 1). There was a significant difference in the IAF of 2.5% and 5% of aqueous extract. The increase in IAF with extract concentration in the initial two concentrations was drastic. With one fold increase in extract concentration, inhibition increased by more than 30 folds from 2.4% to 75.6%. This may be due to increase in the metabolites concentration which is optimum for the inhibition. However, there was not much difference between 5% and 7.5%, possibly because the dead cells fenced the inhibitory chemicals to account for live cells. The other reason could be that the already dead cells might have absorbed most of the inhibitory chemicals from the extract leaving the probability of contact between live cells and inhibitory chemicals low (Zhang et al., 2013). Therefore, even with the increase in extract concentration to 10%, the increase in inhibitory effect is low (75.6% to 78%). Thus, IAF of aqueous extract of *A. adenophora* extract increased with the increase in the extract concentrations. On biomass front, the plate showed numerous colonies and massive growth of fungal mycelium in the control (0% extract), however, as the concentration increased, the fungal biomass and number of colony decreased (Fig 2). Thus, *A. adenophora* extract has a wide spectrum of fungistatic property against *A. candida.* The fungistatic activity of *A. adenophora* against *A. candida* may be due to the presence of secondary metabolites such as (mono-, sesqui-, di-, and tri-) terpenoids, phenylpropanoids, flavonoids, coumarins, sterols, alkaloids (Zhang et al., 2013), flavonoids, chromens, lactones, flavones and flavanones (Torres-Barajas et al., 2013). However, there was no attempt made to understand the phytochemicals of aqueous extracts of *A. adenophora* responsible for such an activity.

3.2. In vivo evaluation of aqueous extract of A. adenophora against A. candida - Disease Severity Index (DSI)

10% aqueous extract was selected for the *in vivo* test. The potted plants labelled as Control (C), Curative Control (CC) and Preventive Control (PC) was used for the test. The test plants (mustard green) infested with *A. candida* inoculums started to show symptoms after four weeks of infestation. The symptoms included distortion of young leaves and flowers, swelling on the stems and whitish lesions on the under surface of the leaves.

Table 2.Disease Severity Index (DSI) and % Reduction (PR) of disease incidence (*A. candida* vs aqueous extract of *A. adenophora*)

Weeks	Disease Severity Index (DSI)			% Reduction (PR) of Disease Incidence	
	C (%)	CC (%)	PC (%)	CC (%)	PC (%)
1	67.9	71.4	25.0		
2	78.6	75.0	32.1	3.6	54.1
3	80.1	77.2	36.8		

(C=Control, CC=Curative Control, PC=Preventive Control)

After symptom development on C and CC, the test plants were further observed for three weeks. In the first week, the highest DSI (71.4%) was recorded in CC test plants followed by control plants (67.9%). The lowest DSI was recorded in the PC test plants (25%). In the second and third week, the highest DSI was observed in the control. In the second week, the DSI were 78.6%, 75% and 32.1% for control, CC and PC respectively. Similarly, the DSI for the third week were 80.1% (Control), 77.2% (CC) and 36.8% (PC); from this it became evident that DSI recorded for three weeks is highest in CC plant as compared to the plants in PC. It is apparent that the infections are less severe in plants pre-treated with aqueous extracts of *A. adenophora* than those attempted to cure of infection. It is because the pre-treatment of the plants with aqueous extract of *A. adenophora* makes the environment around the plants unfavorable (toxic) for the infestation by *A. candida*. It might have also boosted the hosts' defense system by various phytochemicals.

3.3. % Reduction (PR) of disease incidence

The percent reduction was calculated using equation (4). PC was able to reduce infection by 51.4% whereas the CC was able to reduce infection by only 3.6% as compared to control. From this result, it is evident that the preventive control is much more effective than curative control to prevent the *A. candida* infection using aqueous extract of *A. adenophora*.

4. **Conclusion**

The study confirms that the aqueous extract of *A. adenophora* has excellent antifungal activity against *A. candida*. Thus, it demonstrates high potential in its use as alternative eco-friendly agent in controlling, reducing and managing *A. candida* infection and incidences in crucifers. Its antifungal activity is mainly due to the presence biochemicals such as alkaloids, flavonoids, chromens, flavones, diterpenes, sesquiterpenic, triterpenes and flavanones. However, a clear understanding of its bioactive phytochemicals vis-à-vis antimicrobial characteristic is essential so as to further affirm and validate its potential at the chemical and physiological level, and to use it as one of the important plant protection agents in integrated pest management (IPM) programs in subsistence organic farming. Secondly, it is doubly advantageous, in that by using it as an agent of plant disease control, the noxious weed population can also be effectively controlled.

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Comparative Study on Growth and Yield of Oyster Mushroom (*Pleurotus ostreatus*) on Different Substrates

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ABSTRACT

Agriculture in the country is transforming from subsistence to commercial farming system. Most of the time crop residues are left unutilized in the field after harvest. Burning crop debris after harvest is an age old tradition followed by farmers here in Bhutan. Mushroom growers inoculate Oyster mushroom only in rice straw unlike in other countries. Therefore, to diversify Oyster mushroom inoculation to enhance production in the country, and to find best alternative substrates other than rice straw, a comparative study on growth and yield of Oyster mushroom (Pleurotus ostreatus) on different substrates was conducted. Pleurotus ostreatus strain 'PBN' was selected species in the study. A Completely Randomize Design (CRD) with five treatments and five replications including control (Paddy straw) was used. Data were analyzed through one-way ANOVA with SPSS version 22. The treatment mean of five replications were compared using Tukey's Multiple Range Test. The yield and mycelium colonization rate differed among substrates under same climatic condition. There is significant difference in yield between control and T_1, T_3 *&*T_4*, and no significant difference in yield between* T_0 *&*T_2 *(*$P<0.01$*). However, higher yield is obtained from* T_2*. Significant differences in colonization rate were observed among* T_0*,* T_4 *&* T_3 *and in pin head formation among* T_0*,* T_1 *&* T_3*. Yield obtained from each flush from each treatment is directly proportional to the number of pin head formed in each treatment. A total of four effective flushes from five replications were evaluated for yield analysis.*

Keywords: *Oyster mushroom, Mycelium colonization, Yield, Primordia*

1. Introduction

Oyster mushroom is a saprophytic fungus. It can grow on all types of agricultural wastes throughout the year in any agro-climatic zone proper cropping shed is provided. It generates food by decomposing complex organic matter into simple compound (Chang & Miles, 1991). Unlike button mushroom, Oyster mushroom can grow directly in a substrate without composting (Atkins, 2014; Statmets, 1983). It was first cultivated in Germany as a survival mechanism during World War II (Eger, Eden & Wissig, 1976).

Oyster mushroom adapts well in dark environment (Kong, 2014), and has bittersweet smell of benzaldehyde (Beltran-Garcia, Estarron-Espinosa & Ogura, 1997). Lingo-cellulosic substrates

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are good source of growing Oyster mushroom (Sánchez, 2010). *Pleurotus spp*. are rich in medicinal and nutritional properties. Medicinally it has anti-cancerous, anti-inflammatory, antiviral, antibiotic, anti-diabetic and anti-modulator effects (Lavi et al., 2010). Nutritionally it is rich in protein, minerals and vitamin B, C & D (Panjikkaran & Mathew, 2013), and hence the popular reference to mushrooms as poor man's protein.

Pleurotus ostreatus belongs to family *Pleurotacea* and class *Agaricomycetes*. Locally it is called '*Naaky Shamung*' in west and '*Yuelay Bamung*' in eastern part of Bhutan. Like Shiitake mushroom, Oyster mushroom cultivation in the country is also expanding. At present, Oyster mushroom is commonly grown on rice straw as substrate. Oyster mushroom cultivation on rice straw is limited in its scope for expansion since farmers use rice straw as fodder for cattle, and there is also limited rice growing areas, particularly in the east.

The main objectives of this study is to explore Oyster mushroom inoculation in other agricultural wastes (crop residue) beside rice straw and to find out best alternative substrates in order to enhanced Oyster mushroom production in the country. The study also complements finding a solution to better utilization of agricultural waste.

1. Materials and Methods

The experiment was conducted from 1st week of April, 2018 to 2nd week of June, 2018 in Agriculture Research and Development Sub-Centre, Khangma. The five treatments are as follows (replicated 5 times).

T_0: Paddy straw (control)
T_1: Dried banana leaves
T_2: Dried quinoa stubble
T_3: Dried mustard straw
T_4: Dried lemon grass

2.1. Preparation of Spawn

Pure culture was developed in Potato Dextrose Agar (PDA) media (39g PDA in 1 liter distilled water) from fresh fruit body. Later it was transferred to autoclaved wheat grain and developed mother spawn. Inoculated bottles were incubated for a month in incubation room at room temperature 23°Cand 60% relative humidity.

2.2. Preparation of substrate

Different substrates were chopped at 2-5 cm length. Weigh of 10kg dry weight of each substrate were measured and filled in jute sack.

2.3. Soaking

Filled jute sacks were immersed fully under water for 15 minutes. Excess water was drained out for 5 minutes after soaking. Moisture content of each substrate was calculated manually based on

Rahman et al. (2012). Different substrates' moisture absorption capacity are tabulated below (Table 1)

$$MC\ (\%) = \frac{Wet\ wt\ of substrate - Dry\ wt\ of substrate}{Wet\ wt\ of substrate} x100$$

Table 1.Moisture absorption capacity of different treatments

Treatments	Dry weight (kg)	Wet weight (kg)	Weight gain (kg)	MC (%)
T_0	10	27	17	63
T_1	10	28	18	64
T_2	10	26.3	16.3	62
T_3	10	26.8	16.8	63
T_4	10	26	16	62

2.4. Sterilization

Steam treatment was provided to all treatments in steel barrel for 2 hours after ballooning of plastic sheet covering the mouth of barrel. It was monitored and measured with TR-71wf thermo-recorder, and cooled for 30 minutes by spreading on clean plastic sheet before inoculation.

2.5. Inoculation/spawning

Samples of size 2 kg were prepared in clean polypropylene bag from each substrate. Layer method of spawning was followed with spawn rate of 100g per 2 kg substrate. Three holes on each incubated bags were prepared for air circulation.

2.6. Incubation

Inoculated polypropylene bags were transferred to incubation room. Room was treated with 70% ethanol with 3 days interval. Room temperature and relative humidity inside room was maintained at 20-25°c and 65% respectively.

2.7. Experimental design, data collection and statistical analysis

The experiment was laid out in Completely Randomized Design (CRD). Starting date of mycelium growth, days required to complete substrate colonization, days of pin head formation and yield were recorded in MS Excel sheet. Yield was measured with digital physical balance. Recorded data were analyzed by one way ANOVA and treatment means were compared through Tukey test (P<0.01) in SPSS version 22.

3. Result and Discussion

Yield, mycelium growth colonization rate and pin head formation were analyzed. The total yield for analysis was obtained from four effective flushes. Significant difference in yield were observed amongst control and T_1, T_3 & T_4 whereas, there is no significant difference in yield between T_0 &T_2 (P>0.01). However, higher yield was obtained from T_2 compared with T_0 (Table 2), and the lowest yield from T_4. On an average 2kg substrate produced 484g of fresh mushroom, which is in contrast to findings by Jiskani (1999) where he reported that 1 kg fresh mushroom can be generated from 1 kg dry weight of substrate. Unlike Tan (1981) who found each bag to give two flushes, all treatments in this study gave four effective flushes successfully. Highest yield was obtained from second and third flushes compared to first and fourth. However, yield obtained from the substrate may differ with size of substrate: higher the substrate size higher the yield obtained.

Except for T_4, mycelium colonization in all treatments started from 3rd day after inoculation. Colonization in T_4 started only from the fourth day. Significant differences (P<0.01) were observed among T_0, T_4 & T_3 (Table 2). There is no significant between T_0 & T_2, yet, faster mycelium colonization rate was observed in T_0 while healthy mycelium was observed in T_2 (Table 2). Mycelium colonization inside substrate was completed within a span of 21 days after inoculation, as similarly reported in other studies (Mondal, Rehana, Noman & Adhikary, 2010; Tirkey, Simon & Lal, 2017). Rate of mycelium colonization in different substrates may be due to difference in chemical compositions and C: N ratio of the substrate as presented by Bhatti, Mir and Siddiq (1987). Myceliums survive well at room temperatures of 20-25°C and room relative humidity of 60-65% (Gisleröd, 1987; Karacanci, 1997). High humidity inside cropping shed will create favorable environment for green mold (*Penicillium sp.* and *Aspergillus sp.)* that can inhibit mycelium colonization and hence, should be monitored closely.

Significant differences (P<0.001) in the duration of pin head (primordia) formation is observed amongst T_0, T_1 & T_3 (Table 2). Early pin head was formed in T_3 (24 days) and longer duration was observed in T_1 (27 days). Nevertheless, prior to one week after full colonization, primordium was formed in all inoculated bags. Similar result was stated in studies by Ahmed (1988) and Stamets (1983). Number of primordia found in each bags in each flush has direct effect on yield. Higher the number of primordial, higher the yield.

Table 2.Effect of different substrates on yield, mycelium growth, colonization rate and pin head formation

Treatments	Yield (g/bag)	Initial mycelium growth (days)	Fully colonization (days)	Pin head formation (days)
Banana leaves	448[a]	4[a]	20[c]	27[c]
Lemon grass	449[a]	3[b]	19[b]	26[b]
Mustard straw	457[a]	3[b]	17[a]	24[a]
Quinoa straw	533[b]	3[b]	18[b]	26[b]
Paddy straw	530[b]	3[b]	21[c]	25[b]
SE	7.42	0.13	0.63	0.48

[1]Values are means of five replicates. Different letters indicate significant differences between the treatments at $P<0.01$, [2] SE- Standard Error.

4. Conclusion

Oyster mushroom (*Pleurotus ostreatus*) has short cropping duration in that inoculation to harvest cycle can be completed in a month. It can grow in any agro-ecological zone if proper cropping shed is provided. However, success of mushroom farm solely depends on management practices and sanitation maintained inside cropping shed during cropping period. Mycelium colonization rate, primordia formation duration and yield varied among the treatments. Significant differences in these parameters were observed amongst the treatments.

Number of primordia formed in inoculated bags directly affected the yield. Regular watering inside cropping shed is equally essential during primordial formation period so that proper fruit bodies are formed. Though effective production can be generated from first flush to fourth flush, mushroom growers can still harvest few fruit bodies until the colonized substrate get exhausted. For *Pleurotus ostreatus,* optimum room humidity and temperature is 60-65% & 20-25^{0}C respectively during incubation period and 80-90% room humidity should be maintained during fruiting period.

Mushroom growers can utilize agricultural wastes for growing Oyster mushroom in order to use crop debris effectively and economically, and can be an ideal recommendation for utilizing farm waste as well as generate fast cash return.

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Evaluation of Different Sun Drying Methods in Chilli

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ABSTRACT

Chilli is one of the main cash crops for many farmers in Bhutan, including farmers of Kazhi geog under Wangduephodrang dzongkhag. It is either sold or bartered. However, during the peak chilli production season the price drastically drops forcing farmers to resort to drying. Dried chillies during lean season command a very high price. However, drying is challenging in absence of a more efficient and economic dryer. Further, open sun drying is a laborious job in addition to poor quality and contamination by dusts. Therefore, the main aim of the study was to improvise the present traditional method of open sun drying and compare the drying loss and economics of the existing method with other improvised drying methods. The study conducted in Bjaktey village under Kazhi geog, Wangduephodrang dzongkhag followed RCBD with four treatments and three replications each. The parameters measured were final weight, percent loss, cost benefit, drying duration, and water activity of different treatments. The results indicated that T4 (poly tunnel drier) performed better in six of the seven parameters measured, namely loss percent (least at 0.03%), water activity (least at 0.41), drying duration (11 days), temperature (highest at 41.59°C), relative humidity (lowest at 19.54%) and economics (highest net income at Nu. 728.9/m²). The seventh parameter, the final weight lost, in T4 (at 2.47 kg) was significantly different from the control (T1) (at 2.31 kg), but not from the other two treatments T2 (raised bamboo mat) and T3 (raised bamboo mat with plastic roofing). There was no significant difference between T1 and T2 in five of the seven parameters measured, namely loss percent, water activity, drying duration, temperature attained and relative humidity retained. The high temperature and low relative humidity difference facilitated the produce to dry quickly and lower water activity ($_{aW}$) in T4.

It may be concluded that poly tunnel on raised bamboo mat is comparatively convenient, efficient and economical for drying chillies compared to other methods.

Keywords*: Chilli, Economics, Loss percent, Open sun drying, Poly tunnel dryer.*

1. Introduction

Throughout Bhutan chilli (*Capsicum annuum* L.) is grown either in kitchen gardens for home consumption or in larger areas for sale in local markets. Farmers in major chilli growing areas earn good cash income from the sale of chilli in different forms such as seeds, seedlings, green

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tender chilli, green fresh, red fresh, powdered and dried. Dorji, Dema and Euden (2009) found that the potential return per unit area of chilli was high and was achieved in a relatively short period. Therefore, it is favored by most farmers as a cash crop.

Farmers in Kazhi geog under Wangduephodrang dzongkhag grow chilli in large quantities. The geog has a total area of 70.51 acres under chilli cultivation with an annual production of 405.68 Mt (MoAF, 2012). The geog has warm weather conditions with altitude ranging from 1600 to 2400 masl. Most farmers here plant chillies thrice a year, once in March/April, another crop in May/June and the last in July. The geog is also known widely for its indigenous chilli variety Sha-ema.

With increasing production of chilli, particularly in the same season throughout the country, sale of chilli for income is becoming a difficult business. During the relatively short period of peak production season, the glut of chilli in the local market forces the price to dip (Fuller, Lhendup & Lu, 2005). Despite the low price (about Nu 10 per kg), farmers still sell their produce because the traditional sun drying method to preserve chillies is not viable because the harvest season for the first and second crops coincides with the monsoon rain. Drying perishables such as chilli during continuous heavy rain with low solar radiation results in low quality produce and incurs huge loss (Women and Environment Programme, 2013). However, towards the end of the third harvesting season, autumnal dry and sunny weather replaces monsoon rains. It is at this time of the year that farmers choose to sun-dry their chillies in huge quantities.

Open sun drying is the only method preferred by the farmers of Kazhi because of its convenience and low cost. Open sun drying of agricultural produce is cheap and a popular practice adopted by farmers for a while (Chavan, Yakupitayage & Kumar, 2011; Chavda & Kumar, 2009; Navale, Thorat, Harpale & Mohite, 2013). The vegetable dryer developed by the National Post Harvest Centre (NPHC), Paro, which can also be used for drying chilli is not used by farmers because this dryer cannot dry large volumes of chilli, and also because it involves high electricity cost. Navale et al., (2013) reported that use of dryers heated by coal, gas, fossil fuels, wood and electricity depends on the availability of energy, increases the production cost and is not environment friendly.

Desai, Palled and Anantachar (2009) found that chilli can be dried by solar drying, hot-air, freeze drying or osmotic dehydration. Muhidin and Hensel (2012) defined drying as a process of thermally removing moisture from a product that involves complex process of simultaneous heat and mass transfer. It has been reported that open sun drying is associated with many problems that reduce both the quality and quantity (drying recovery) that ultimately affects the storage life and household income. Browning of chilli was the main problem encountered in sun drying of chilli in major production areas of the world (Chavda & Kumar, 2009; Manjula, Ramachandra & Nidoni, 2013).

Difficulties in drying chilli during monsoon and the use of inappropriate drying methods remains an issue for chilli farmers in Bhutan. On the other hand many success stories of low cost technology such as solar dryer for drying fruits and vegetables elsewhere have been reported (Chavan et al., 2011; Parikh & Agrawal, 2011). Perumal (2007) and Tiwari et al. (2013) found that solar dryer was highly preferred for its many advantages over other drying methods. Solar dryer can generate higher air temperature and lower relative humidity which help to improve both drying rate and quality of the dried product (Amunugoda, Senanayake, Wijeratnam & Kulatunga, 2013; Chavan et al., 2011). It also saves energy, time, occupies less area, improves product quality, makes the process more efficient and protects the environment (Chavan et al., 2011; Fudholi, Ruslan, Othman & Sopian, 2012). Therefore, where sun shine is abundant solar dryer could immensely help marginal farmers (Tiwari et al., 2013).

Given the several advantages of solar dryer, this study aims to investigate and compare the traditional drying method with three other improvised sun drying methods in terms of drying loss, economics and product quality.

2. Materials and Methods

This study was carried out in Bjaktey village in Kazhi geog under Wangduephodrang dzongkhag. It is 30 km south east of the College of Natural Resource, Lobesa. The site at an altitude of 1900 masl has a warm temperate type of climate with frequent rainfall from September to October. The farmers in the village normally dry chillies from October till the end of December. Hence, the experiment for the study was carried out from September to October 2013.

Bamboo mats (1 x 5 m), transparent polyethylene sheet, bamboo basket (for harvesting), data logger (HOBO U-100-003), digital weighing balance (Eagle), measuring tape (Freemans Fibre Glass- 100 m), bamboo pegs and poles were used for the experiment.

The experiment was conducted under ambient conditions adjacent to the chilli production field. There were four treatments with three replications each. To avoid bias and influence of one treatment over the other, the replications were placed 2-3 m apart through drawing lots. Each replication (bamboo mat size) was 1 x 5 m that accommodated 15 kg of fresh chilli in one layer (180 kg of chilli, in total).

Control (T1) was the local method of sun drying, in which chilli was dried on the bamboo mat placed on the ground. In the second treatment (T2) chillies were placed on a bamboo mat that was raised 1.5 feet above the ground. In the third treatment (T3) chillies were placed on a bamboo mat that was raised 1.5 feet above ground with transparent polyethylene roofing placed 2 feet above the mat, and in the fourth treatment (T4) chillies were placed on a bamboo mat that was raised 1.5 feet above ground and a poly tunnel made out of bamboo frame covered by transparent sheet was placed on top of the mat entirely enclosing the chillies. This latter method is similar to the solar tunnel dryer model used by Pattanasethanon (2009) to study hot pepper drying by using solar tunnel dryer.

Fully mature red chillies were harvested during clear day and sorted. Fifteen kilograms of chillies were spread in one layer on each bamboo mat. The chilli in each replication was stirred twice a day as practiced by the farmers for uniform drying except for T4. T4 was kept intact without any disturbance until properly dried. As practiced by farmers, after the first week, everyday at sunset the control (T1) and T2 were covered with bamboo mat leaving some space between the mats to protect chillies from dews.

Data loggers were placed in each treatment in the beginning of the experiment to record relative humidity and temperature within the treatments and under ambient temperatures for comparison. Data recorded were downloaded. In the process of drying, spoiled chillies were collected in a separate basket for each treatment and weighed to determine loss percent. Once all the chillies were dried properly to a safe water activity they were collected, weighed (both good and spoiled) and recorded to compare the effect of treatments.

Water activity was determined using a water activity meter. A mean of three measurements were reported in the report. The duration required for drying in each treatment was also recorded. All the inputs (bamboo mats, poles, plastic sheets) and activities (preparation, displaying, stirring, folding) required for the whole drying process and weather parameters of each day were recorded on daily basis for analysis.

Data were statistically analyzed using analyses of variance (*ANOVA*). Bonferroni *Post hoc* tests were performed at $p<0.05$ to test for significant differences between the main effects and interactions using the SPSS-16.0 software.

3. Results and Discussion

3.1. Final weight and weight loss (%) of chilli

There was significant effect of drying methods on final weight and loss percent of dried chilli ($p< 0.05$. However, paired comparison using Bonferroni *post hoc* test indicated no significant differences amongst T2, T3 and T4, but were all significantly different from control (T1) (Table 1). It was observed that there was higher percentage of spoilage, seed lost and stems detached from chilli in T1 as compared to other treatments, which must have affected the final weight. Similiarly, Tiwari et al. (2013) also found more spoilage and weight loss in open sun drying in comparison to solar dryer.

In terms of loss percent (spoilage), there was no significant difference between T1 and T2, but they were significantly different from T3 and T4 (Table 1). Moreover, T3 and T4 were also significantly different from each other with T3 showing high loss percent at 0.31 compared to 0.03% in T4. There was 21.64% loss in T1 and a comparatively lower loss with 1.21% in T4. The total spoilage in control (T1) was comparatively (93.13%) higher than T4. This was due to short drying time because of high temperature and low relative humidity in T4. T4 was also totally protected from ambient weather, night dew, dust and insects.

Rajeshwari and Ramalingam (2012) also found that open sun drying method leads to more spoilage of product and greater loss due to adverse climatic conditions. This result indicates that T4 (poly tunnel dryer) was a better drying method in terms of quality product, short drying time and lower loss (spoilage) percent compared to open sun drying and modified open sun dryers.

Table 1.Paired comparison of final weight and Loss/spoiled percent of dried chilli

Treatment	Final weight (kg)	Loss/spoiled weight (%)
T1- Traditional open sun drying	2.31 b	0.50 a
T2- Raised bamboo mat	2.59 a	0.43 a
T3- Raised bamboo mat with plastic roofing	2.57 a	0.31 b
T4- Raised bamboo mat with poly tunnel	2.47 a	0.03 c
Significance	**	***
CV%	2.51	7.30

***$p < 0.05$; Means with different letters are significantly different

3.2. Water activity

Table 2.Mean comparison of water activity (a_W) between the treatments

Treatment	Mean
T1- Tradition open sun drying	0.54ab
T2- Raised bamboo mat	0.56a
T3- Raised bamboo mat with plastic roofing	0.44bc
T4- Raised bamboo mat with poly tunnel	0.41c
Significance	**
CV%	8.14

**significant at $p<0.05$, Means with different letter indicates significant difference

There was significant effect of drying methods on water activity $p< 0.05$. Paired comparison test indicated that T4 was significnatly different from the control (T1) and T2, but it was not significantly different from T3 (Table 2). The control was not significantly different from T2 and T3, but T2 and T3 were significantly different from each other. The highest (0.56 a_W) water activity was observed in T2 and lowest (0.41a_W) in T4 (Table 2). Paul and Sing (2013) also found similar result. Higher temperatures and low relative humidity in T4 must have brought down the water activity. Perumal (2007) on the other hand observed that there was no significant difference in the water activity among different drying methods.

To curve microbial spoilage and prolong storage life of dried chilli, the minimum water activity of dried chilli must be below 0.6 a_W (Fellows, 2000). Since the water activity of all the treatments in the study were below 0.6 a_W, those dried products may be assumed to be safe and shelf stable with respect to microbal growth and spoilage.

3.3. Drying Duration (days)

There was comparatively high difference in drying duration among the treatments (Figure 1). T4 took the least duration of 11 days whereas T2 took the longest (22 days). This translated into a net time saving of 50% in T4 compared to T2. This result was similar to the finding of Fudholi et al. (2012) and Palled et al. (2012) where they found 49% and 52.38% time saved respectively in solar dryer over open sun drying due to decrease humidity since solar radiation and air temperature considerably increased inside the tunnel. Similiarly, Wazed, Islam and Uddin (2009) also found considerable reduction of drying time in solar tunnel dryer (simialr to T4) as compared to open sun drying (T1) because in open sun drying significant amount of energy was lost to the environment. In a study conducted by Paul and Singh (2013), solar drier took 10-15 days to dry chillies, which is more efficient than traditional open sun drying.The drying duration (19 days) in control T1 was better than T2. Akarslan (2012) observed that there was a convective heat loss due to the blowing wind over and beneath the product surface,which must have contributed to delayed drying in T2 as compared to T1. On the other hand, ventilation is also necessary for water to escape.

Despite the study period it was observed that drying rate is dependent on drying conditions, which are influenced by factors such as weather conditions during the drying period. Similar observation was made by Perumal (2007). Several researchers found much higher percent reduction in drying time of 68-83% in solar dryer compared to open sun drying (Perumal, 2007; Paul & Sing, 2013; Sacilik, 2004; Nwokoye & Okeke, 2008).

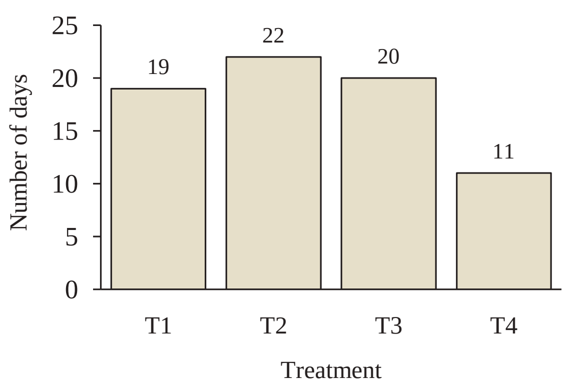

Figure 1.Drying duration (days)

3.4. Temperature and relative humidity variations among treatments

The maximum temperature recorded in each of the treatments (T1, T2, T3 and T4) were 28.20^{o}C, 28.23^{o}C, 28.29^{o}C and 41.59^{o}C, respectively (Fig. 6-A). Perasiriyan, Karthikadevi and Sivakumar (2013) recorded maximum temperature of 43.4^{o}C in solar tunnel dryer and so did Fadhel et al. (n.d.) at 55.1^{o}C. They however worked under different climatic conditions. The final drying temperature was recorded highest in T4 with 38.99^{o}C and lowest in T2 with 23.45^{o}C.The drying rate was comparatively higher in solar dryer due to better absorption of solar energy by the product since the energy is trapped in the structure, facilitating absorption as observed by Perumal, (2007). This difference in temperature must have contributed to faster drying rate of chilli in T4. This result was in line with the findings of Desai et al. (2009).

It was found that drying temperature can be easily raised to 5-30°C under solar dryer, however, raising temperatures above 60^{o}C can be harmful for vegetables, including chillies as this rise in temperature can induce loss of volatile nutrients through the excessive loss of moisture (Basunia et al., 2011).

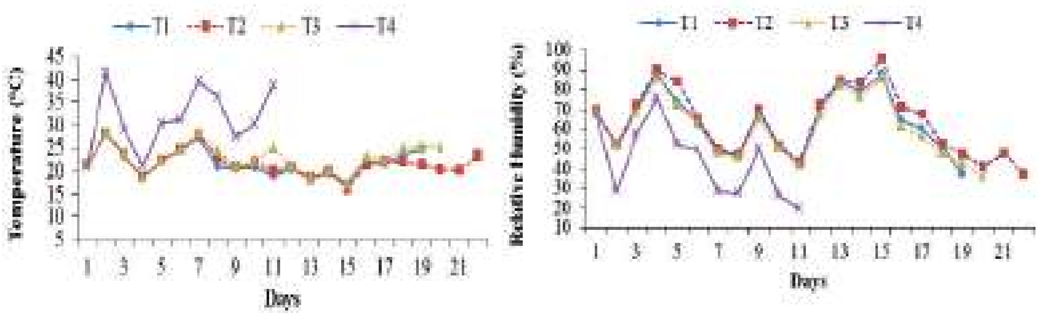

Figure 2.A-Temperature (°c); B-Relative Humidity (%) variations among treatments

The minimum relative humidity for T1, T2, T3 and T4 were 36.87%, 37.21%, 35.87% and 19.54%, respectively (Figure 2-B). It was also similar to the finding of Perumal (2007) where he recorded 16% and 31.9% in solar and open sun dryer respectively. Perasiriyan et al. (2013) recorded much higher relative humidity both in solar tunnel and ambient conditions at 25-40% and 41-54% respectively. The relative humidity difference of 47.49% between the highest (T2) and the lowest (T4) must have been responsible for lower water activity (aw) in T4 and the product under this treatment to dry faster as compared to rest of the drying methods. Fudholi et al. (2012) reported a difference of only 23% between solar and open sun drying methods.

The inside of the solar dryer was observed to be comparatively warmer than outside, clearly indicating that the drying rate in solar tunnel would be higher than open sun drying. Similiarly Fadhe et al. (2013) found that evaporating capacity of the air increases with low relative humidity in solar dryer which favours faster drying. Chavan et al. (2011) reported that energy from solar radiation was trapped inside the poly tunnel, while significant amount of solar energy was lost to the environment in open sun drying. Akarslan (2012) observed that there was a convective heat loss due to wind blowing over the product surface, besides radiation loss due to reflection from the surface.

3.5. Economic

Table 3.Economic comparison between the treatments (Cost per 5 m^2 (area) bamboo mat)

TMT*	Bamboo mat Nu	Bamboo pole Nu	Plastic Sheet	Preparation Nu	Stirring Nu	Folding Nu	Displaying Nu	Total VC* Nu	Gross Income Nu	Net Profit Nu
T1	120	0	0	3.15	6.3	15.75	15.75	160.95	1088	476.9
T2	120	50	0	9.45	9.45	12.6	12.6	214.1	1297	633.1
T3	60	100	53	37.8	5.04	0	0	255.84	1358	652.2
T4	60	100	66	56.7	0	0	0	282.7	1461.6	728.9

*TMT- Treatment; VC-Variable cost, Nu- Ngultrum (Bhutanese currency, 1Nu =72 USD).

Although the variable cost incurred in T4 was much higher (43%) than in T1, the net profit was 34.03% higher in T4 thanT1. This net profit in T4 with an average market price of dried chilli at Nu 600/kg that would translate to Nu 728.9/5m² as compared to Nu 476.9/5m² in T1. The total spoilage in T1 was 93.13% higher than in T4 that must have contributed to the drastic reduction in total net profit in T1. The cost of plastic, bamboo mat and pole contributed 79.94% to the variable cost in T4, whereas bamboo mat and after care (displaying, stirring and folding) involved contributed 98.04% to the variable cost in T1.

Similarly, Chavda and Kumar (2009); Rigit, Jakhrani, Kamboh and Tiong Pe (2013) reported that although the initial cost of solar dryers was high compared to open sun drying, it is more economical than traditional open sun drying and dryers based on conventional fuels. Fadhel, Koolo, Farhat and Belghith (2014) also reported high labor cost in open sun drying over solar drying. Preparation cost was comparatively lower (Nu. 3.15) in T1 as compared to T4, which was Nu. 56.7.

4. Conclusion

The results of this study reveal that chilli dried on a raised bamboo mat with solar tunnel drying method is comparatively better in retaining final weight due to minimum loss/spoiled percent as compared to traditional open sun drying. There was also significant difference in water activity between the treatments with solar tunnel dryer having the lowest at 0.41a_W. Low water activity extends product shelf life as the biological activity that is responsible for accelerating quality deterioration is reduced. In T4, drying time was considerably reduced as compared to all the three methods because of the enclosure that trapped the solar heat and protected against all environmental hazards. The high heat inside the enclosure also significantly reduced the relative humidity as compared to all other methods, which not only led to faster drying of produce, but also maintained the quality of the final product. The solar tunnel drying method also reduced the labor cost as no constant stirring of produce and frequent supervision were required unlike in the traditional open sun drying. Due to lower labor cost and loss percent (spoilage) solar tunnel drying method is more economical.

It can be concluded that raised bamboo mat with solar tunnel made out of polythene/plastic sheet is more economical and efficient in drying chilli as compared to traditional open sun drying and the other two methods involved in the study. However, because the solar tunnel method used in this study was raised, it involves cost and farmers' time. Therefore, similar method, but without raising off the ground may have to be studied in the future to determine whether similar benefits can be derived from the proposed slightly modified method.

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Analysis of SPAD Values and Chlorophyll Content of Organic Vegetables under Protected System

Bimal K. Chetri[i]

ABSTRACT

Physiology of plants is greatly influenced when exposed to various environmental conditions including induced factors such as protected systems, soil condition and nutrient application. This study aimed to evaluate the change in leaf greenness given the vital role that plant pigments play in the photosynthetic process, physiological condition and eventually their role in plant growth. Other objective was to develop organic management option for organic growers in protected system (S) like in Polyhouse (S_1) and Shadenet (S_2) focusing on use of organics on three different green leafy vegetable crops viz. Indian spinach (C_1), Fenugreek (C_2) and Amaranthus (C_3) employing split-split plot design. The SPAD readings and chlorophyll content analysis were taken at 3 different growth stages (20, 30 and 40 days after sowing). Analyses were performed in triplicates (n= 3) using agricolae package in R statistical software. Two-way ANOVA with mean separation by Tukey's Least Significant Difference (LSD) at α =0.05 was used to compare differences between systems ($S_{1/2}$) organic treatments ($NM_{1\text{-}5}$) and their interactions. Results showed SPAD values, chlorophyll content and chl.a./.b. ratio were significantly higher in S_1 than in S_2. Similarly, system-wise and treatment-wise total dry weight were significantly higher in S_1 than in S_2 at the level of $p<0.001$ for all 3 growth stages (GSs). Mean comparison between ($S_{1/2}$:$C_{1\text{-}3}$) and ($S_{1/2}$: $NM_{1\text{-}5}$) interaction was significant at the probability level of $**p<0.01$. Treatments (NM_{1-5}) effect on mean SPAD value and total chl. content was insignificant. Tukey's HSD method was used for pair-wise comparisons. LSD test for SPAD values and total chl. were significant over GSs and between interactions at $p<0.001$ level. Chl.a., chl.b. and total chl. content varied significantly between (($S_{1/2}$: NM_{1-5}) and C_{1-3}: $S_{1/2}$) interaction at the level of $p<0.01$. Chl.a./.b. ratio was higher in S_2 compared to S_1. Chl.b. was significantly higher than chl.a. in both the systems ($S_{1/2}$) and between systems it was higher in S_2. This study suggests positive correlation between SPAD values, chlorophyll and total dry weight (DW) ($p<0.0$) for all three crops.

Keywords: *SPAD, Chlorophyll Content, Organics, Vegetables, Protected System*

1. Introduction

In recent decades, use of polyhouse for growing vegetable crops has increased worldwide. Partially or fully protected cultivation under polyhouse and shade net with different shade factor (%) is preferred by urban growers with small holdings as crops produced are of better quality and

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higher productivity (Sabir & Singh, 2013) as disease and pest can be managed efficiently (Nordey et al., 2017) and vegetables are grown all round-year irrespective of climatic condition which fetches premium price for the growers (Singh & Sirohi, 2004). Even at higher altitudes protected cultivation has been proven successful in meeting regular food supply (Mishra, Singh, Kumar & Singh, 2010). Obviously, the other reason could be to ensure food safety. One of the factors for better productivity is daytime temperature increases in polyhouse house (relative to outside) due to characteristics of the cladding material, outside wind velocity, incident solar radiation and transpiration of the crop grown inside the polyhouse (Kittas, Katsoulas, Bartzanas & Bakkar, 2013). Temperature and relative humidity in shade net house are slightly higher than outside condition although outside weather condition has direct influence on it. Shade net houses are suitable for warm climatic condition for growing vegetables during summer months. Such factorial influence on physiogical processes in protected systems with organic treatments has been found to be strongly correlated with the dry biomass produced (Acatrinei, 2010).

Organic treatments have been reported to increase biomass in field crops like wheat by Saikia, Bhattacharya & Baruah (2015), in maize (Agegnehu, Bass, Nelson & Bird, 2016), in vegetables like cabbages (Islam et al., 2006). Vermicompost, neem cake, rock phosphate and compost are important organic treatments available to organic growers. Vermicompost is a nutrient-rich organic manure readily available to plants, with a low C:N ratio, high porosity and high water-holding capacity, which can enhance soil fertility and improve plant growth significantly (Hernández et al., 2010; Lazcano & Dom´ınguez, 2011; Lim, Wu, Lim & Shak, 2015; Saadatian et al., 2017). Neem cake is another potential source of organic manure which is the by-product obtained after neem oil extraction, with adequate quantity of NPK including essential micronutrients. It is also used as an effective bio-control agent. Compost although not rich in essential nutrients is a soil conditioner (Rashid, 2011) and an important source of organic nutrients.

Crops under protected cultivation with organic nutrients perform better in terms of their yield and production. With this assumption it is also important to study underlying factors that attribute crop production in micro-environment such as in protected system with organic management practices would perform better in terms of their physiological parameters including water and selective nutrient absorption, greenness of the leaf, photosynthesis and growth parameters.

Therefore, three dark green leafy vegetables were chosen for the experiment as consumption of vegetables are the main source of source of minerals (including iron, calcium, potassium, and magnesium) and vitamins, including vitamins K, C, E, and many of the B vitamins. Indian spinach (*Beta vulgaris* var. *bengalensis* Hort.) with succulent leaves is one of the most popular leafy vegetables of tropical and subtropical region and is grown widely in India (Padmanabha, Umesh and Krishnappa, 2008). The others were Fenugreek and Amaranthus. *Trigonella foenum-graecum* L. (English name: fenugreek) is native to Asia and southern Europe renowned culinary and medicinal uses in the history of old civilizations (Khan, Naz, Farooq & Tahira, 2014).

Amaranthus sp. (commonly called amaranth) is an annual or short-lived perennial plant used as leafy vegetables, grain, ornamental, leafy vegetable, medicinal and forage crop in many countries including India (George, Kumar & Chakraborty, 2014; Hauptli & Jain, 1977).

Since this short duration leafy vegetables are predominantly grown by farmers with small landholdings, choosing these vegetables were appropriate for the experiment that aimed to compare the effects of organic treatments under two types of protected system. Since the experiments were conducted using different combination of organic nutrients, care was also taken to use *Trichoderma harzianum* which is reported to be effective against *Rhizoctonia solani* (Elad, Chet & Katan, 1980) and also as a growth stimulator (Yedidia, Sirvastva, Kapulnik & Chet, 2001) as reported in cucumber. Experiments pertaining to physiological parameters were carried out with three green leafy vegetables (Indian spinach, Fenugreek and Amaranthus) under organic management practices with soil application of organic treatments in the form of vermicompost, combination of vermicompost+neem cake vermicompost+neemcake+rock phosphate and compost against control under two separate protected systems viz. polyhouse and shade net (40% shade factor). Split-split plot design was used to perform this trial. This study tried to analysis SPAD (Soil Plant Analysis Development) values and chlorophyll content of three green leafy vegetables and how they attributed to total DW production.

2. Materials and Methods

2.1. Field site and crops

This experiment was conducted in two different protected systems (polyhouse and green colored shade-net) during summer 2018 at Bio-resource Farm of Institute of Organic Farming, University of Agricultural Sciences, Dharwad, Karnataka, India. The organic field site (Plot No: C 52-A; Coordinates: 15° 29’50.695” N; 74°58’40.96” E, elevation 768.0 m) was allocated to evaluate the effect of protected system and organic nutrients and their interaction on physiological parameters and growth of Indian Spinach (*Beta vulgaris* L. var. *bengalensis* Hort.), Fenugreek (*Trigonellafoenum graecum* L.) and Amaranthus sp. Sprinkler-Can was used for normal irrigation. Local organic seeds of Indian Spinach (C_1), Fenugreek (C_2) and Amaranthus (C_3) were collected from organic farmer of Dharwad. The three green leafy vegetables were chosen on the basis of their phenological period of one-month duration approximately.

2.2. Growing condition

Protected cultivation is a cropping technique wherein microclimate to alleviate one or more of abiotic stresses for optimum plant growth was controlled partially under the constant care of gardener.

2.3. Soil condition

It is important to know the soil health by analyzing available nutrients including its chemical, physical and biological properties. Chemical soil analysis determines the content of basic plant nutrients; nitrogen (N), phosphorus (P_2O_5), potassium (K_20), pH, EC, organic matter, trace elements and other physical characteristics (water holding capacity, permeability, and density). Pre-treated soil analysis was carried for available N, P, K, EC, pH and organic carbon (OC) and available NPK in both the protected systems is given in detail (Table 1). The experiment was laid out on red soil and medium black loam soil in S_1 and S_2 respectively. In Dharwad, bulk density, per cent pore space, moisture content, organic matter content, EC and available K are considered higher in black soil compared to red soil with soil pH ranging from neutral to alkaline and acidic.

2.4.Weather condition

The average recorded temperature and relative humidity (%) in polyhouse (S_1) and shadenet (S_2) were 35.2 ˚C, 43% and 36.2˚C; 41% for the month of March and 39.5˚C, 34.5% and 38.5, 35.3% for the month of April respectively. Temperature in S_1 was comparatively higher than S_2 and outside temperature while relative humidity being opposite in trend during the study period. The average temperature was maximum-34.9˚C (March), 36.2˚C (April) and minimum 19.3˚C (March), 21.1˚C (April) in Dharwad. Average relative humidity was (max (65.3%) & (79.6%)) and (min (27.8%) & (36.7%)) in the month of March and April respectively.

2.5. Experimental design

The experimental design was a factorial split-split plot design (Figure 1) with S_1 and S_2 as the two main plots. Type of crops (C_1, C_2 and C_3) as the split plot, and organic treatments∗ as the split-split plot (Control (NM_1), Vermicompost (NM_2), VC+Neem cake (NM_3), VC+NC+Rock phosphate (NM_4) and compost (NM_5). Organic nutrients were applied on the basis of recommended dose for each type of crop under trial. Each plot length of 18x1(m) in 3 replications was equally divided into 5 sub-sub plots. Seeds were shown in four rows with outer row spacing of 11.5 cm and inter-row spacing of 22.5 cm.

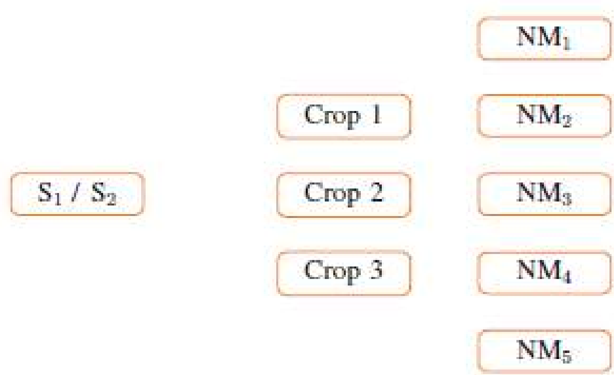

Figure 1.Split-split design

Table 1.Nutrient status of the soil in two protected systems before sowing

System	N (kg/ha)	P(kg/ha)	K(kg/ha)	OC (%)	EC (ds m1)	pH
Polyhouse (S1)	207.00	62.00	540.00	0.70	0.31	7.90
Shade-net(S2)	210.00	51.50	520.00	0.59	0.29	7.30
Crop	Recommended dose of organic nutrients added with respect to crops					
Spinach (C1)	150.00	100.00	100.00			
Fenugreek(C2)	100.00	50.00	0.00			
Amaranthus (C3)	100.00	50.00	50.00			

2.6. Soil and seed bed preparation

Soil beds were prepared using simple tools like spade and hand-held harrow. Coarse sand and stones were separated and removed from the beds. Organics were applied 7 days before sowing of seeds.

Previous crop history**:** In S_1 trial plot tomato and cucumber where grown. There was no immediate record of previous crop grown in S_2 trial plot.

2.7. Data gathering

All three crops were sown by hand dibbling method on 10 March and were harvested on 20 April. SPAD readings and chlorophyll estimation were recorded at 20 DAS, 30 DAS and 40 DAS (harvest) for 3 growth stages (GSs). Lab-based analysis of chlorophyll content and growth parameters were carried out during field observations interval.

2.8. SPAD (Soil Plant Analysis Development) values

The SPAD meter (SPAD-502, KONICA MINOLTA, INC.) is a widely used hand-held device for the instant and non-destructive measurement of leaf chlorophyll content in the field in agriculture research (Ling, Huang & Jarvis, 2011). The SPAD measures the transmittance of red (650 nm) and infrared (940 nm) radiation through the leaf, and calculates a relative SPAD meter value Corresponding to the amount of chlorophyll present in the sample leaf (Minolta, 1989). During this study leaf SPAD value obtained was the average of 4 readings (2 on each side of leaf midrib). Readings were taken from 9 am-12 noon for all the samples in the field.

2.9. Chlorophyll content

The photosynthetic pigments and their abundance vary among plants. In vegetable species chl.a. is present to achieve oxygenic photosynthesis while other pigments help in the light absorption and in the transference of light radioactive energy to reaction centers (Costa, Calvete, Schons & Reginatto, 2010). Leaves sample used for SPAD readings were weighed to 0.05g and used for chlorophyll content analysis. 0.05 g of fresh plant leaf samples were placed in a test tube added with 5 mL of DMSO (Sudhakar, Latha & Reddy, 2016) and kept overnight. Absorbance was recorded at 645 nm for chl.a. and 663 nm for chl.b. using Bio Spectrophotometer with Touch Screen and Built-in PC (Elico Limited) for further chlorophyll content estimation. The amount of chlorophyll present in the extract is mg chlorophyll per g (mg g^{-1}f.wt.) tissue was measured as per formula given by Arnon (1949).

2.10. Statistical analyses

According to Ledolter (2010), incorrect analyses[jk] are often overlooked in split-split plot designs. Split-split plot design was used in two different protected systems (greenhouse and shade-net) separately for studies. The statistical analysis focused on the relating factors of the factorial split plot design which is considered to be not only less expensive but also statistically proven to be efficient (Jones & Nachtsheim, 2009, and references therein) was employed.

All analyses were performed in triplicates (n= 3) and corresponded to both the protected systems (S_1 and S_2). The differences between the mean were analysed by two-way analysis of variance(ANOVA) with mean separation by Tukey's Least Significant Difference (LSD) at α = 0.05 to determine whether there were significant differences between systems ($S_{1/2}$) and treatments (NM_{1-5}) and their interactions ($S_{1/2:}$ NM_{1-5}). Mean among ($S_{1/2}$), (NM_{1-5}) and their ($S_{1/2}$: NM_{1-5}) were compared using LSD at $P \leq 0.05$. Significant level set at the probability level of $*p<0.05$; $**p<0.01$; $***p<0.001$ for all the analysis in this study. Data analysis was done in R statistical software (R Core Team, 2018) using agricolae package (Mendiburu, 2017) except for Pearson correlation coefficient which was analysed using SAS System.

3. Results

The results from field experiment conducted at Bio-resource farm, Main Agricultural Research Station (MARS), University of Agricultural Sciences, Dharwad during March-April, summer 2018 are presented in this section. Brief statements of the results in the text are explained followed by the appropriate graphs in the sequence of the methodology as written in the report. Two-way analysis of variance (ANOVA) was carried out with mean separation by Tukey's Least Significant Difference (LSD) at α = 0.05 at the significant level.

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[j]Finding whole-plot factors significant too often (even if they are not present)
[k]Failing to uncover sub-plot factors (even though they are present)

Table 2.Effect of protected systems and organic nutrients on SPAD and chl. content of greenleafy vegetables

Values (Mean of	SPAD	Chl.a.	Chl.b.	T.chl.	Chl.a/b.
Polyhouse (S_1)	42.95[a]	1.16[a]	1.51[a]	2.70[a]	0.79[a]
Shadenet (S_2)	36.77[b]	1.10[a]	1.32[b]	2.45[b]	0.83[b]
LSD at 5%	1.88	0.08	0.15	0.23	0.03
Crops (C)					
Spinach (C1)	31.61[c]	0.80[c]	0.91[b]	1.71[b]	0.85[a]
Fenugreek (C2)	51.77[a]	1.25[b]	1.63[a]	2.93[a]	0.78[b]
Amaranthus (C3)	36.21[b]	1.36[a]	1.70[a]	3.09[a]	0.80[b]
LSD at 5%	1.90	0.08	0.12	0.20	0.01
Nutrient management (NM)					
Control (NM1)	38.69[a]	1.14[a]	1.42[ab]	2.57[a]	0.80[bc]
VC (NM2)	40.16[a]	1.18[a]	1.50[a]	2.70[a]	0.79[c]
VC + NC (NM3)	40.02[a]	1.15[a]	1.46[ab]	2.63[a]	0.81[ab]
VC+NC+RP	40.43[a]	1.11[a]	1.39[ab]	2.53[a]	0.80[bc]
Compost (NM5)	40.01[a]	1.10[a]	1.31b	2.44[a]	0.83[a]
LSD at 5%	1.76	0.12	0.18	0.30	0.01

Values (column means) with the different small letter (superscript) in a column are significantly different according to the Tukey's LSD test at p<0.01.

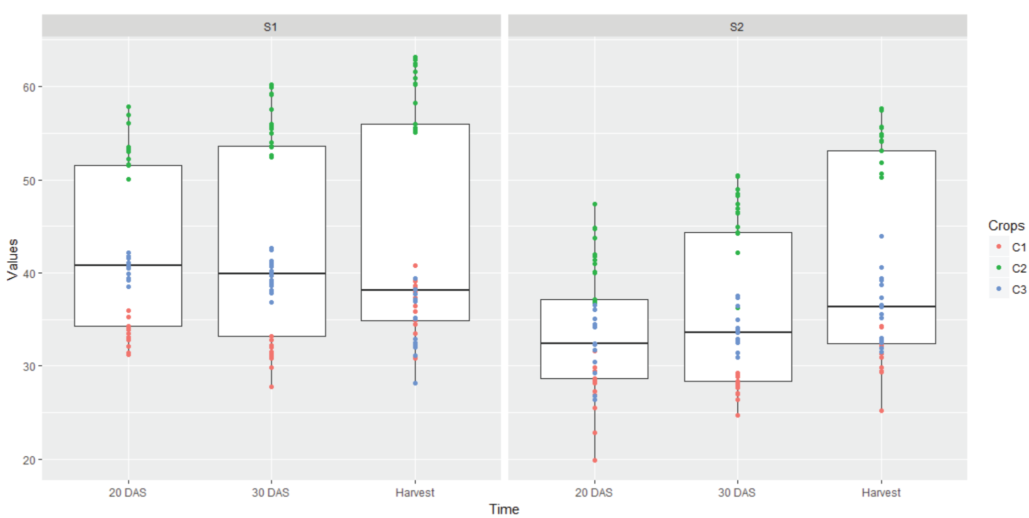

Figure 2.Mean SPAD values showing ($S_{1/2}$: C_{1-3}) and ($S_{1/2}$: NM_{1-5}) interactions at for 3 GSs

Table 3.Effect of system: treatment interaction on SPAD values and chl. content of green leafy vegetables at 3 GSs (20, 30 and 40 (DAS) harvest)

Values (Mean of 3 GSs	SPAD	Chl.a.	Chl.b.	T.chl.	Chl.a/b.ratio
	Interactions (S:NM)				
NM1:S1	42.20^{a}	1.15^{a}	1.49^{ab}	2.64^{abc}	0.79^{de}
NM2:S1	42.89^{a}	1.20^{a}	1.63^{a}	2.83^{a}	0.76^{f}
NM3:S1	42.52^{a}	1.17^{a}	1.60^{a}	2.77^{ab}	0.79^{de}
NM4:S1	42.80^{a}	1.16^{a}	1.51^{ab}	2.67^{abc}	0.79^{e}
NM5:S1	44.38^{a}	1.13^{a}	1.48^{abc}	2.60^{abc}	0.81^{cd}
NM1:S2	35.18^{c}	1.12^{a}	1.39^{abc}	2.51^{abc}	0.82^{bc}
NM2:S2	37.43^{bc}	1.15^{a}	1.41^{abc}	2.57^{abc}	0.83^{abc}
NM3:S2	37.52^{bc}	1.13^{a}	1.38^{abc}	2.50^{abc}	0.84^{ab}
NM4:S2	38.07^{b}	1.07^{a}	1.32^{bc}	2.39^{bc}	0.82^{bc}
NM5:S2	35.65^{bc}	1.07^{a}	1.21^{c}	2.29^{c}	0.85^{a}
LSD at 5%	2.48	0.17	0.26	0.43	0.0
Growth Stages (GSs)					
20 DAS	37.99^{c}	1.029^{b}	1.23^{c}	2.26^{b}	0.83^{a}
30 DAS	39.45^{b}	1.184^{a}	1.60^{a}	2.76^{a}	0.76^{b}
Harvest (40DAS)	42.14^{a}	1.19^{a}	1.41^{b}	2.68^{a}	0.84^{a}
LSD at 5%	1.36	0.09	0.14	0.24	0.01

Values (column means) with the different small letter (superscript) in a column are significantly different according to the Tukey's LSD test at $p<0.01$.

3.1. SPAD and chlorophyll content analysis

Multiple correlations were performed (Table 4), and two-way ANOVA and Tukey's HSD with ggplot was used to compare the means of SPAD values and chlorophyll content. Higher the SPAD values higher were the chl. content between systems and crops, however, among treatments result was insignificant (Table 2).

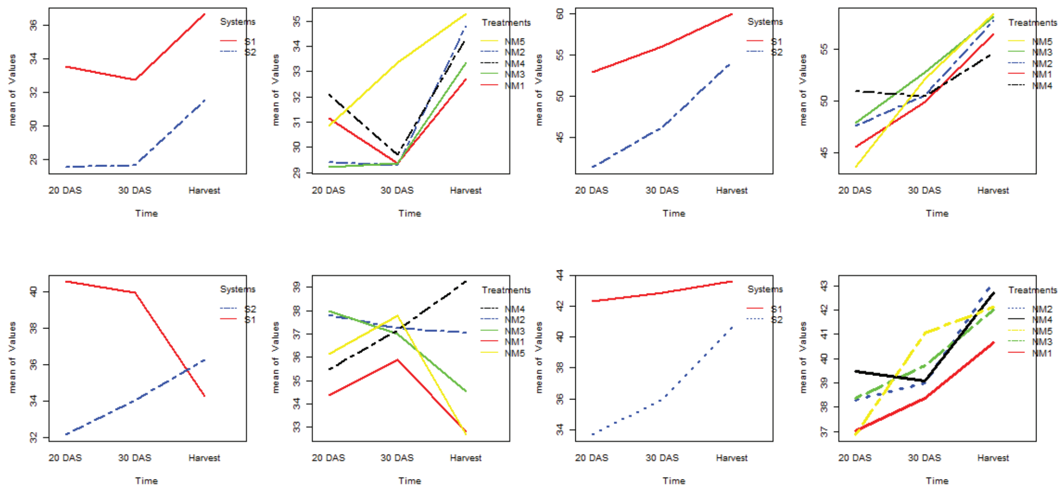

Figure 3.Mean SPAD values with time and interaction: (S1/2: NM1−5) at 20, 30 and 40 DAS (harvest.) where 1st row is for C_1 (1st two plots) and C_2 (2nd two plots); 2nd row is for C_3(1st two plots) and mean of 3 crops (2nd two plots).

3.2. SPAD

The SPAD readings were taken at 3 different growth stages in two protected systems for 3 different green leafy vegetable crops. Significant difference is observed with system ($S_{1/2}$): Crop ($C_{1–3}$) interaction (Figure 2). Mean comparison with the ($S_{1/2}$: $C_{1–3}$) and ($S_{1/2}$: $NM_{1–5}$) is significant at the probability level of $***p<0.001$. Between treatments mean SPAD value is not significant (Table 2). SPAD values varied and increased over 3 growth stages (Table 3) at $***p<0.001$ level except for C_3 (Figure 3). C_3 was ready for harvest by 30 DAS in S_1. Tukey's Honest Significant Difference (Tukey's HSD) was used to make all the pair-wise comparisons (Figure 4). Interactions between system, treatment and crop ($S_{1/2}$: $NM_{1–5}$ and $C_{1–3}$: $S_{1/2}$) for SPAD values and total chl. content are significant at the probability level of $p<0.01$ over all 3 growth stages.

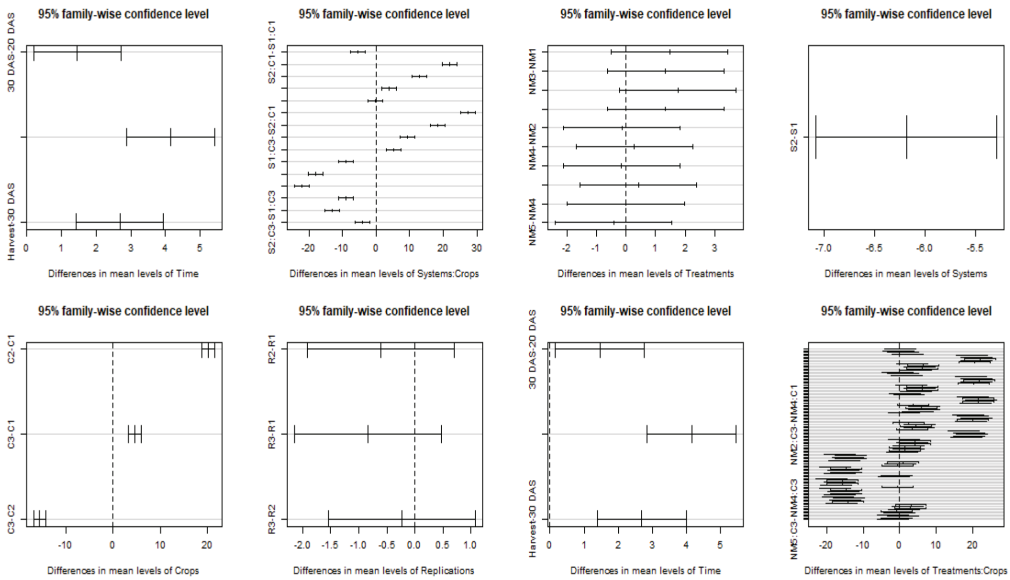

Figure 4.Differences in mean SPAD values of 95% confidence level

Row 1: ($S_{1/2}$: C_{1-3}) and Row 2: ($S_{1/2}$: NM_{1-5}) interaction at 3 GSs. Any confidence intervals that do not contain 0 provide evidence of a difference in the groups.

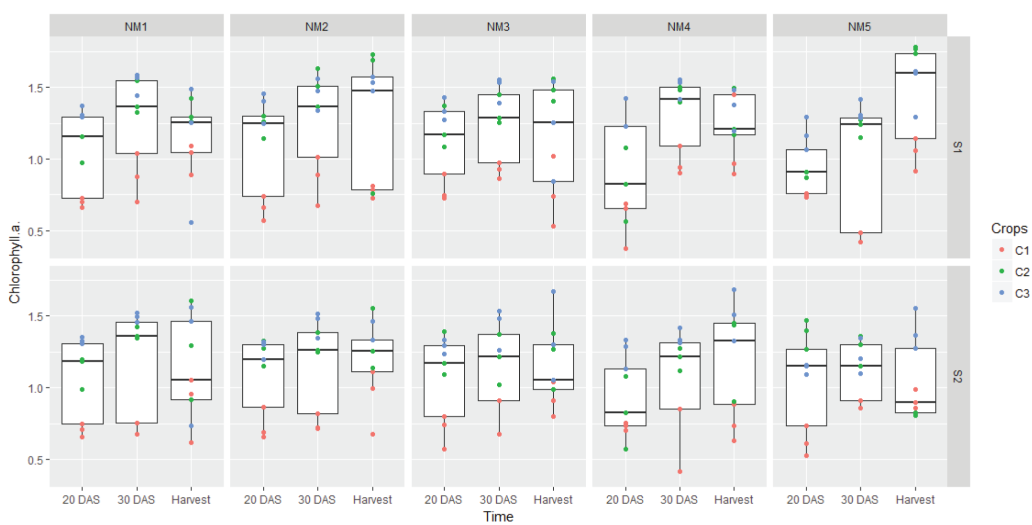

Figure 5.Mean chl.a. content (mg g^{-1}f.wt.) showing ($S_{1/2}$: NM_{1-5}) interaction for 3 different GSs

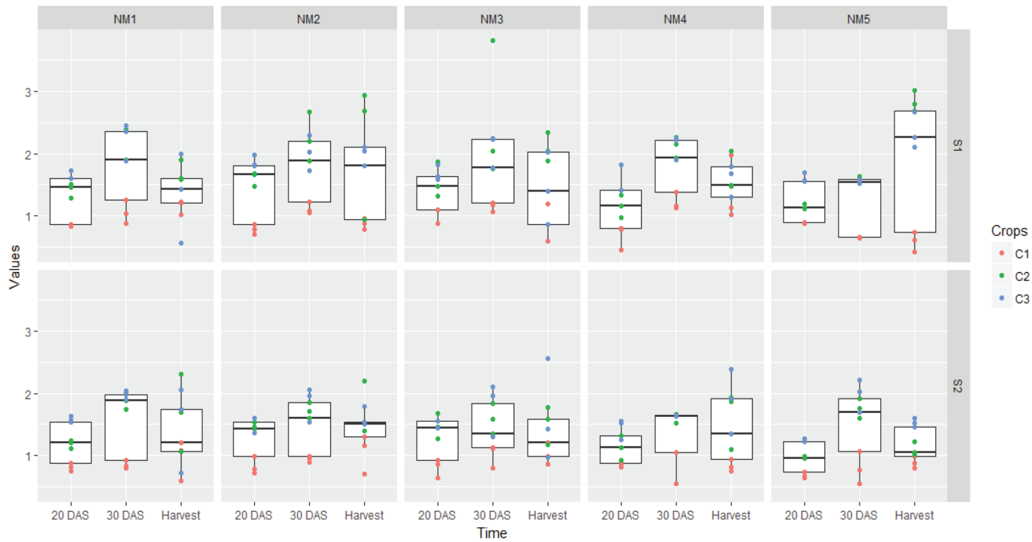

Figure 6.Mean chl.b. content (mg g^{-1}f.wt.) showing ($S_{1/2}$: NM_{1-5}) interaction for 3 different GSs

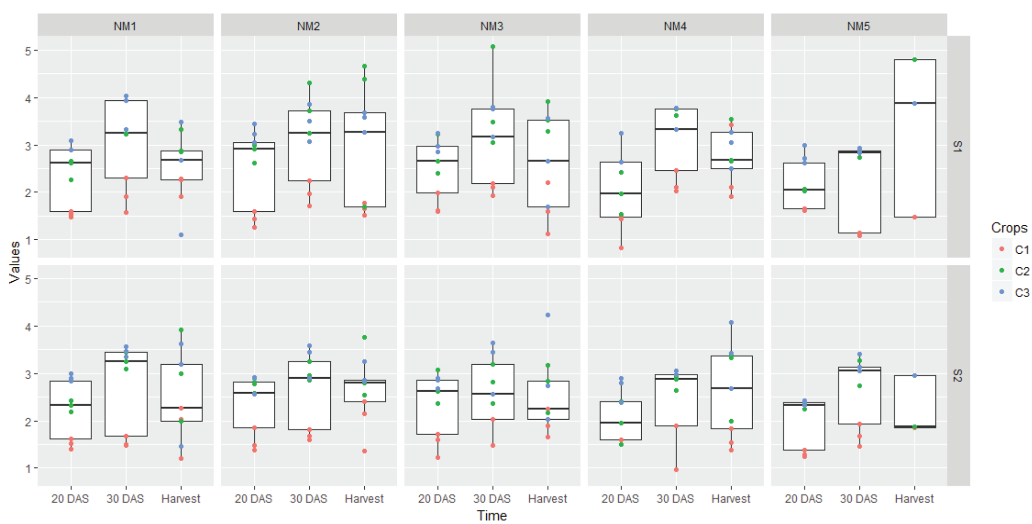

Figure 7.Mean total chl. content (mg g^{-1}f.wt.) showing ($S_{1/2}$: NM_{1-5}) interaction for 3 different GSs

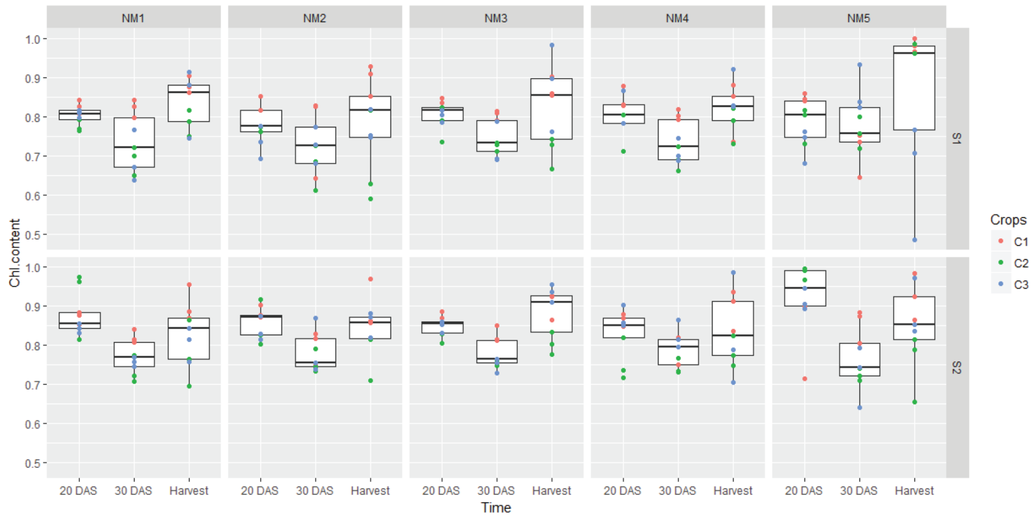

Figure 8.Mean chl.a./.b. ratio (mg g^{-1}f.wt.) showing ($S_{1/2}$: NM_{1-5}) interaction for 3 different GSs

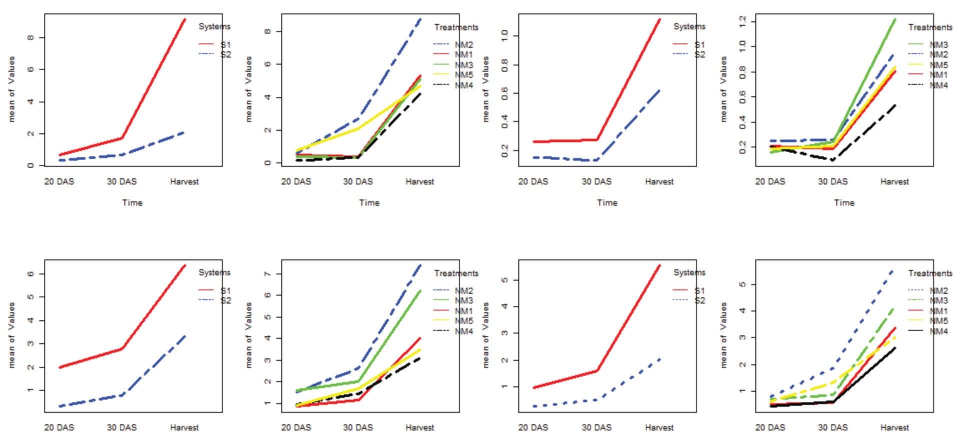

Figure 9.Mean total DW (gm) with time: ($S_{1/2}$: NM_{1-5}) at 20, 30 and 40 DAS (harvest) where 1st row is for C_1(1st two plots) and C_2(2nd two plots); 2nd row is for C_3(1st two plots) and mean of 3 crops (2nd two plots).

3.3. Chlorophyll content

Chl.a., chl.b. and total chl. content (Figure 5, Figure 6 and Figure 7) was significant between system and interaction between ($S_{1/2}$: NM_{1-5} and C_{1-3} : $S_{1/2}$) at the level of $**p<0.01$.

Chl.a./.b.ratio is analyzed basically to compare how their content varies under different treatment (NM_{1-5}) and systems ($S_{1/2}$). Chl.a./.b. ratio is higher in S_2 compared to S_1 (Figure 8).

The correlation between 5 variables, viz., SPAD values and chl.a., chl.b., total chl. and Chl.a/b ratio was analysed. SPAD values were positively correlated with chl.a. (0.40), chl.b. (0.49) and total chl. (0.47), whereas SPAD value was negatively correlated with chl.a./b. (-0.29) at $p<0.01$.

3.4. Total DW (gm) of the plant

Total DW of all 3 crops (C_1, C_2 and C_3) is significantly higher in S_1 compared to S_2. Treatments- and system-wise total DW increased significantly over the growth stages, however, interaction between system: treatment within same system ($S_{1/2}$: NM_{1-5}) is not significant (Figure 9).

4. Discussion

SPAD mean values for all 3 crops in S_1 (polyhouse) was significantly higher than in S_2 (shadenet). Steady increase in SPAD values can be attributed to factorial effects (Abdelhamidg, Horiuchi & Oba, 2003). SPAD mean values in C_3 in S_1 have been observed to decrease sharply in C_3 as crop maturity was advanced by 10 days in S_1 and was ready for harvest (Row 2: C_3 in Figure 4).

Similarly, C_1 and C_2 crop maturity advanced by a week in S_1 compared to S_2. This trend was also recorded by Parvej, Khan and Awal (2010) in tomato varieties in polyhouse micro-climate. However, higher than 50 in SPAD readings does not necessarily increase the photosynthesis efficiency (Nyi, sridokchan, Chai-arree & Srinives, 2012). Higher SPAD values also indicate the higher level of N content in the leaf (Nyi et al., 2012; Uchino et al., 2013) which has strong correlation with higher chl. content in all three crops. SPAD readings and total DW have been reported strongly correlated except for later growth stages of rice and spinach by Yang, Yang, Lv& He (2014) and Liu et al. (2006) respectively. It could also mean that there is a strong correlation with SPAD values and DW of the crops (Figure 4, Figure 10). SPAD values were significantly different for C_1 (Indian spinach), C_2 (Fenugreek) and C_3 (Amaranthus). SPAD values of C_2 were recorded higher compared to C_1 and C_2. Similar variation in SPAD readings in different crops were also reported by Netto, Campostrini, de Oliveira and Bressan-Smith (2005) in coffee leaves and in Jatropha leaves by Nyi et al. (2012).

Leaf chlorophyll content in the crops in S_1 and S_2 consistently increased from 20 DAS to harvest. Chl.a. content in S_1 was comparatively higher to S_2, so as the total DW production in S_1as compared to S_2. This could be as a result of ch.a. and b content which are essential pigments in converting light energy to chemical energy (Steele, Gitelson & Rundquist, 2008) for primary products in plants. Similar relation was observed by Nyi et al. (2012) in *Jatropha curcas* L. As expected chl.b. in plants acclimated to low light intensities, its content was comparatively higher in S_2 and S_1 thanchl.a. But between two protected systems, chl.b. content was much higher in S_2 (Table 2). Similarly chl.a./.b. ratio was significantly higher in S_2 across all three types of crops. Chl.b. content was higher than chl.a. so does the chl.a./b. ratio as reported by several researchers

(Bhatt,1999; Boardman, 1977; Kosma et al., 2013; Murchie & Horton, 1997; Žnidarčič, Ban & Šircelj, 2011). With respect to texture of the leaves, crops in shade-net have thinner lamina with softer texture as compared to polyhouse crops. This could have been as a result of light intensities variation in polyhouse and shade-net (Ilić, Milenković, Šunić & Fallik, 2015). SPAD values and chl. content had consistently increased over the 3 growth stages till harvest (40 DAS). However, in Amaranthus (C_3) which had attained maturity earlier by 10 days in polyhouse compared to shade-net, a sharp disease in SPAD values and chl. content after 30 DAS was observed. This trend was reported by Riccardi et al. (2014) in quinoa and amaranth during reproductive and senescence periods. Combined effect of organic treatments and protected system on total DW production was significant. Similar effect was reported by Kumar and Arumugam (2010) under nitrogen treatment in sunflower. There was significant effect on total DW as a result of system: treatment interactions which could be attributed not only to available soil nutrient but also to growth condition inside protected system which has influenced SPAD values and chlorophyll content.

5. Conclusion

Protected cultivations have been lauded by many agriculturists and have been recommended as a solution to produce fresh vegetables throughout the year irrespective of climate and weather conditions. Growing vegetables in polyhouse and shade-net have been proven to optimize the agricultural productivity both in terms of quality and quantity. The presence of higher chlorophyll content and SPAD values in green leafy vegetables indicated better yield in polyhouse compared to shade-net could be attributed not only to nutrient management but also to physiological parameters as influenced by controlled-environmental conditions of protected cultivation. Crops under polyhouse have performed significantly better than crops in shade-net in short course of time for marketable yield. Total DW of 3 types of crops which was significantly higher in polyhouse treated with vermicompost responded faster at all 3 growth stages compared to combined treatments of vermicompost+neem cake+rock phosphate. If further research on effects of organic treatments including physiological parameters on different horticultural crops under protected cultivation has to be carried out, knowledge on the soil nutrient status, previous crop history with pest and diseases and cropping system need to be taken into account to ascertain multi-factorial influence on vegetable production.

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Adaptation of Quinoa in Bhutanese Cropping Systems

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ABSTRACT

Quinoa, a new Andean crop, was introduced to Bhutan in 2015. Rapid evaluation of quinoa was carried out in 2015 and 2016 to assess its adaptability and to generate cultivation and crop management information under the Bhutanese low external input subsistence mountain farming systems. Ten different varieties were evaluated at six different locations across the country from which four varieties have been released by the national Variety Release Committee (VRC) of the Ministry of Agriculture and Forests (MoAF). Crop maturity and grain yield of different varieties from two years of trials and demonstrations under different agro-ecological zones and cropping systems indicated good adaptability of Quinoa for cultivation as an alternative crop. Varieties Amarilla Marangani (Ashi Heychum - AM) and Amarilla Saccaca (Ashi Heychum – AS) produced an average grain yield of 2.31 and 2.24 t ha^{-1} in 2015. In 2016, of the 10 varieties evaluated in six locations, mean yield ranged from 1.22 to 2.57 t ha^{-1}which was statistically not significant. There was, however, significant difference in days to maturity among the test varieties. The information generated is used to rapidly promote this new protein rich cereal under existing cropping systems to enhance household food and nutritional security. Based on the on-station and on-farm observation trials, varieties and crop management practices have been recommended for different cropping systems and agro-ecology.

Keywords: *Cropping system, Quinoa varieties, Maturity, Yield, Sowing time*

1. Introduction

The Food and Agriculture Organization (FAO) of the United Nations has recognized quinoa (*Chenopodium quinoa* Willd) as a strategic crop that can contribute to global food security because of its high nutritional quality, genetic variability, adaptability to adverse climate and soil conditions, and low production cost (FAO, 2011). Quinoa is proven to possess high tolerance to different abiotic stresses and there is growing interest to introduce quinoa in marginal agricultural production systems for food security (Choukr-Allah et al., 2016). Quinoa is also known for its broad genetic diversity which makes it a highly versatile crop that can successfully adapt to different types of growing environments. Several studies have established that quinoa can be cultivated in different growing environment with humidity range of 40 to 90%, at

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altitudes varying from sea level to 4500 meters above sea level (masl) and has the ability to tolerate temperature variation from $^{-}8°C$ to 38°C (Jacobsen, Jensen and Liu, 2011).

Quinoa was introduced to Bhutan in 2015 from Peru by the Department of Agriculture (DoA) with the support of the FAO (Katwal, 2018). The primary objectives of introducing Quinoa in Bhutan are to diversify the farmer's traditional cropping systems, adapt this versatile crop to different growing environments as a climate resilient crop, and to enhance the food and nutritional security of the Bhutanese people. Quinoa was never grown in Bhutan before 2015 and hence no information on crop cultivation and management was available. Information on the quinoa cultivation in the South Asia region is very scanty and not easily accessible. The challenges to the DoA was to assess the adaptability of this new crop in unique Bhutanese mountain agriculture systems where most of the farming is done in steep slopes with minimum use of external inputs and generate cultivation and crop management information for the rapid promotion of quinoa.

In 2015, FAO introduced two varieties, Amarilla Marangani and Amarilla Saccaca. Six more varieties were again received in 2016 from FAO, one variety was introduced from India and one was received from an informal source making a total of 10 varieties. Following the introduction of this new crop, observation trials were planned and conducted in different locations. The objectives of such trials were to assess the adaptability of quinoa under different agro-ecological zones and cropping systems; generate basic information on crop management, maturity, sowing time, yield, and to the package the cultivation and crop management information for rapid promotion of quinoa in Bhutan. Research and development was conducted by four different Agriculture Research and Development Centers (ARDCs) at Yusipang, Bajo, Samtenling and Wengkhar. The first experiences of acclimatizing and adapting a new crop of quinoa under diverse agro-ecology and cropping systems are discussed in this paper.

2. Materials and Methods

The evaluation and adaptation of quinoa varieties was started in 2015 with two introduced varieties, namely, Amarilla Marangani and Amarilla Saccaca. The evaluation was continued with 8 new varieties in more locations in 2016.In 2015, due to the limited seed, observation trials were conducted at Yusipang (2600 masl), Phobjikha (2900 masl) and Khangma (2100 masl). Yusipang and Phobjikha represented the cool temperate agro-ecology with potato based cropping system while Khangma represented the dry-subtropical agro-ecology where maize based cropping system is dominant. All the trials were conducted in rain fed dry land cropping systems where crops are not irrigated. The time of sowing and harvesting differed according to the locations and are summarized in Table 1. Quinoaseeds were sown in line at a row spacing of 50 cm. The plant to plant spacing was maintained by thinning at 25 cm. Seeds were sown uniformly in line and covered with a thin layer of soil using a locally made broom. In all the locations sufficient quantities of Farm Yard Manure (FYM) was applied. In Phobjikha and Khangma, one top dressing of Nitrogen was given at the rate of 70 kg ha^{-1}. In Yusipang, Suphala (16:16:16 NPK) was applied at the rate of 70 kg ha^{-1} at the time of sowing. Weeds were controlled by three hand weeding. At Yusipang, the trial was irrigated twice using small sprinklers. To measure yield, three crop cuts for each variety were taken from an area 6m^2. The crop was manually harvested and kept in the shade for 10 days for curing. Threshing and winnowing was done manually.

In 2016, ten varieties were evaluated at six different locations. The general experimental conditions of trials sites are summarized in Table 2. Depending on the locations, seed sowing was started in the second week of March and completed by 15th April. Harvesting was done depending on the maturity of the varieties which started in September and was completed by end October for all the 10 varieties. The plot size used in all the location was 10 m^2. At crop maturity, whole plot was harvested manually and the samples from the trial were tied into bundles and dried by hanging in the shade for 10-15 days for curing. The samples were manually threshed and cleaned using local winnowers to obtain the grains for yield estimation. The data from the trials were analyzed using MS Excel and SPSS software.

Further, to rapidly demonstrate and assess the performance of quinoa crop and varieties, on farm trials were also conducted in the farmer's fields in large single observation plots at different locations. To estimate the yield from such demonstrations, three crop cuts were taken and grain samples were processed following standard threshing and cleaning procedures. In some locations, farmer's field day was conducted to create awareness on the new crop. In the low altitude areas of Chhukha and Samtse, demonstrations were established in the second week of October and harvested in January and February.

Table 1.Details of quinoa observation trial conducted in 2015

Variety	Locations and Altitude					
	Yusipang (2600 masl)		Phobjikha (2900 masl)		Khangma (2100 masl)	
	Date of Sowing	Date of Harvest	Date of Sowing	Date of Harvest	Date of Sowing	Date of Harvest
A. Marangani	26th March	1st Oct.	27th March	24th Nov.	7th April,	18th Sept.
A. Saccaca	2nd April	7th Oct.	27th March	24th Nov.	25th April	2ndOct.

Table 2.Cropping system and basic weather data of trial sites, 2016

Location	Dominant Cropping System	Temperature and Average Rainfall of Trial Sites during Sowing and Harvesting Months (Source: SYB, 2017)				
		Weather Parameters*	Sowing Months		Harvesting Months	
			March	April	Sept.	Oct.
Yusipang	Potato	Max. Temp. °C	26.0	27.0	29.0	28.5
	Based	Min. Temp. °C	0.0	3.0	11.0	0.0
	Dryland	Av. Rainfall (mm)	42.2	23.5	154.4	72.7
Katsho	Potato	Max. Temp. °C	19.0	18.5	20.5	20.5
	Based	Min. Temp. °C	-4.0	0.50	10.0	-1.0
	Dryland	Av. Rainfall (mm)	70.1	44.9	157.0	116.2
Dawakha	Potato	Max. Temp. °C	22.5	25.5	25.0	21.0
	Based	Min. Temp. °C	0.0	4.0	11.0	0.0
	Dryland	Av. Rainfall (mm)	255.0	506.0	294.0	0.0
Khangma	Maize	Max. Temp. °C	27.5	28.5	30.5	31.5
	Based	Min. Temp. °C	6.5	8.0	13.0	8.0
	Dryland	Av. Rainfall (mm)	47.8	150.8	89.6	73.2
Mertsham	Maize	Max. Temp. °C	27.5	28.5	30.5	31.5
	Based	Min. Temp. °C	6.5	8.0	13.0	8.0
	Dryland	Av. Rainfall (mm)	47.8	150.8	89.6	73.2
Trashiyangte	Maize	Max. Temp. °C	25.5	26.0	28.5	29.5
	Based	Min. Temp. °C	2.0	5.5	13.0	2.5
	Dryland	Av. Rainfall (mm)	47.1	136.5	326.3	133.7

Max. = Maximum, Min. = Minimum, Temp. = Temperature, Av.= Average. °C= degrees Centigrade

3. Results and Discussion

In 2015, the two quinoa varieties, Amarilla Marangani and Amarilla Saccaca which were cultivated for the first time in three different locations matured successfully and produced good yields. Due to limited seeds, replicated trial could not be carried and hence only mean data are presented. Further, as quinoa is an introduced crop no traditional varieties were available to make comparison. The vegetative growth in all the three locations was profuse with plant heights in the range of 1.63 m to 2.52 m for both varieties (Table 3). The days to maturity ranged from 155 to 230 days while the yield produced varied from 1.63 t ha^{-1} to 2.75 t ha^{-1} (Table 3) for both the varieties in all three locations. The mean yield recorded for A. Marangani was 2.31t ha^{-1} and for A. Saccaca was 2.24 t ha^{-1}. The mean yield did not show huge difference because both the varieties are genetically very similar as they come from a close pedigree.

Table 3.Plant height, maturity and yield of two quinoa varieties from three locations, 2015

Variety	Yusipang (2600 masl)			Phobjikha (2900 masl)			Khangma (2100 masl)			Mean Yield t ha^{-1}
	Plant Height (m)	Maturity (days)	Yield t ha^{-1}	Plant Height (m)	Maturity (days)	Yield t ha^{-1}	Plant Height (m)	Maturity (days)	Yield t ha^{-1}	
Marangani	2.07	195	2.75	1.82	225	1.99	2.37	165	2.19	2.31
Saccaca	1.69	182	2.00	1.63	230	2.42	2.52	155	2.31	2.24

In 2016, all the ten quinoa varieties, of which seven were cultivated for the first time, matured successfully in all six locations that represented the warm temperate and cool temperate agro-ecological zones (Table 4). One way ANOVA showed that there was significant difference among the varieties for days to maturity while the varieties were not significantly different in terms of mean yield t ha^{-1} (Table 4). DoA-1-PMB-2015 matured earliest in 112 days while Blanca de Junin took the longest (187 days) to mature. The varieties can be grouped into three maturity groups :DoA-1-PMB-2015, Ivory 123 and Salcedo INIA (early); INIA 420 Negra Collana, INIA 415 Pasankalla, Hualhuas, INIA 427 Amarilla Saccaca, Amarilla Maragani and Huancayo (medium) and Blanca de Junin (late). The mean grain yield was not significantly different for all the varieties, which ranged from 1.22 t ha^{-1} to 2.57 t ha^{-1} (Table 4). The highest mean yield of 2.57 t ha^{-1} was produced by INIA 427 Amarilla Saccaca while the lowest mean yield of 1.22 t ha^{-1} was recorded for variety Ivory 123.

In the lower elevations (< 1200 masl) when three selected varieties were sown in the maize based dry lands in March, the vegetative growth and flowering was good but there was no grain setting in all the varieties. The foliage was damaged by heavy infestation of Armyworm (*Spodoptera frugiperda*) and Locust (*Shistocerca gregaria*). However, when the crop was sown from October to January, there was good grain setting and yield (Table 5). The crop maturity was also hastened due to low rainfall during winter months and higher temperature at the sites.

Table 4.Maturity and mean yield of 10 Quinoa varieties from six locations, 2016

Variety	Days to Maturity*	Grain Yield t ha^{-1}
DoA-1-PMB-2015	112[a]	2.52
Ivory 123	133[ab]	1.22
Salcedo INIA	154[ab]	1.24
INIA 420 Negra Collana	164[bc]	1.33
INIA 415 Pasankalla	168[bc]	1.51
Hualhuas	174[bc]	2.39
INIA 427 Amarilla Saccaca	173[bc]	2.57
Amarilla Maragani	174[bc]	1.91
Huancayo	175[bc]	2.23
Blanca de Junin	187[c]	1.77
P	<0.001	ns
SE ±	13.79	

***means followed by same letters are not significantly different

Table 5.Maturity and mean yield of three Quinoa varieties cultivated in low altitude areas, 2016

Variety	Location	Altitude masl	Date of Sowing	Date of Harvest	Days to Maturity	Yield t ha^{-1}
Amarilla Marangani	Samphelling	300	21.11.15	13.3.16	112	1.52
Amarilla Saccaca	Samphelling	300	21.11.15	13.3.16	112	2.42
Ivory 123	Samtse	800	5.1.2016	16.4.16	133	1.22
Amarilla Saccaca	Lingmethang	600	27.10.16	13.2.17	109	2.63

Quinoa is a new crop to Bhutan and there is no information on local adaptability. The results from the first observation trials on two varieties at three different locations gave a good indication on the potential adaptability of quinoa under different agro-ecology and Bhutanese cropping systems.

The ideal temperature for quinoa growth is between 15 to 20°C and it is known to withstand temperatures from -4 °C to 38 °C. Quinoa is a water efficient plant and can also produce acceptable yields with rainfall of 100 to 200 mm (FAO, 2011). The temperature and average rainfall data of 2016 showed that in the six locations the temperature in the sowing months varied from -4 to 28.50°C while at harvesting it varied from -1 to 31.50°C. The average rainfall during sowing and harvesting months in the trial sites ranged from126.88 to 131.66 mm. This indicates that quinoa can be successfully grown as a rain fed crop under the dry land potato and maize based cropping systems.

The crop maturity and yield data from 2015 and 2016 indicate that quinoa can adapt well and could be grown as an alternative crop in both potato and maize based cropping systems. Bhutanese farmers practice three distinct cropping systems which are rice, maize and potato based systems (Katwal, 2013). Bhargava, Shukla, and Ohir (2006) found that the potential of Quinoa to adapt under ecologically extreme conditions provides immense opportunities for diversification in the high altitude area of the Himalayas and North Indian Plains.

Despite being an introduced crop, all varieties produced appreciable yields ranging from 1.22 to 2.57 t ha^{-1}. These yields are comparable to the yield of other locally established cereals like maize (3.62t ha^{-1}), wheat (1.70t ha^{-1}), buckwheat (1.34 t ha^{-1}), and millets (1.32t ha^{-1}) grown by farmers in the dry land (DoA, 2016).

Various estimates of quinoa yield are reported from different Asian countries. In India, Bhargava et al. (2006) have reported quinoa seed yield for different germplasm from 0.32 to 9.83 t ha^{-1}. In Turkey, where quinoa was introduced as a new crop and cultivated during the period 2009 - 2013, average grain yield of 0.50 t ha^{-1}a to 1.5 t ha^{-1} has been recorded (Yazar, Sezen, Tekin & İncekaya, 2016). In the Middle East and North African (MENA) countries, average yield varies between 1.2 and 1.4 t ha^{-1}, while the maximum attainable yield is predicted to go up to 8–10 t ha^{-1} (Choukr-Allah et al., 2016).

In the lower elevations when crop is sown in February and March, the time of flowering tends to coincide with the start of the summer season and summer temperature in these subtropical areas goes above 30°C. For quinoa air temperature above 35°C is known to cause dormancy or pollen sterility (AAFRD, 2005).

4. Conclusion and Recommendations

Quinoa is a new crop and there is urgency for information need on cultivation techniques, time of sowing, crop management and productivity for planning and promoting this nutritious cereal. Trial results from 2015 and 2016, and additional information collected from several demonstration trials which are not reported in this paper form the following recommendations.

Bhutanese Name: Quinoa is an exotic crop introduced to Bhutan and there was no local name. Absence of local name made it difficult to promote this crop with the farmers. To popularize this new crop among the farmers, the DoA has given local Bhutanese names to quinoa (Table 6) in different languages popularly used in Bhutan (Katwal, 2018).

Table 6.Local name of quinoa in four common languages used in Bhutan

No	Language	Name
1	English	Royal Quinoa
2	Dzongkha	Ashi Heychum
3	Sharchop	Ashi Mo
4	Lhotsham	Rani Bethu

Source: Katwal, 2018

Variety: Based on the data from two years of evaluation, the 20th Variety Release Committee (VRC) of the Ministry of Agriculture and Forests (MoAF) released four varieties for cultivation under different cropping systems and agro-ecology (Table 7). Of the four varieties two are from the early maturity and two from medium maturity groups.

Table 7.Information on four released varieties

Bhutanese Name	Original Name	Origin	Plant Height (cm)	Maturity (Days)	Grain Colour	Mean Yield t ha^{-1}
Ashi Heychum- AM	Amarilla Marangani	Peru	188	173	Yellow	1.88
Ashi Heychum- AS	Amarilla Saccaca	Peru	165	170	Yellow	2.25
Ashi Heychum- 123	Ivory 123	India	122	150	Brownish	2.25
Ashi Heychum- TW	DoA-1-PMB-2015	Unknown	120	140	Brownish	1.88

Source: Katwal, 2018

Time of Sowing**:** General recommendations on the time of sowing for different agro-ecological zones and cropping systems are made. In the high altitude areas above 1200 masl where potato and maize based cropping systems are dominant, Ashi Heychum- AM and Ashi Heychum- AS are more suitable. For autumn planting after harvest of potato in areas above 1200 masl Ashi Heychum- 123 and Ashi Heychum-TW are recommended as they are early maturing. In areas below 1200 masl where the dry land maize based cropping system is predominant; Ashi Heychum- 123 and Ashi Heychum-TW are recommended (Table 8). The suggested seed rate is 3-5 kgs $acre^{-1}$ and can vary with sowing methods.

Table 8.General sowing time for different agro-ecological and cropping systems

No	Altitude (m asl)	Agro-Ecosystem	Predominant cropping System	Sowing Time	Remarks
1	<3600	Cool Temperate	Barley, wheat or Potato Based	Mid April-Mid May	Main Crop
2	1800-2600	Warm Temperate	Potato Based	Mid March- Mid April First fortnight of August	Main crop Second crop after potato
3	1200 -1800	Dry Subtropical	Maize Based	Mid July- Mid August	After Maize
4	Below 1200 masl	Warm Subtropical, Dry Subtropical Humid Subtropical	Maize Based Rice Based	October-November	After Maize and Rice

Source: Katwal, 2018

In just over two years since its introduction, quinoa, a new Andean crop has successfully acclimatized and adapted to the Bhutanese cropping systems. From the trials and on-farm demonstrations, information on the agronomy, crop management and productivity have been generated. Using this information, package of practices for quinoa has been developed. Bhutanese farmers are rapidly learning the art and skill of growing this new crop. For the rapid development of this nutritious and healthy food, the DoA has accorded quinoa a commodity status which is at par with other major staples like rice and maize. The research centers are promoting quinoa in different agro-ecological zone and cropping systems in collaboration with extension program and farmers. The four recommended varieties, their sowing time and seed rates will further enhance the promotion of this crop. To provide more choices of quinoa varieties popular in the export market, new varieties have been introduced and are being evaluated. To meet the increasing seed requirement and to encourage farmers to grow this new crop, the DoA has put in place a provisional buy-back mechanism of quinoa grains from farmers at Nu 100 per kilogram. The utilization and consumption of quinoa is also promoted through local food fairs. Based on the research outputs, a Japanese company called Euglena Co. Limited, has also started the commercial cultivation of variety Ashi Heychum- AM in Haa and Paro dzongkhags.

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Reaction of Wheat Varieties to Rust Diseases at Mid and Low Altitudes in Bhutan

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ABSTRACT

Wheat rusts are the important fungal diseases that limit the production and downgrade wheat grain quality. This study was conducted to determine the reaction of wheat varieties to rust diseases at different altitudes. Field experiments were conducted from December 2016 to March 2017 at Mendagang for mid altitude (1332 masl) and at Samtenling for low altitude (378 masl). The experiment followed a randomized complete block design with three replications and 15 varieties. Of the 15 varieties three were from Bhutan, eight from SAARC, and four from ICARDA. Disease assessments were performed approximately at 60, 90 and 120 days after sowing following the modified Cobb's disease rating scale. Only leaf rust was observed at both the sites and all 11 germinated varieties were evaluated. Leaf rust incidence ranged from 2.5 to 10% and 2.5 to 16% at mid and low altitudes respectively. Disease severity of 5 to 20%, corresponding to field response of immune to moderately resistant was observed at mid altitude; while 5 to 100%, with immune to susceptible was observed at low altitude. There was a significant difference in disease incidence by site (p=.038) but not in disease severity (p=.129). The disease severity was positively correlated (r=.359); (r=.034) with mean minimum and maximum temperature respectively and (r=.361) with mean minimum relative humidity. Correlation was highly significant (p=0.003). This study found variety ICARDA 1, with 100% severity as highly susceptible to leaf rust at low altitude while Bajosokha Kaa remained immune in both the sites. The results indicate that leaf rust can occur in both low and mid altitudes; however, selection of suitable leaf rust tolerant varieties requires more extensive studies.

Keywords: *Altitude; Resistant, Rust, Severity, Susceptible, Temperature, Relative humidity*

1. Introduction

Wheat (*Triticum aestivum* L.) is the world's most extensively grown cereal. Wheat ranks third to rice and corn in total production globally. It is one of the main staple food in both developed and developing countries (Getie, 2015) providing food for over 10 billion people (Ahanger, Gupta, Bhat & Dar, 2014). In Bhutan, wheat together with barley comprises the third important cereal in term of consumption and production. However, the production of wheat is low with the current productivity of 1.76 Mg ha^{-1} (DoA, 2014), which is lower than global average yield of 3 Mg ha^{-1} (Tshewang, Park, Chuahan & Joshi, 2017).The total production of wheat was 4286 Mt from an area of 5441 acres in 2013 (MoAF, 2015). This resulted in importation of 2,326 Mt worth Nu

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416 million to meet the demand in 2014 alone (NSB, 2015). Wheat is used mainly for human consumption and supports nearly 35% of the world population (Dreisigacker, 2004). According to Doty (2012), because of its rich content of gluten, wheat is used for making flour which is used in production of baked goods and cereal products. Sustainable productivity of wheat is important to meet future demand for grains. However, wheat production is limited significantly by several abiotic and biotic stresses.

Wheat rusts are one of the most important diseases that limit wheat production worldwide (Aquino, Carrion & Calvo, 2002; Singh, Sethi & Chaudhary, 2004). Wheat rust diseases are caused by fungi belonging to the class Basidiomycetes and order Pucciniales (McIntosh, Wellings & Park, 1995; Agrios, 2005). The three rust diseases of wheat are stem rust or black rust caused by *Puccinia graminis* Pers. f. sp. *Tritici* Eriks.; stripe rust or yellow rust caused by *Puccinia striiformis* West end. f. sp. *Tritici* Eriks*.*, and leaf rust or brown rust caused by *Puccinia triticina* Eriks. Rust pathogens differ in morphology, life cycle and environmental preferences for growth and development. *Puccinia graminis* f. sp. *tritici* develops well under hot and humid climatic conditions, while *Puccinia striiformis* f. sp*. Tritici* prefers a cooler climate. In contrast, *Puccinia triticina* is adapted to a relatively broader range of conditions, making it the most widespread of the three rust diseases (Bariana et al., 2007; Getie, 2015).

The three species of rust pathogens in wheat cause the most destructive diseases affecting cereals (Stubbs 1985; Kolmer, 2005). The yield loss is usually high when the disease becomes severe before grain formation. Disease severity, however, depends on the resistance level of the cultivar grown, environmental conditions and the time of onset of the disease (Afzal et al., 2008; Brar, 2015).

In Bhutan, with the exception to stem rust, stripe and leaf rusts have been a recurrent threat to wheat production. Stripe rust epidemics in 1985 and 1986 caused losses of more than 50% of wheat production in *Punakha-Wangdue* valley (Mann and Hobbs, 1988; Tshewang, 2014). A survey conducted in April 2012 in 46 sites of *Gasa, Punakha, Wangdue, Paro*and *Haa dzongkhags* detected stripe rust in 13 sites and leaf rust in 11 sites (Om et al., 2012). The same study did not record stem rust incidence in any of the sites. Similarly, the results of the annual rusts surveillance of National Plant Protection Centre (NPPC) has repeatedly recorded stripe and leaf rusts as the most common rust diseases of wheat in Bhutan (Tshewang, 2014). The recurrent problem of stripe and leaf rusts in Bhutan coupled with wide cultivation of a susceptible variety necessitated the assessment of resistance in both local and global wheat germplasm against rusts. Although improvement of wheat varieties has been initiated and progress made, varietal improvement research needs to be extended in respect to performance of such varieties at different altitudes. Therefore, the current study is done to determine the reactions of wheat varieties to rust diseases at mid and low altitudes. This study has significance in selection of varieties for a particular cropping zone as a part of rust management strategy.

2. Materials and Methods

Field experiments were conducted at two locations; in farmers field at *Mendagang* (27.5886°N; 089.8711°E), representing mid altitude (1,332 masl) and at Agriculture Research and Development Centre (ARDC), *Samtenling* (26.9058°N; 090.4308°E), for low altitude (378 masl) in the cropping season of 2016-2017. Mendagang which is under *Dzomi gewog* of Punakha district experiences dry sub-tropical climatic conditions with annual average rainfall of 880 mm. While Samtenling under *Sarpang* district falls under Wet-subtropical agro-ecological zones and is humid with heavy rainfall of 2,500-5,500 mm per year.

Field temperature and relative humidity during the study period were recorded using tinytags (PLUS 2-TGP-4500, Omni Instrument Australia Pty Ltd) which was set to log (record) every hour. The Tinytag Plus 2 is a self-contained temperature and relative humidity data logger that has a high reading resolution and accuracy.

Experiment in each site was laid out in randomized complete block design (RCBD) with fifteen treatments and three replications. An area of 40 m^2 comprising 45 plots was established in each site. Each plot measured 1.1 m × 0.8 m and was assigned a treatment randomly. Plots were separated by 0.3 m wide spacing between the plots (treatments). Replicates were separated by 0.5 m spacing. Treatments were represented by 15 wheat varieties (Table 1). The wheat varieties consisted of three released Bhutanese varieties, eight SAARC varieties (four Bangladeshi, three Indian and one Nepali) and four varieties from International Center for Agricultural Research in the Dry Area (ICARDA). Seeding rate used was 100 Kg ha^{-1} with planting spacing of 10-16 cm by 20-25 cm. Therefore, around 50 gram of each variety was sown with a planting distance of 11 cm × 14 cm in each plot. Experimental plots were irrigated four times with first irrigation at 15 days after sowing (DAS). Subsequent irrigations were done at an interval of two weeks. Three hand weedings were done to keep the plots free of weeds.

Table 1.Details of Wheat varieties used in the study

Varieties	Description	Reaction type to rust	Reference
Bajosokha Kaa	Improved wheat variety released by ARDC, Bajo in 2014.	resistant to stripe rust & leaf rust	Joshi (2014); Tshewang et al. (2017); Tshewang. pers.comm.
Gumasokha Kaa	Improved wheat variety released by ARDC, Bajo in 2014.	resistant to stripe rust & leaf rust	Joshi (2014); Tshewang et al. (2017); Tshewang. pers.comm.
Bumthang Kaa Drukchu (Danphe)	Improved wheat variety released by ARDC, Bajo in 2015.	resistant to stripe rust, leaf rust & stem rust	Joshi (2014); Tshewang et al. (2017); Tshewang. pers.comm.
BARI GOM-25	Bangladesh variety received thorough SARRC Regional	resistant to leaf rust	Hossain et al (2013); Malaker& Reza (2011)
BARI GOM-26	Bangladesh variety received thorough SARRC Regional	resistant to leaf rust & moderately resitant to stem rust (Ug99)	Hossain et al (2013); Malaker& Reza (2011)
BARI GOM-27	Bangladesh variety received thorough SARRC Regional	resistant to leaf rust & resistant to stem rust (Ug99 & its variants)	Hossain et al (2013); Malaker& Reza (2011)
BARI GOM-28	Bangladesh variety received thorough SARRC Regional	resistant to leaf rust	Hossain et al (2013); Malaker& Reza (2011)
DBW-39 (Bajokaa 2014)	Indian variety received thorough SARRC Regional	NA	
Bajoka 2967	Indian variety received thorough SARRC Regional	resistant to leaf rust & strip rust	http://ztmbpd.iari.res.in/technologies /varietieshybrids/cereals/wheat/
Bajoka 107	Indian variety received thorough SARRC Regional	resistant to leaf rust	Singh et al. (2014)
NL-1073 (Francolin; BARI GOM-27)	Nepal variety received thorough SARRC Regional	resistant to Ug 99 and its variants	https://www.cimmyt.org/francolin-ug99-tolerant-wheat-variety-released-in-bangladesh/

NA= information not available

Table 1contd… Details of Wheat varieties used in the study

Varieties	Source and Description
Bajosokha Kaa	Bhutan, Improved wheat variety released by ARDC, Bajo,(2012-2013
Gumasokha Kaa	Bhutan, Improved wheat Variety released by ARDC, Bajo, (2012-2013
Bumthang Kaa Drukchu	Bhutan, Improved wheat variety released by ARDC, Bajo, 2015
BARI GOM-25	Bangladesh variety received thorough SARRC Regional
BARI GOM-26	Bangladesh variety received thorough SARRC Regional
BARI GOM-27	Bangladesh variety received thorough SARRC Regional
BARI GOM-28	Bangladesh variety received thorough SARRC Regional
DBW-39 (Bajokaa 2014)	Indian variety received thorough SARRC Regional
Bajoka 2967	Indian variety received thorough SARRC Regional
Bajoka 107	Indian variety received thorough SARRC Regional
NL-1073	Nepal variety received thorough SARRC Regional
ICARDA 1	Varieties adapted in dry areas
ICARDA 2	Varieties adapted in dry areas
ICARDA 3	Varieties adapted in dry areas
ICARDA 4	Varieties adapted in dry areas

Disease incidence and severity were assessed three times starting from tillering to ripening stage according to Zadoks, Chang and Konzak (1974). The first assessment commenced at approximately 60 days after sowing (DAS) followed by two assessments at 90 and 120 DAS. Sample size of 45 plants from each variety was assessed for both disease incidence and severity. Disease incidence was determined by using the following formula after recording the total number of infected plants from the sample size during each assessment date.

$$\text{Disease incidence} = \frac{\text{Number of diseased plants}}{\text{Total plants assessed}} \text{ x } 100$$

The same plants were also used for assessment of disease severity. Disease severity which is the percentage of plant tissue covered by the disease was determined by using the modified Cobb's disease rating scale (Roelfs et. al, 1992). Disease scores were interpreted based on the descriptions and the reaction type of each score (Table 2.) that was developed by Research Institute for Plant Protection (IPO) and International Maize and Wheat Improvement Center (CIMMYT).

The prevailing rust disease for each site was further assessed to determine the level of infection. In both sites, only leaf rust was recorded. The level of infection was determined by considering the severity of the disease for varieties inspected in the respective sites. The Coefficient of Infection (C.I.) was calculated using the methods outlined by Roelfs et al. (1992) in which the values of severity were multiplied by a constant number of host response such as immunity:

(O) = 0.05, resistant (R) =0.1, moderately resistant (MR) = 0.2, intermediate (M) = 0.4, moderately susceptible (MS) =0.6, and susceptible (S) = 1(Table 2.).

Table 2.Rust reaction rating scale and field response developed by IPO and CIMMYT

Descriptions	Disease severity (%)	Reaction type (field response)
No visible infections on plant	5	No reaction (O)
Visible chlorosis or necrosis, no *uredia* are present (Few minute lesions on leaves)	10	Resistant (R)
Small *uredia* are present and surrounded by either chlorotic or necrotic areas	20	Moderately resistant (MR)
Variable sized *uredia* are present, some with chlorosis, necrosis or both	40	Intermediate (M)
Medium sized *uredia* are present and possibly surrounded by chlorotic areas. (typical lesions surrounded by distinct chlorotic halos covering)	60	Moderately susceptible (MS)
Large *uredia* are present, generally with little or no chlorosis and no necrosis	100	Susceptible (S)

Data was compiled in Microsoft Excel 2013 spreadsheet and organized for analyses using Statistical Packages for Social Science (SPSS). The data was subjected to normality test using box plot method. The disease incidence and severity between the sites were assessed using non-parametric Kruskal-Wallis test that determined the significant difference ($p<0.05$). The spearman's rho correlation test was conducted to determine the correlation between disease severities and mean monthly minimum and maximum temperature and relative humidity according to Khan, Yaqub and Nasir (1998).

3. Results and Discussion

3.1. Weather data during the study period and development of rust diseases

Weather data on field temperature and relative humidity were recorded from December 2016 to March 2017 using Tinytags (data logger). Average monthly minimum and maximum temperature and relative humidity for each site are given in Figure 1 (1 denote Mendagang and 2 Samtenling). Overall, the average monthly minimum temperatures at mid altitude in Mendagang are lower than at low altitude in Samtenling. However, average monthly maximum temperatures in both sites are comparable except for the month of December. Based on the average monthly minimum temperature, the lowest temperatures observed were in January with 3.2°C and 14.2°C temperatures at mid and low altitude sites respectively. The average monthly maximum temperatures ranged from 30.5°C to 32.4°C and 30.2°C to 30.8°C in mid and low altitudes respectively. The overall mean minimum relative humidity is higher in low altitude site compared to mid altitude. The average monthly relative humidity for both sites were lowest in January with 18.6% at mid altitude and 29.4% at low altitude.

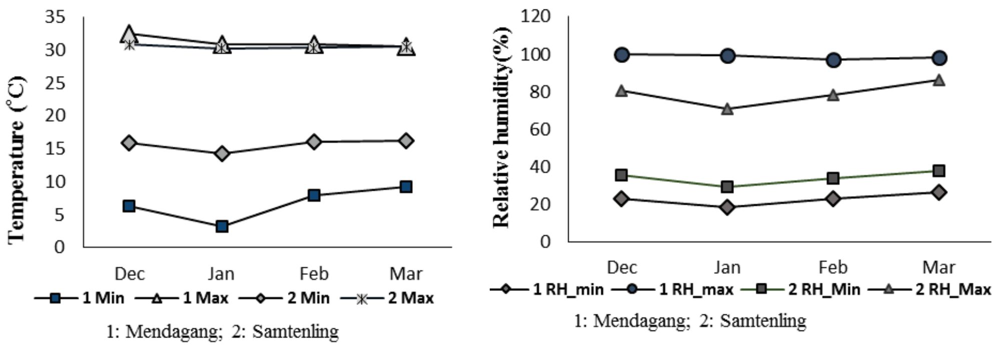

Figure 1.Mean minimum and maximum temperature (left) and relative humidity (right) recorded at mid and low altitudes sites

These results indicate that the mean temperatures and relative humidity in both sites were within the conditions favourable for the development of rust pathogens. Urediospores of stem rust pathogen are reported to germinate at a minimum temperature of 2°C and maximum of 30°C though optimum temperature requirement range from 15°C to 24°C (Roelfs et al., 1992) and high relative humidity near to 100% (Line, 2002). Similarly, spores of stripe rust pathogen germinate at minimum of 0°C and maximum 23°C (Chen, 2005) with optimal temperature range of 9°C to 13°C and relative humidity of 100% (Kansu, 2011). The leaf rust pathogen develops at about 20°C with dew periods of three hours or less (Roelfs et al., 1992).

3.2. Observation of wheat rust

Rust was scored from only 11 varieties out of the 15 varieties sown in both the sites. The varieties that did not germinate were Bajoka 2967, NL-1073, ICARDA 2 and CARDA 4. Hence all observations and data analyses were based on those 11 varieties. Plant responses were measured based on disease incidence and severity of stem, stripe and leaf rusts. Among the three wheat rust diseases studied, only leaf rust was observed in both the study sites. Stem rust and yellow rusts did not occur during the study period. Stem rust occurs only in summer with high relative humidity in hot places (Kolmer, Chen & Jin, 2009). Also Om et al. (2012) did not record any incidence of stem rust in Bhutan during their survey in April 2012. It is possible that wheat grown in hot places such as Samtenling escapes stem rust infection either due to absence of inoculums or due to unfavourable weather conditions. The present study was conducted from December to March which is a relatively cooler period with low relative humidity compared to humid and high temperatures of spring and summer months. As for stripe rust, the disease is common in higher elevation of temperate region with altitude of more than 2,500 masl. Previous surveys have recorded leaf rust in warm places in the mid to low altitude areas and stripe rust in areas of high altitudes (Tshewang & Ghimirey, 2016; Om et al., 2012). The results of the present study support the earlier observations of leaf rust in mid to low altitude areas. The leaf rust incidences ranged from 2.5 to 16% in both sites and disease severity of 5 to 20% at mid altitude and 5 to 100% at low altitude were recorded.

3.3. Leaf rust incidence and severity at mid-altitude site in Mendagang

At mid altitude site, eight of 11 varieties were evaluated for leaf rust. The leaf rust incidence ranged from 2.5 to 10% and severity of 5 to 20%. Among the 11 varieties; BARI-GOM 25, 27 and 28 were infected at 60 DAS, each with incidence and severity levels of 2.5% (Figure 2) and 10% (Figure 3) respectively. Highest incidence was observed in variety Gumasokha Kaa and BARI-GOM 28 at 90 DAS but the incidences in these two varieties decreased at 120 DAS (Figure 2). While highest disease severity was recorded at 90 DAS on BARI-GOM 26 and 28 with severity score of 20% each (Figure 3) the lowest severity (5%) was recorded for Bajosokha Kaa which remained immune throughout the assessment.

The observation of low disease incidence and severity of leaf rust at mid altitude could be due to dry weather during the study period and resistance within the varieties. According to Tshewang et al. (2017) and Joshi and Tshewang (2015), Bajosokha Kaa, Gumasokha Kaa and Bumthang Kaa Drukchu have good disease resistance in addition to having yield advantage and water stress tolerance. The present study did not analyze yield data, however, low disease severity observed in these varieties support the earlier findings.

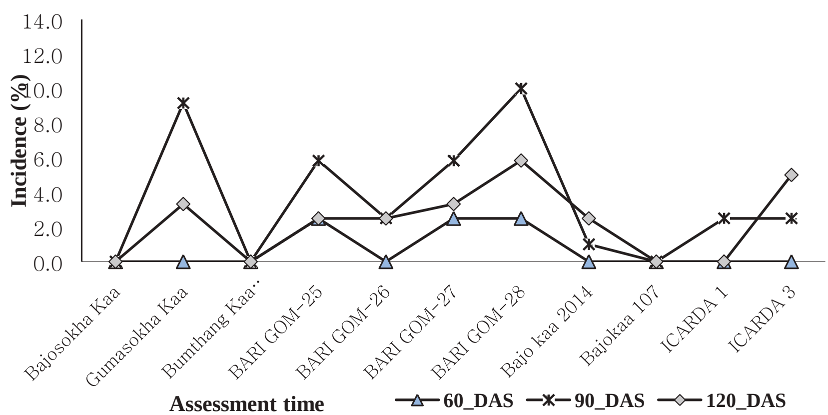

Figure 2.Leaf rust disease incidence (%) during different assessments at Mendagang

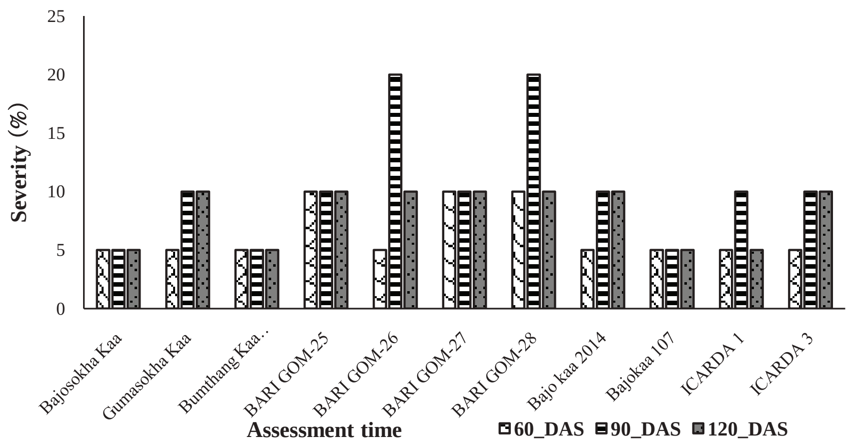

Figure 3.Severity (%) of leaf rust disease in the varieties at Mendagang

3.4. Leaf rust incidence and severity at low altitude in Samtenling

Leaf rust disease incidence and severity at Samtenling ranged from 2.5 to 16% (Figure 4) and 5 to 100% (Figure 5) respectively and all 11 germinated varieties were infected. At 60 DAS, leaf rust incidence was recorded only on two varieties, BARI GOM-27 and 28, though most varieties exhibited leaf rust by 90 or 120 DAS. Highest leaf rust incidence was observed in variety BARI-GOM 25 with 16% at 90 DAS followed by BARI-GOM 27 with 8% (Figure 4.). However, at 120 DAS leaf rust incidence decreased in BARI-GOM 25. In contrast, leaf rust incidence in ICARDA 1 increased from 6% at 90 DAS to 15% at 120 DAS.

As for leaf rust severity differences among the varieties, ICARDA 1 showed the most severe infection with severity score of 60% and 100% at 90 and 120 DAS respectively. The observation of increase in leaf rust severity on ICARDA 1 could be due to the late germination of this variety and exposure of young plants to more favourable weather conditions and inoculums in the fields. The remaining varieties showed comparable disease responses to leaf rust.

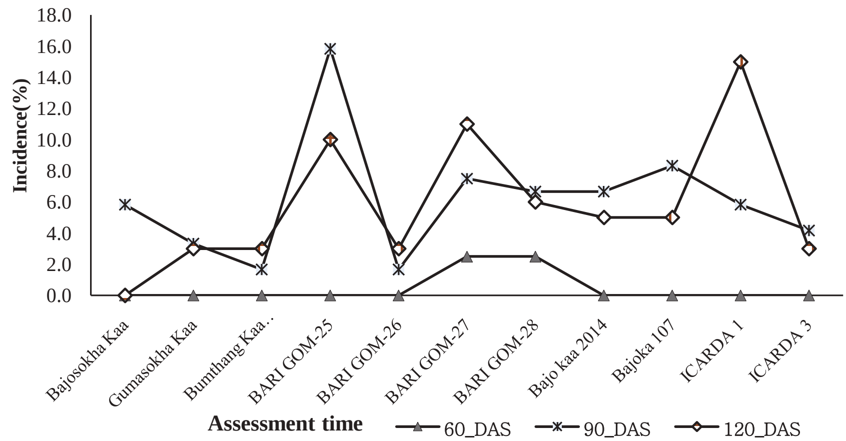

Figure 4.Leaf rust incidence (%) at 60, 90 and 120 DAS during different assessments at Samtenling

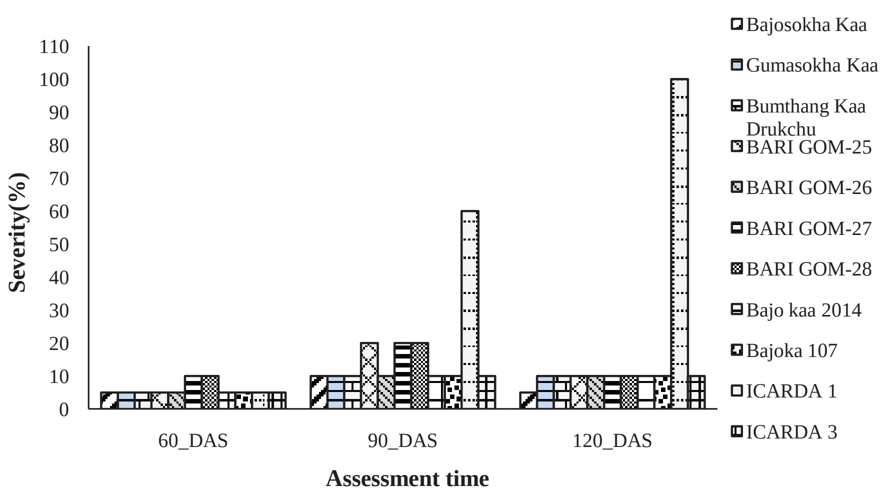

Figure 5.Leaf rust severity of the varieties grown at Samtenling

3.5. Comparison of leaf rust incidence and severity between sites

The mean of the three assessments were computed for comparison between the sites. Significant difference in disease incidence between the two sites was observed ($\chi2$ *(1)* = 4.311, *p*=0.038). However, disease severity was not significant between the sites ($\chi2$ *(1)* = 2.307, *p*= 0.129). Likewise based on the disease severity scores determined at 120 DAS, disease severity ranged from 5 to 20% and 5 to 100% at Mendagang and at Samtenling respectively. This indicates that the plant responses to leaf rust in the mid altitude correspond to immune (5O) to moderately resistant (20MR) while that of the low altitude corresponds to immune (5O) to susceptible (100S) (Table 3.). This seems to suggest that though leaf rust is prevalent in both mid and low altitude, it is more severe in low altitude. Based on disease reaction types all the three local varieties Bajosokha Kaa, Gumasokha Kaa and Bumthang Kaa Drukchu can be categorized as immune (5O) to resistant (10R) while BARI-GOM varieties are moderately resistant (20MR) to resistant (10R) in both the sites. Bajosokha Kaa is said to possess good resistance to rust but ICARDA varieties are still being evaluated at ARDC, Bajo (Tshewang, 2014). Interestingly, IACRDA 1 showed an immune (5O) field response when grown at mid altitude but became susceptible (100S) to leaf rust at low altitude with severity scores of 60% and 100%. The results seem to indicate that while most of the varieties are suitable for both low and mid altitudes; ICARDA 1 may not be suitable for low altitude areas due to its high severity scores. However, the high severity of ICARDA 1 in low altitude site in Samtenling could be due to late germination.

Table 3.Severity and reaction of leaf rust at 120 days after sowing

	Mendagang			Samtenling		
Varieties	% Severity	Reaction type[1]	C. I values	% Severity	Reaction type[1]	C. I values
Bajosokha Kaa	5	5O	0.25	5	5O	0.25
Gumasokha Kaa	10	10R	1	10	10R	1
Bumthang Kaa Drukchu	5	5O	0.25	10	10R	1
BARI GOM-25	10	10MR	1	10	10R	1
BARI GOM-26	10	10R	1	10	10R	1
BARI GOM-27	10	10R	1	10	10R	1
BARI GOM-28	10	10MR	1	10	10R	1
Bajokaa 2014	10	10R	1	10	10R	1
Bajoka 107	5	5O	0.25	10	10R	1
ICARDA 1	5	5O	0.25	100	100S	100
ICARDA 3	10	10R	1	10	10R	1

C.I=coefficient interval

[1]O=immune; R= resistant; MR=moderately resistant; MS= moderately susceptible; S= susceptible

3.6. Relation of disease development to weather (Temperature and Humidity)

The leaf rust disease incidence was positively correlated with mean minimum temperature (r=.454) and mean minimum relative humidity (r= .455) (Table 4.) and the correlation was significant (p= 0.01). However, it is negatively correlated (r= -.153) with mean maximum relative humidity but the correlation was not significant (p>0.05). Similarly, disease severity was positively correlated with mean minimum temperature (r=.359) and mean minimum relative humidity (*r*= .361) with significant (*p*=0.003). As for correlation between leaf rust incidence and severity with mean maximum temperature and relative humidity, leaf rust incidence was negatively correlated with mean maximum relative humidity (*r*= -.128) but the correlation was is not significant (*p*= 0.307). In contrast, leaf rust incidence (*r*=.006) and severity (*r*= .034) were positively correlated with mean maximum temperature but were not significant (*p*>0.05).

Infections of leaf rust can occur at a temperature range of 3°C to 25°C with more than three to six hours of leaf wetness (Roelfs et al., 1992; Bolton, Kolmer & Garvin, 2008). Grabow (2016) found that temperature, leaf wetness, and relative humidity variables were the most highly correlated variables in the disease development. The latent period of rust disease decrease with increase in temperature thus increasing the amount of spore production over time (Tomerlin, Eversmeyer, Browder & Kramer, 1983; Eversmeyer & Kramer, 2000). The current study also found that increase in temperature and relative humidity from January till March increased the disease severity due to condition conducive for the disease development in both the sites.

Table 4.Correlation between disease incidence, severity and weather data (temperature/relative humidity) at mid and low altitudes

		Mean_Tmin	Mean_Tmax	Mean_RHmin	Mean_RHmax
Spearman's rho	Incidence (%)	.454**	.006	.455**	-.153
		.000	.960	.000	.220
	Severity (%)	.359**	.034	.361**	-.128
		.003	.787	.003	.307

**. Correlation is significant at the 0.01 level (2-tailed)

Mean_Tmin/ Tmax= mean minimum and maximum temperature; Mean_RHmin/RHmax = mean minimum and maximum relative humidity

4. Conclusion

The results from this study indicate that reactions of the wheat varieties to leaf rust differed at different altitude zones. Leaf rust of wheat was observed on eight of 11 varieties in mid-altitude, while all 11 varieties showed leaf rust infection at low altitude. Disease severity varied from immune to moderately resistant in mid altitude site while in the low altitude site, plant responses varied from immune to susceptible. This seems to indicate that low altitude site is more conducive for leaf rust development.

In both the sites, the local released varieties showed more resistant than the varieties from SAARC and ICARDA. At mid altitude, Bajosokha Kaa, which is a local variety and widely cultivated by farmers remain immune to leaf rust while SAARC varieties and ICARDA varieties depicted resistant to moderately resistant field responses. Exceptionally, ICARDA 1 developed severe rust symptom resulting in susceptible field response at low altitude but of moderately resistant at mid altitude. This, however, could be due to differences in germination time, as ICARDA 1 germinated late and was exposed to inoculums load. The study also analysed the relationship between leaf rust severity, temperature and humidity which were found to be positively correlated.

The assessment of yield performance for different varieties could not be undertaken as the wheat plant did not reach full maturity during the study period (December to March). Also the varieties included in the study comprised mostly resistant varieties, as shown in Table 1 and no comparison could be made with a susceptible line or variety. Therefore, it is necessary to include susceptible lines/checks which would be useful in detecting the inception of disease and level of severity in a particular site. Moreover, the study was conducted for only one season and comprised only of two altitudinal zones. As such, the study does not provide sufficient evidence for choosing varieties at different sites. Hence, similar studies that include sites in high altitude area would be required to have more comparative and reliable results to draw definitive conclusions.

Acknowledgement

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Postharvest Damage and Losses of Mandarin Fruits in Bhutan

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ABSTRACT

Postharvest damage and losses in the horticultural production chain is a major challenge in a developing country like Bhutan. Though there are significant damages and losses of mandarin fruits in Bhutan, no reliable data is available. This study through field surveys assessed the extent of damages and losses due to postharvest conditions in the field as well as natural factors. Data were randomly collected from the fields of eight districts (Samdrup Jongkhar, Pema Gatshel, Zhemgang, Sarpang, Tsirang, Dagana, Chukha and Samtse). Postharvest damages of mandarin fruits due to postharvest handling was found to be 25.57% while the complete loss of mandarin due to same factor stands at 5.63%. Partial damages from natural causes such as diseases, birds, pests and physiological disorders stands at 10.26% while, 3.82% were completely damaged in the field. This accounts to 31.20% of the total mandarin fruits harvested being damaged (including losses) due to postharvest handling operations and 14.08% of the mandarin fruits were damaged (including losses) at the time of harvesting due to natural causes making the total damage to the mandarin fruits at 45.28%. Only about 54.73% of the mandarin fruits were marketed without damages or defects. Harvesting operations contributed to about 9.37% while, physiological disorders accounted for the maximum damages with 11.63% among the natural causes. Lack of proper storage and transportation facilities were the leading factors. Mandarin growers need to be supported with proper storage and transportation facilities as well as educate players in the value chain.

Keywords: *Mandarin fruits, Postharvest damages, Losses*

1. Introduction

Agriculture is the main source of livelihood in Bhutan as about 66 % of the Bhutanese population live in rural areas and depend on agricultural resources for their livelihood (MoAF, 2014). The diverse agro-climatic conditions of Bhutan are favourable for production of wide-range of horticultural crops. Citrus is the most widely grown fruit plants in Bhutan along with numerous other horticultural crops. The most common and widely grown types of citrus in Bhutan are local mandarin (*Citrus reticulata*) (Dorjee, Bockel, Punjabi & Chhetri, 2007). Currently about 60% of the Bhutanese farmers are directly or indirectly involved in mandarin farming (Joshi & Gurung, 2009).

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The integrated and systematic production of mandarin fruits in Bhutan started in early 1960s with the establishment of the Department of Agriculture (Gyeltshen, Grifith, Lakey & Dorji, 2015). Prior to this, cultivation of mandarin fruit was limited to backyard orchard for self or family consumption (Dorji, 1999). Over the years mandarin fruit cultivation was identified as a potential source of income for famers and by 1980s Bhutan began exporting mandarin fruits to India and Bangladesh (Dorji & Yapwattanaphun, 2011). Presently, mandarin in Bhutan is the main source of foreign exchange and livelihood for farmers in the citrus growing districts besides providing employment opportunities during harvesting and marketing seasons (Joshi & Gurung, 2009).

The main production areas in Bhutan are concentrated in subtropical-southern and central districts of Zhemgang, Sarpang, Dagana, Tsirang, Samtse, Chukha, Pema Gatshel and Samdrup Jongkhar (Gyeltshen et al., 2015). Mandarin orchards are mainly found in the foothills at altitudes between 300 – 1500 masl (Tashi, 2007). Mandarin fruit production over the last few decades has seen substantial increase both in number of trees as well as production. From 29,6161 metric tons of mandarin produced in 2000, the production reached 72,071 metric tons in 2007 (Dorjee et al., 2007).

Bhutanese mandarins are not just high-income earning cash crop but also are superior and unique in taste as it comes from clean environment (Dorjee et al. 2007). In general, mandarins are low in calorie and contain no saturated fats or cholesterol but rich in dietary fibres and pectin (Ladanyia & Ladaniya, 2010). Mandarins, like other citrus fruits are an excellent source of vitamin-C and also contain a good level of vitamin-A. Apart from other B-complex vitamins, mandarin fruit also contain some amount of minerals like potassium and calcium (Liu, Heying & Tanumihardjo, 2012).

Nonetheless, this high value cash crop production and marketing in Bhutan is hindered by number of constraints. Some of these include high cost of production, low yield, weak input delivery system, lack of accessibility and high cost of transportation, and weak market information system (Dorjee et al., 2007). Moreover, there is a significant postharvest loss of mandarin fruits due to lack of proper postharvest knowledge among the growers, exporters and retailers. It is estimated that about 30 % of the mandarin fruits are lost due to poor postharvest activities though the figure needs to be assessed with proper research on post-harvest loss of mandarin fruits in Bhutan (Dorjee et al., 2007).

Since accurate data on postharvest damages and losses of mandarin fruits in Bhutan is not available, this preliminary study is aimed to assess postharvest damages and losses of mandarin fruits from harvest till export to the international markets, besides the damages and losses from natural factors. With clear information on damages and losses of mandarin fruits at different stages of postharvest handling operations and natural causes; interventions can be developed and designed to minimize such damage and losses that will ultimately generate more income to the mandarin growers.

2. Materials and Methods

2.1 Study area

The survey was conducted to collect the data on postharvest damage and losses of mandarin fruits due to postharvest handling activities in the field viz. preparation for market and transportation to the market. The survey data was collected through random sampling from eight mandarin fruit growing districts of Bhutan (Samdrup Jongkhar, Pema Gatshel, Zhemgang, Sarpang, Tsirang, Dagana, Chukha and Samtse). The livelihood of most farmers in these districts depends on small scale growing and marketing of mandarin fruits.

2.2 Data Collection

The National Post Harvest Center (NPHC) technical staff collected the data from mandarin orchards, collection centres/depots and the major international marketing exit points (Samdrup Jongkhar, Gelephu and Phuntsholing).

2.3 Primary data

Primary data were collected through field surveys in the prescribed standard format. Two random samples were collected from each data collection area with three replications. Four independent factors viz. harvesting-handling operations, field to depot transportation, sorting/grading/packaging and depot to market transportation were used for data collection from each of these postharvest activities. Further, damages and losses of mandarin fruits due to natural factors were also assessed by collecting information on bird and insect damages, diseases and physiological disorders on mandarin fruits.

2.4 Data analysis

Data were analyzed using Web Agri Stat Package (WASP 2.0) and Microsoft (MS) Excel Spreadsheet.

3. Results and Discussion

Survey findings on damages and losses due to each of these factors are presented in the following sections.

3.1 Damages and losses due to postharvest handling operations

The percent gross damage of mandarin fruits at different stages of postharvest handling operations are shown in Figure1. In almost all the orchards, mandarin fruits were harvested by seasonal labourers hired by the contractors. As shown in the Figure 2, it was observed that 7.89% of the mandarin fruits were damaged due to harvesting operations but marketable, whereas 1.48% were damaged beyond marketability during harvesting and thus without any economic value. Mandarins were harvested manually and thus factors such as dropping of fruits, injury by the harvesters' hands, bruises from branches and storage bags/baskets and abrasions contributed to the damages and losses. Thus gross damages of mandarin due to harvesting operations stands at 9.37%.

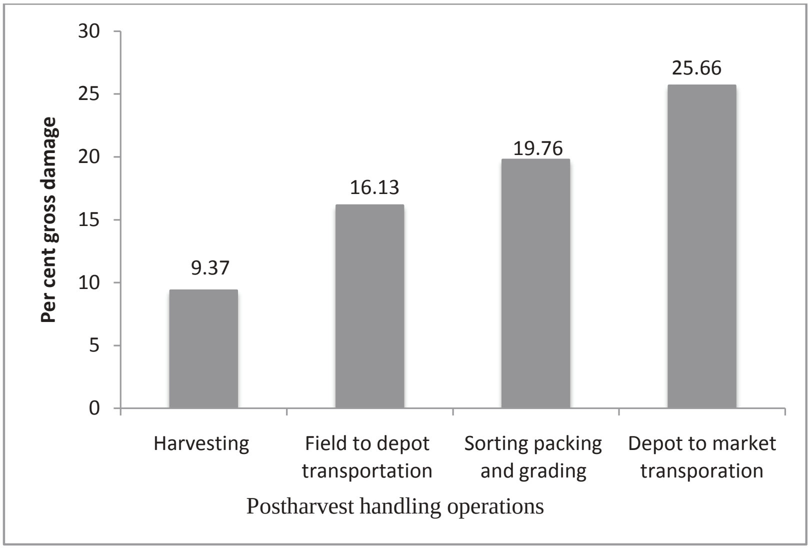

Figure 1.Per cent damages of mandarin fruits at different postharvest handling operations.

Harvesting time and natural conditions are also crucial in minimizing the post-harvest damages and losses. Mandarins harvested at the right time of season with the use of proper equipment will reduce damages substantially (Sudheer & Indira, 2007). Moreover, mandarin contractors and seasonal harvesters can be trained on good harvest handling practices.

The harvested mandarin fruits are then transported to collection points or depots for sorting, grading and packaging. Mandarin fruits were again assessed for damages and losses after reaching the depots from orchards. It was observed that another 6.93% of mandarin fruits were damaged during transportation to depot making the total per cent of damaged mandarin fruits at 16.13% (Figure 1). During this operation, 1.19% of mandarin fruits were completely damaged beyond marketing conditions and thus assessed as the total loss. Damages and losses of mandarin fruits during the transportation can be attributed to poor mode of transportation such as in open-air trucks, poor road conditions, long distance transportation and rough handling of fruits by the transporters. Since the major nature of transportation damages and losses were due to bruising, peeling and shrinkage of fruits; the transporters could be advised to use locally available cushioning materials in the trucks and avoid transporting mandarin in open trucks exposing the fruits to sun and rain.

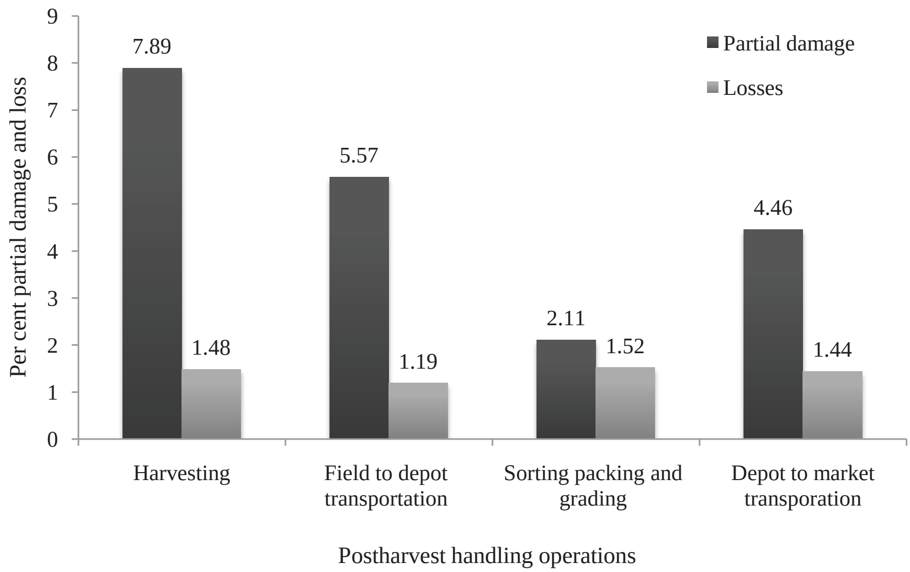

Figure 2.Percent partial damage and complete loss of mandarin due to post harvest handling operations

In the collection depots, mandarin fruits were then sorted, graded and packed for both domestic and international markets. Most of the packers and graders were Indian labourers from across the border. Damages during sorting, grading and packing were mainly due to improper handling of fruits. The percent damage of fruits during this operation stands at 19.76% indicating that additional 3.63% of the mandarin fruits were damaged during sorting, grading and packing as shown in Figure 1 out of which 1.52% fruits were completely damaged (i.e. without economic value). It was observed that the graders did not follow proper grading of fruits, but sorted the fruits based only on shape, size and colour. Rough handling of fruits while sorting, grading and packaging and use of inappropriate containers and poor storage conditions lead to these damages (Singh & Reddy, 2006). Facilitation with automatic grading machines along with appropriate containers and professional training of the graders could minimize the damages of fruits during these operations.

Fruits after grading and packing were transported to bigger markets, mainly in India and Bangladesh. The damage to mandarin fruits during this operation was also assessed. About 4.46% of the mandarin fruits were partially damaged while 1.44% of them were totally damaged during the transportation. When mandarin fruits reach the final market or export entries, 25.66% of them were either partially or completely damaged (Figure 1).

3.2 Damages and losses due to natural causes

Fruits were assessed for damages and losses due to natural causes such as birds, insect pests, diseases and physiological disorders at the time of harvest. As shown in Table 1, 10.26% of the fruits were partially damaged and another 3.82% were completely damaged due to natural causes. Among the natural causes, physiological disorders contributed to highest damage with 11.63% followed by diseases at 1.42% and birds and insects damages at 1.03% (Figure 4).

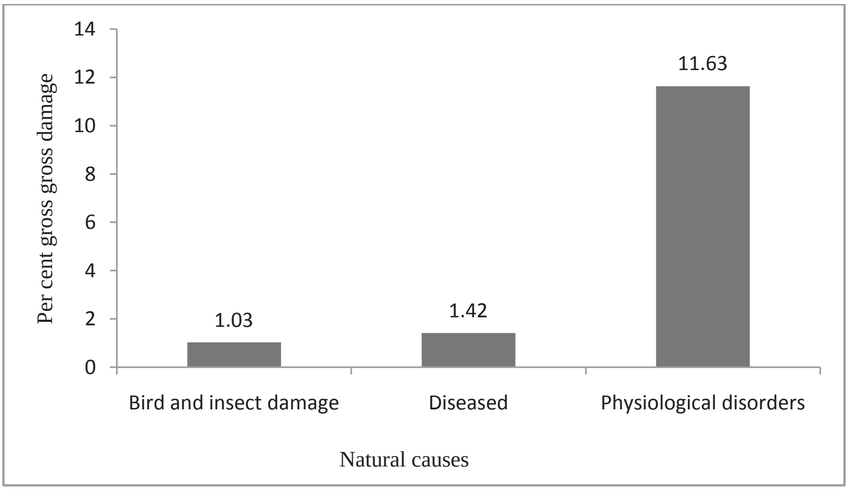

Figure 3.Percent gross damages of mandarin fruits due to natural causes at the time of harvesting

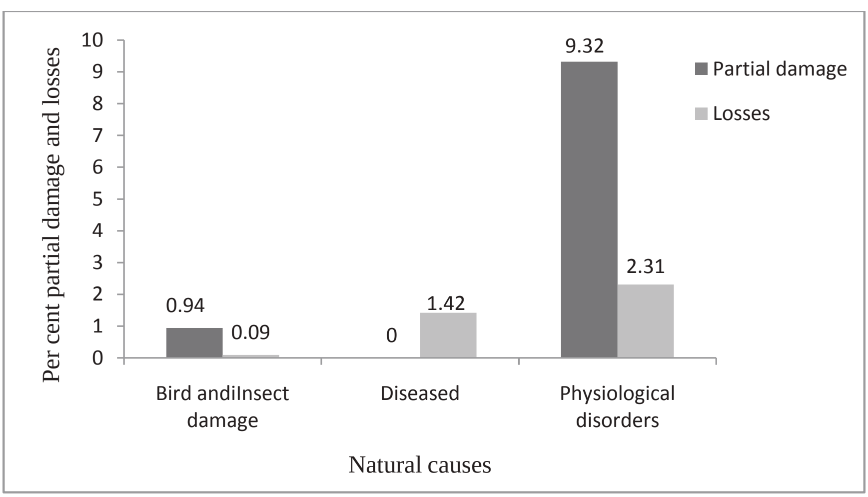

Figure 4.Partial damage and complete loss of mandarin fruits at the time of harvest due to natural causes

Adoption of good pre and post-harvest management technology can minimize damages and losses of mandarin due to natural causes. Proper orchard management practices will keep insect/pests away and use of certain fungicides needs to be considered to minimise fungal diseases in the orchards (Eckert & Eaks, 1989; Ladanyia & Ladaniya, 2010).

Table 1.Final results of post-harvest losses from handling and natural causes

Sl. No	Factors	Partial damage (%)	Loss (%)	Damage total (%)
1.	Handling operations	25.57	5.63	31.20
2.	Natural causes (diseases, birds and insects and physiological disorder)	10.26	3.82	14.08
3.	Total	35.83	9.45	45.28

4. Economic impact of post-harvest damage and losses of mandarin fruits

Like any cash crop, both quantitative and qualitative losses of mandarin fruits will not only have economic impact on the mandarin farmers and the players in the supply chain but also impact the food security of the nation (Murthy et al., 2009). Though it is difficult to exactly calculate the monetary impact of damage and losses of mandarin fruits in Bhutan due to complexity of the mandarin supply chain; efforts have been made to estimate the economic losses from the recent market data and the total postharvest losses available for the mandarin fruits in this study.

Table 2**.**Mandarin fruit production and export statistics (Bhutan, 2016-17)

Sl. No	Mandarin production (MT)	Quantity exported (MT)	Value (Million Nu)
1.	28,017.00	16,141.61	471.51

Source: (DRC, 2017; MoAF, 2017)

Table 3.Estimate of economic losses due to postharvest losses of mandarin fruits (Bhutan, 2016-17)

Sl. No	Mandarin production (MT)	Postharvest partial damage (MT)	Economic losses (Million Nu.)	Postharvest losses (MT)	Economic losses (Million Nu.)
1.	28,017.00	10,038.49	NA	2,647.60	77.33

Source: (DRC, 2017; MoAF, 2017)

Bhutanese mandarin growers produced 28,017.00 MT of mandarin fruits in 2017, out of which 16.141.61 MT of mandarin fruits were exported to Bangladesh and India thus earning Nu. 471.51 million in revenue (DRC, 2017; MoAF, 2017). The earning from the export of mandarin fruits could be much more if not for the revenue loss of Nu 77.33 million from 9.45% (2,647.60 MT) of mandarin fruits lost due to postharvest handling operations as shown in Table 1. Bhutanese mandarin fruits were exported at an average price of Nu. 29.20 per Kg (DRC, 2017).Thus, income generation for mandarin growers, retailers and exporters could be enhanced if appropriate interventions are designed and put in place to reduce postharvest losses of mandarin fruits.

Though there could be considerable financial losses incurred from the partially damaged fruits, it is beyond the scope of this study to track and obtain data for the assumed depreciated price fetched by 35.83% or 10,038.49 MT of partially damaged mandarin fruits in the local and export markets. Thus, economic losses from the partially damaged fruits could not be determined.

5. Conclusion and Recommendations

Numerous factors lead to damage and loss of mandarin fruits in Bhutan. These causes include birds, insect/pests, diseases and physiological disorders besides poor postharvest handling practices. Poor production practices, improper temporary storage facilities and lack of appropriate physical infrastructures (transport, storage facilities and access to roads) also contributed to the damage and postharvest losses. Minimizing postharvest damages and losses of mandarin fruits can generate better economic returns for farmers and others who are engaged in the market value chain (Wills et al*.*, 1999).

The total postharvest damage of mandarin fruits in Bhutan during 2017-18 was very high at 45.27% of the total production. Postharvest damages from handling activities such as harvesting, transportation from the field to depot, sorting, grading and packaging and transportation to markets accounted for 9.37%, 6.76%, 3.63% and 5.90% respectively. On the other hand, damages from natural causes such as birds and insects, diseases and physiological disorders accounted for 1.03%, 1.42% and 11.63% respectively. Only about 54.73% of the mandarin fruits were undamaged and reached the final markets. About 14.08% of the total productionwas completely damaged. Reduction of this total loss will not only help to reduce the cost of production, trade and distributions but will also lower the price for consumers and increase the income to famers through larger sales volume (Bhattarai et al., 2013).

Postharvest damages and losses of mandarin fruits were found to be the major problem faced by Bhutanese mandarin growers, retailers, contractors and exporters. It was observed that certain specific interventions could be made in the production systems/orchard management as well as postharvest management practices. In light of this study finding, physical facilities such as proper storage facilities and improved fruit transport systems need be explored for mandarin growers and contractors. The National Post Harvest Center could scale up mass training of farmers, support and facilitate in providing proper handling and harvesting equipment and continue providing technical support to relevant stakeholders involved in mandarin production and marketing system to minimize postharvest losses.

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Status of Field Crops Diversity in Dewathang and Orong Geogs, Samdrupjongkhar

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ABSTRACT

Status of on-farm diversity of field crops was assessed in Dewathang and Orong geogs under Samdrupjongkhar dzongkhag (district) by Samdrupjongkhar Initiative (SJI) to document and understand the status of field crops diversity, reasons for maintaining agro-diversity, farmers perception on emerging issues like climate change, and crop production constraints and challenges faced by the farming communities. A total of 131 households were randomly sampled and surveyed using a structured questionnaire. The change in a number of households cultivating the different field crops that comprised cereals, legumes and some underutilized crops were compared 20 years before and now to compute the percent change. Paired t-test was used to analyze the data to assess the change in on-farm agro-biodiversity. This study revealed that the overall percentage of households cultivating different field crops has reduced by 27% in the span of twenty years. Paired t-test analysis of the data shows that there is a significant change (P<0.01) in the number of households cultivating different or field crops 20 years before and now. Three critical reasons assigned by farmers for cultivating different crops and varieties were for food security and livelihood (53%), to meet different needs (28%) and meet religious needs (18%). Three most important challenges on the cultivation of different crops listed by respondents were farm labour shortage (26%), human-wildlife conflict (24%) and pest and disease attack (17%). Some 44% of the respondents observed that climate change has occurred over the last twenty years. This study indicates that the on farm-agro diversity plays a very critical role for food security and livelihood. To help the communities to sustainably maintain the in-situ conservation, linking on farm agro-diversity to other income generating enterprises like agro-tourism, organic production and value addition of products for income generation should be pursued. Findings from this study were used as baseline to design interventions to address community's challenges in field crops conservation, development and utilization.

Keywords: *Agro-biodiversity, On-farm, Conservation, Community, Decline, Climate change*

1. **Introduction**

Agro-biodiversity is a sub-set of biodiversity. It is a result of natural selection processes and the careful selection and inventive development of farmers, herders and fishers over millennia. Many

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people’s food and livelihood security depend on the sustained management agro-biodiversity (FAO, 1999). Agro-biodiversity refers to interaction between agricultural management practices, farmers’ resource endowments, bio-physical resources, and species (Brookfield & Stocking, 1996). Agricultural biodiversity, includes harvested crop varieties, livestock breeds, fish species and non-domesticated (wild) resources within field, forest, rangeland including tree products, wild animals hunted for food and in aquatic ecosystems ; Non-harvested species in production ecosystems that support food provision, including soil micro-biota, pollinators and other insects such as bees, butterflies, earthworms, greenflies; and non-harvested species in the wider environment that support food production ecosystems (agricultural, pastoral, forest and aquatic ecosystem) (FAO, 1999).

Agro-biodiversity can contribute to food and agriculture in several different ways. Experiences and research have revealed that agro-biodiversity among many other benefits is very vital for increasing productivity, food security, and economic returns of the farmers; helps to reduce the pressure of agriculture on fragile areas, forests and endangered species and can help make farming systems more stable, robust, and sustainable (Thrupp, 1997). Agro-biodiversity continues to serve as the source of livelihood for local communities in the different agro-ecosystems such as the drylands, high elevation areas and low-lying humid tropical areas (Abdelali-Martini, et al., 2008). In Bhutan where subsistence farming is still dominant, agro-biodiversity plays a pivotal role for meeting household food security and poverty alleviation (Katwal, et al., 2015). In the Bhutanese self-sustaining, integrated and subsistence agricultural production system, agro-biodiversity is the cornerstone for household food security and livelihood. Bhutanese farmers continue to cultivate many traditional crops and varieties and preserve their seeds using their own seed selection criteria which help in the conservation of on-farm agro-biodiversity. In Nepal, Upreti and Upreti (2002) have recorded that farmer’ indigenous knowledge is one of the major factors contributing to conservation of agro-biodiversity.

Generally, Bhutanese farming system and diet is dominated by nine important cereals (*Dru-na-gu)* which are the main source of energy and nutrition (NBC, 2016). The nine different cereals or *Dru-na-gu* arealso essential for religious rituals *(rimdos*) and annual religious ceremony *(loche*) that are integral part of the culture and tradition of the Bhutanese people (Lhendup, 2008).

Considering the important role of on-farm agro-biodiversity, the Samdrup Jongkhar Initiative (SJI) a local community based organization initiated the assessment of the status of field crops diversity in Dewathang and Orong geogs. The SJI operates under the umbrella of the *Lhomon* Society (LMS), a registered civil society organization that aims to promote community development in keeping with the principles of Gross National Happiness. The conservation, development and utilization of field crops was initiated in 2016 with support of the Global Environment Facility (GEF) Small Grant Project (SGP) of the UNDP. In order to establish a baseline for the GEF project, a household survey to document the status of field crops was carried out by the SJI.

The objectives of the survey was to document and understand the status of field crops diversity, challenges faced by the farming communities in crop production, and establish and use the baseline data to design relevant interventions to address challenges faced by farmers in crop production and conservation. This survey documented the status of field crops diversity, crop production issues and challenges faced by farmers. Further, some of the interventions initiated and challenges for crop rehabilitation, conservation and utilization programs are also discussed.

2. **Materials and Methods**

A simple random sampling was done to collect data using a structured questionnaire survey. To ensure that an individual has equal opportunity of being selected for the interview from the target population we decided the sample size using standard deviation(σ), margin of error (E) and confidence level with known population $(N= [(z_{a/2})^2*\sigma^2]/E^2)$. There are six villages with 310 households. From total households the standard deviation was 29.2. Thus, at confidence level of 95%, we sampled 131 household. Based on the objective of the study, a structured questionnaire was prepared and field tested at Gayzor village. Primary data was collected through personal interview with the farmers of different villages using structured questionnaire. Data collected through the household survey was compiled using Microsoft Excel 2013 and analyzed using SPSS (Statistical Package for the Social Sciences) version 23.

3. **Results and Discussion**

3.1. Demographic profile of study sites

There are 870 households in Dewathang and Orong geogs and from the total households 131 households were randomly sampled and surveyed for this study. The numbers of households surveyed in each chiwogs are presented in Table 1. Of the total 131 respondents 87 were female and 44 male.

Table 1.Number of respondents by geog and chiwogs

Geogs	Chiwogs	Number of Respondents
Dewathang	Bangtsho	13
	Martang	24
	Domphu	14
	Rekhey	23
Orong	Mencheri	17
	Philuma	40
	Total	131

3.2. Status of field frops diversity in Dewathang and Orong geogs

Dewathang and Orong geogs largely fall under the dry and wet sub-tropical agro-ecology. Farmers of two geogs have grown different cereals crops since time immemorial. The major field crops cultivated in the two geogs are rice, maize, wheat, barley, buckwheat, millets, oilseeds, legumes and some underutilized crops. Farmers mostly practice subsistence farming and continue to grow different crops and varieties in their farms to meet their household food security and livelihood. The extent of cultivation of different was assessed by comparing number of households cultivating the field crops 20 years before and the percent change was estimated for each crop. The overall decline in the number of household cultivating different field crops in Dewathang and Orong geogs compared to 20 years before is 27%. The highest percentage of households who have discontinued the cultivation was for wheat and barley (Figure 1). Paired T-test analysis of the data shows that there is a significant change (*P<0.01*) in number of household cultivating different or field crops 20 years before and now (Table 2). The analysis indicates that the number of households cultivating rice, wheat, barley, buckwheat, millet, and mustard has significantly declined. The increase in the number of households cultivating the crop is only in maize but the change is not significant (Figure 1).

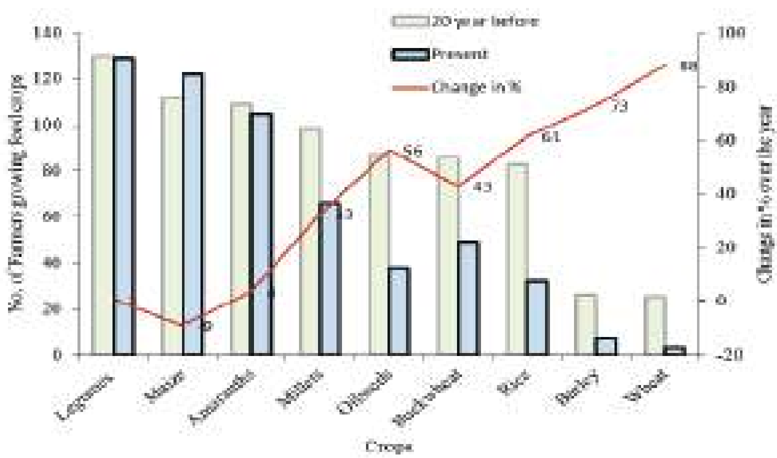

Figure 1.Different field crops cultivated and percent change in numbers of farmers cultivating 20 years before and now.

The survey found that the community of two geogs cultivates all the nine traditional crops (the *Dru-na-gu*) that are prevalent and recognized in the farming systems. The nine crops are rice, maize, wheat, barley, buckwheat, millets, legumes, mustard oilseeds and amaranth. The successful cultivation of all the nine crops is an indication of the agricultural suitability of a farming community (NBC, 2016). Amongst the field crops, maize and rice is the most popular crop cultivated by maximum number of households. Farmers cultivated different traditional varieties and have assigned local names to the crops and varieties cultivated by them (Table 3). The main reasons for the increase in the number of households cultivating maize are attributable to maize being a staple food, its good adaptation in the dry land rain fed farming system of both the geogs, and availability of seed of good varieties promoted by the Department of Agriculture (DoA). In maize, out of six varieties currently cultivated, five are farmer's traditional varieties. This is a very good sign of farmers still maintaining their traditional varieties. However, as farmers have raised pest and disease incidence as the third important challenges for crop production (Fig. 4), traditional varieties could be vulnerable to diseases like Gray Leaf Spot (*Cercospora zea maydis)*. Katwal et al., (2013) have reported that traditional maize varieties are highly susceptible to Gray Leaf Spot.

Rice is also the main staple in some chiwogs of Dewathang and Orong geogs. This study found that farmers cultivate rice under irrigated rice and upland rice ecosystem. This study showed that the household cultivating rice in the Dewathang and Orong geogs has decreased by 61 % (Fig. 2) over the past twenty years. The cultivation of upland rice referred locally as *Pangbara* is slowly declining in both the geogs. Currently there are three varieteis of upland rice cultivated by farmers which are are *Sambara*, *Zerbar* and *Khetsala bar* (Table 4). The reasons for decline of upland rice cultivation are attributed to legislation on the ban on slash and burn system (*Tseri*), wild animals attack and labour shortage.

Table 2.Paired t-test on the number of respondents growing major food crops before 20 years and now in Dewathang and Orong geogs, 2017

	Test	Mean	N	Std. Error Mean	P value
Rice	Before 20 years	.634[a]	131	.0423	
	Present	.244[b]	131	.0377	**
Maize	Before 20 years	.855[a]	131	.0309	
	Present	.855[a]	131	.0309	ns
Wheat	Before 20 years	.191[a]	131	.0345	
	Present	.023[b]	131	.0131	**
Barley	Before 20 years	.198[a]	131	.0350	
	Present	.053[b]	131	.0197	**
Buckwheat	Before 20 years	.656[a]	131	.0416	
	Present	.374[b]	131	.0424	**
Millet	Before 20 years	.748[a]	131	.0381	
	Present	.504[b]	131	.0439	**
Mustard	Before 20 years	.664[a]	131	.0414	
	Present	.290[b]	131	.0398	**
Amaranth	Before 20 years	.832[a]	131	.0328	
	Present	.802[a]	131	.0350	ns
Legumes	Before 20 years	.992[a]	131	.0076	
	Present	.985[a]	131	.0108	ns

ns = not significant **highly significant at P <0 .01

Wheat (Bong) is one of the components of dru-na-gu. Wheat flour is used making tormas (idols or images of local deities). Cultivation of this crop has drastically declined mainly due to lack of farm labour. The extent of barley cultivation (Phemong) has also significantly declined. According to the farmers, such decline is attributed to the change in the food consumption pattern as rice is preferred over wheat and barley. Farmers cultivate sweet and bitter buckwheat. Sweet buckwheat is called Brema or Guntshung while bitter buckwheat is called Khala. Buckwheat is consumed in the form of cooked dough (Bokpe) and noodles (Puta).

Amongst the oilseed crops, mustard (Memba) is most popular. Farmers of Dewathang and Orong geogs grow two types of mustards locally known as Memba serbu (yellow seed colour) and Memba tshalu (red seed colour). Mustard is mainly grown by the farming communities for the extraction of oil for the house hold consumptions, and is the major source of cooking oil. The primary challenge faced in mustard cultivation is the lack of suitable oil expellers.

Different types of millets are cultivated by the farmers. The finger millets are commonly called Kongpu. Millets are grown to meet the supplementary dietary requirements. It is consumed in the form of cooked dough made from its flour. It is also used for brewing local alcoholic drinks (Bangchang and Ara). Compared to other cereals, majority of the farmers continue to cultivate this crop in remote areas and marginal lands. There are two types of finger millet grown by farmers in Dewathang and Orong. They are compact and loose type. According to the respondents, these millets are less prone to damage by wild the animals and infection by pest and diseases.

Fox-tail millet (Yangra) literally means the essence of prosperity or being precious. This is because foxtail millet is ready for harvest during the lean-food season. Some varieties of this crop help address household food needs during the lean season. Farming communities of Dewathang and Orong geogs cultivate five varieties of foxtail millet, namely Khang Yangra, Danishampi Yangra, Rongshong Yangra, Yangra Changlu and Pusoktang/Busum Yangra. This study revealed that cultivation of all five varieties has declined compared to the last twenty years.

The little millet or common millet is quite a popular crop in the study site. Two varieties of little millet cultivated are Chera Balingbu and Chera Tshalu. Farmers grow little millet mainly for household consumption and according to their local belief, the little millet is considered offensive in making offerings during religious ceremony and other special events. There are two types of amaranth varieties grown by the farming communities which are Lhasomo and Sharang mo. Lhasamo are of two types, Lhasmo Tsalu and Lhasamo Balingbu (red and white varieties). Amaranth is grown in very small quantities in small patches of land by almost all the household. It is consumed in the form of roasted snacks with tea. It is also used for making local brews called Changkoey/Nagpa.

Different types of legumes and pulses are cultivated by the farming communities of Dewathang and Orong geogs. Grain legumes are mostly grown on dryland; however, it can be grown in wetland and kitchen gardens. Most farmers grow traditional varieties and maintain their own seeds. Respondents mentioned that they cultivate grain legumes for food, as source of income, and for maintaining soil fertility. Compared to other cereals, there is also very little change in the extent of grain legume cultivation between 20 years before and now (Figure 1). Farmers continue to cultivate different 10 traditional varieties of beans which are listed in Table 4. Brokchey oray is the most common beans grown by the farmers in the locality while Ngangshing Orey is the least cultivated variety. Farmers also cultivate different types of pulses. The pulses cultivated are mostly the varieties of Vigna species which are locally known as Gagpu Changlu, Gagpu Tshalu, Gagpu Yanglu and Gagpu Singay. Additionally, farmers also grow soybean and peas in small areas. Soybean is grown to make traditional fermented soya cheese. The cultivation of different legumes by the farmers is a good practice for sustainable soil fertility management. There is also a very good scope for commercial cultivation of legumes and pulses for income generation.

Table 3.Varieties of rice, maize, millets and amaranth cultivated at the study site

	Crops							
	Rice	Maize	Wheat &Barley	Foxtail Millet	Little Millet	Buckwheat	Oilseed	Amaranth
	Pang Bara	Yangtsepa Ashom	Bong	Khang Yangra	Chera Balingbu	Brema/Guntshung	Memba Serbu	Lhasamo
	Sam Bara	Betpai Ashom	Phemong	Dani Shampi Yangra	Chera Tshalu	Khalla	Memba Tshalu	Sharang Mo
Var.	Zerbara	Barma Ashom		Rongshong Yangra				Lhasa Mo Tshalu
	Khetshala Bara	Baipo Ashom		Yangra Changlu				Lhasamo Balingbu
	Bhur Kamja	Zetpo Ashom		Pusoktang Yangra/Busung				
	Khangma Maap	Baipo-zetpo Ashom						

Table 4.Types and traditional varieties beans cultivated in Dewathang and Orong geogs.

Type	Name of Local Variety of Beans
Climber	1. Brok`chey oray 2. Ngangshing oray 3. Martshala oray 4. Pengkulung oray 5. Wangchelingpa oray 6. Pheshanpu oray 7. Ney-nga oray 8. Jog oray
Dwarf	1. Choktor oray 2. Brokchey oray

Other less popular or underutilized crop cultivated by the communities included Perilla (Perilla fructescens) or locally known as known as Nam. Unlike other crops, Nam is adapted to various elevation and climatic conditions. It is used as ingredient to prepare traditional Bhutanese tea and Ezey (Pickle). There are two types of Perilla, namely Nam balungbin (white Perilla) and Nam changlu (black perilla).

3.3. Seed flow pathways at the study sites

The farming communities of the two geogs continue to depend on formal and informal seed systems. The seed flow diagram (Figure 2) indicates that seed exchanges occur within and outside the communities. The informal seed system continues to play a critical role in perpetuating on-farm diversity but the management of informal seed system is left to the farmers alone. To preserve the seed and facilitate the seed exchange of traditional crops and varieties, SJI has started a Community Seed Bank (CSB) in Dewathang geog.

The implementation of Plant Genetic Resources for Food and Agriculture (PGFRA) emphasizes the need to strengthen the local seed supply system and germplasms currently available in the national gene bank or in the community seed banks (NBC, 2012). Farmers report Dzongkhag Agriculture office as one of the main source of new seeds.

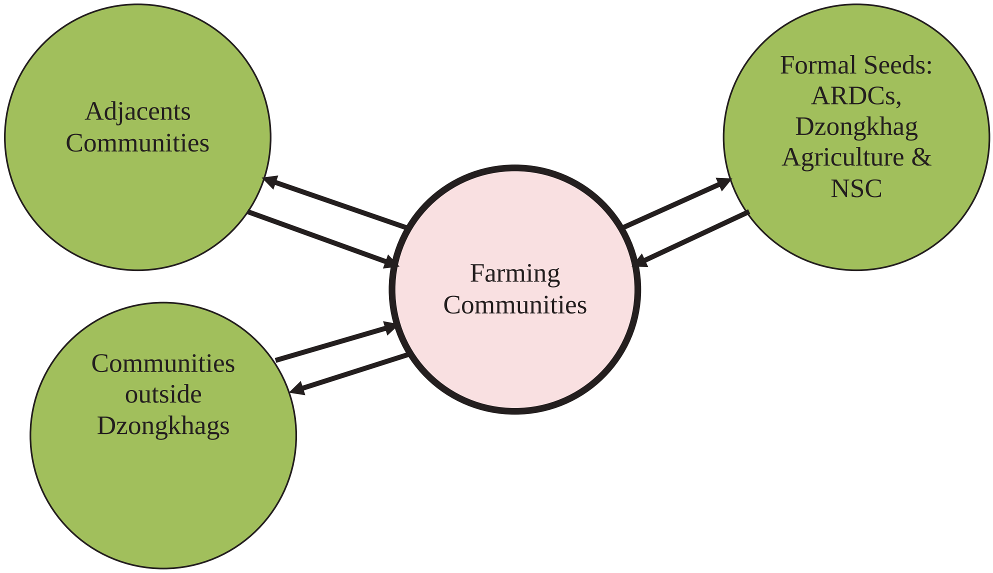

Figure 2.Seed flow diagram prepared by respondents

3.4. Reason for cultivating different crops

Majority of the respondents agree that they cultivate different crops for food security and livelihood. Three critical reasons assigned by farmers for cultivating different crops and varieties are for food security and livelihood (53%), to meet different needs (28%) and meet religious needs (18%). A similar study that assessed the farmers reason for maintain on-farm agro-biodiversity in Bhutan found that an overwhelming 93% of the respondents manage and use agro-biodiversity for household food security and livelihood (Katwal et al, 2015). Many resource-poor farmers in Nepal and Vietnam adapted crop varieties to serve as the main resource to meet their needs and secure livelihood (Sthapit, Rana, Eyzaguirre & Jarvis, 2008) Farmers cultivate different field crops as one of the coping strategies against crop failures through on-farm crop diversity, and to meet their religious and cultural needs (Figure 3).

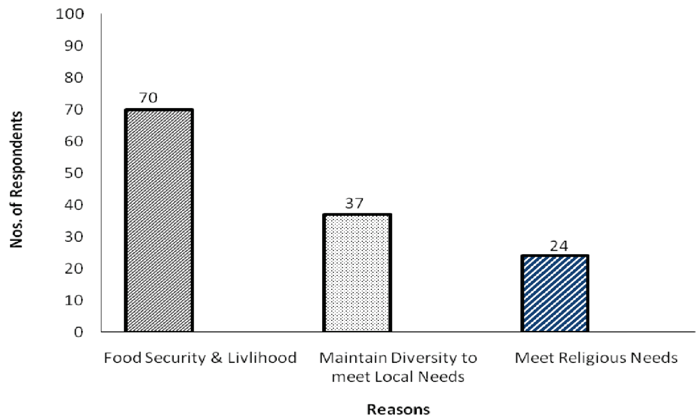

Figure 3.Reasons for cultivating different field crops

3.5. Challenges faced by Farmers in Cultivation and Conservation the Field Crops

Farmers listed numerous challenges they faced in crop production. The three most important challenges listed by respondents are farm labour shortage (26%), human wildlife conflict (24%) and pest and disease attack (17%). Crop damage caused by wildlife is seen as serious threats to livelihood and crop diversity loss, as communities tend to give up crop cultivation in wildlife prone areas (NBC, 2014). Introduction of new seeds is seen as the least important challenges faced in field crops cultivation and conservation in Dewathang and Orong. This study is in-line with the finding of Katwal et al (2015) that the threat of displacement of the traditional crops and varieties by introduction of new seeds is very minimum. However, NBC (2012) reported that displacement of land races by new and genetically uniform cultivars has been recorded as a major threat for PGFRA in Bhutan.

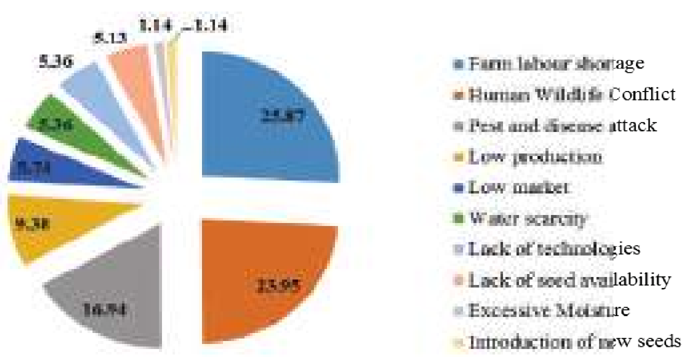

Figure 4.Different challenges listed by respondents for field crops cultivation and conservation

3.6. Farmers perception on local climate

Farmers consider rainfall as the most important weather parameter which affects their livelihood. About 44% of the respondents felt that there was high variation in the rainfall pattern while 41% felt that there was no change in the rainfall pattern. Remaining 19% observed changes in rainfall pattern (Figure 5). In general farmers' understanding and awareness on climate change is poor. A survey done in 2010 to document the impact of climate change on biodiversity reported that community's understanding on impacts of climate change and preparedness on any potential impacts is poor (MoAF, 2011).

Majority of farmers in the two geogs have dryland based rainfed cropping system and depend on rainfall for production of crops and hence timely rainfall is very critical. Majority of the respondents (83%) reported that they have not observed significant changes in the sowing and harvesting pattern of the field crops. Adjusting sowing time in relation to the changes in rainfall pattern could be a good adaptation strategy for farmers to cope against the observed variation n rainfall pattern.

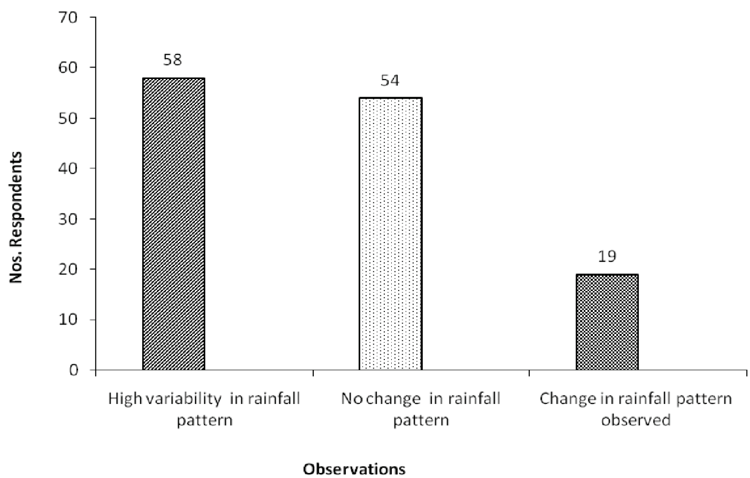

Figure 5.Famers Perception on rainfall pattern

4. **Conclusion and Recommendations**

This study has established that farmers in the two geogs continue to depend on agro-biodiversity for food security and livelihood. Farmers still cultivate different cereals, pulses and some underutilized species like amaranth and perilla. Farmers produce, maintain and exchange seeds within and outside their geogs. They continue to cultivate many traditional crop varieties and the rate of displacement by other new crops and seed is very minimum. Apart from maize, legumes and underutilized crops like amaranth, the percentage of households cultivating different crops is significantly declining. Growing different crops and varieties that can adapt well under marginal environment continues to be the only sustainable coping strategy of farmers against potential risks of crop failures. It is apparent that farmer's awareness and understanding on the potential impacts of climate change on agriculture is very poor.

There are many challenges for the sustainable of *in-situ* conservation of field crops in the two geogs. Making *in-situ conservation* programs more sustainable, remunerative and attractive to the communities is challenging. Low yield of traditional crops and varieties makes them vulnerable to crop displacement by other improved varieties and commercial crops. Managing pest and diseases in a sustainable manner without the use of agro-chemicals and increasing shortage of farm labour needs to be addressed to ensure the continuity of current on-farm agro-biodiversity. Crop damage by wild animals and birds is forcing farmers to leave their land un-cultivated. Limited understanding of the communities on the long-term value of on-farm agro-biodiversity and inability to link agro-biodiversity to other important remunerative enterprises like agro-tourism and organic farming are urgent issues that have to be addressed. Mainstreaming CSO lead initiatives on agro-biodiversity with government agencies for recognition and support is currently weak.

The SJI has initiated work on rehabilitation of traditional crops and varieties from 2012 with the support of GEF SGP project, and is closely working with Agriculture Research and Development Center (RDC), Wengkhar through six lead farmers in Dewathang geog with the support of International Fund for Agriculture Development (IFAD) project. Some of the potential interventions to enhance the conservation, development and utilization of on-farm agro-biodiversity are through value addition and marketing of traditional crops and their products for income generation. There is a need to focus on product development, diversification, and marketing of local produce to sustain farmer's livelihood through income generation. There is an urgent need to explore and promote labour saving devices to address the needs of small subsistence farmers and women farmers in particular – who are the actual custodians of seed. Sustainable on-arm agro-biodiversity conservation initiatives will require designing women and youth-focused enterprises. Linking of agro- biodiversity to other important rural enterprises such as agro-tourism, organic seed production and production of organic fertilizers has good potential for long-term sustainable on-farm conservation of field crops.

Participatory and voluntary approaches for the conservation, development, and utilization of agro-biodiversity and its benefits through a long term community visioning and participation has to be initiated. Further, mainstreaming of community-based agro-biodiversity programs initiated by CSOs like SJI with the National Bio-diversity Center, Dzongkhag Agriculture Sector and Research and Development Centers will lead to more sustainable *in-situ* conservation.

Acknowledgement

SJI and authors are grateful to Small Grants Project, Global Environment Facility of the UNDP for funding the traditional crop rehabilitation project in Dewathang and Orong geogs. We also thank the community of the two geogs of Dewathang and Orong for their support during our field visit and for their feedback.

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Post-harvest Losses of Rice in Paro Valley

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ABSTRACT

Rice production in Bhutan was recorded at 86,385t in 2017 with self-sufficiency estimated at 45%. The current post-harvest loss of rice in Bhutan is estimated as high as 30%. Addressing post-harvest losses in rice through poor post-harvest handling and management of rice crop can significantly increase rice availability. Apparently, there is insufficient data on post-harvest losses of rice at different stages of post-harvest operations in Bhutan. For an effective reduction in losses, it is imperative to estimate the losses and the stages at which they occur. This study was conducted in Paro, which is one of the main rice growing districts in Bhutan. The study was aimed to assess the post-harvest losses in rice during harvesting, in field drying, in field transportation of harvested paddy to threshing floorand threshing process.Five commonly grown rice varieties (No. 11, Yusi Ray Maap-2, Yusi Raykaap-2, KhangmaMaap and Dum Ja (local) were assessed to determine the losses occurring during the different post-harvest operations. The total post-harvest loss, irrespective of the different varieties is estimated at 5.50% of the total production. The Dum Ja variety exhibited the maximum post-harvest loss of 11.60 % of the total production, while the No. 11 variety exhibited the minimum post-harvest loss of 1.33% of the total production.

Keywords: *Post-harvest loss, Rice varieties, Self-sufficiency*

1. Introduction

Rice (*Oryza sativa* L.) is the main staple food crop of the Bhutanese with per capita consumption of 172 kg per year and the current rice self-sufficiency in the country is estimated at 45% (Gautam et al., 2013). Rice is cultivated in all agro-ecological zones of Bhutan except the alpine zone in the north. The crop is grown at an elevation of 200 meter above sea level in the southern foothills to 2800 meter above sea level in the north (Shrestha, 2004). The mainrice growing districts by production are Punakha, Paro, Wangdue, Dagana, Sarpang, and Tsirang. Lhuntse, Samdrup Jongkhar and Tashigang are the other main rice producing dzongkhags in Bhutan (MoAF, 2017). Rice in Bhutan is cultivated under both irrigated and rain-fed systems (Chhogyel, Ghimiray, Wangdue & Bajgai., 2015).

Rice production in Bhutan was recorded at 83,332 t and 86,385 t in the year 2016 and 2017, respectively (RNR statistics, 2017). To achieve 60% rice self-sufficiency in the 12th FYP, the

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Department of Agriculture is assertively promoting high altitude and spring paddy cultivation across the country (Karchung, 2017; Gautam et al., 2013).

This preliminary study on post-harvest losses of rice grain in Bhutan with focus in Paro dzongkhag (district) was conducted to obtain the baseline data on post-harvest losses. The data on post harvest losses then could be used to develop interventions required to reduce losses at different stages of post harvest handling that could contribute to rice self sufficiency.

Rice availability can be improved by increasing production, introducing improved varieties, improving distribution, and reducing losses. Reduction of post-harvest losses in rice is an essential factor in safeguarding future global food security and it has been estimated that almost one third (1.3 billion t) of global rice production is lost annually (FAO, 2015). Poor methods of harvesting and handling of the produce, use of inappropriate container while packaging, poor storage conditions, poor transportation and distribution system are some of the major factors contributing to these losses (Kiaya, 2014). The constraints in cultivating rice in Bhutan are low soil fertility, prevalence of pest and diseases, cold temperature and high labour requirement (MoAF, 2001).

Proper post-harvest management techniques can be applied to reduce the post-harvest loss in rice. Timely harvest of paddy at optimal moisture percentage is critical. The right stage for harvesting paddy is when about 80% panicles have about 80% ripened spikelet. At the time of harvest the upper portion of the spikelet should be straw coloured and grain should have 20% moisture content (Guisse, 2010). While early harvest lead to immature grain, deterioration of quality and broken rice during milling, delay in harvesting will result in grain loss caused by rodents, pests, shattering and lodging (Guisse, 2010). Use of suitable technique while harvesting and avoiding too much drying, fast drying and wetting of grains can help in significant reduction of post-harvest loss in rice (Kader, 2002). The losses in threshing and winnowing can be minimized using improved mechanical methods. Proper storage after milling can help in minimizing losses caused by rodents and pests (Patil, 2011). Effective post-harvest handling and management of the crop can help in minimizing rice imports and achieving rice self sufficiency in the country. For effective reduction in loss of rice, it is essential to assess and estimate the post-harvest loss in rice occurring during the different stages of post-harvest operations in rice (Appiah, Guisse & Darty, 2011).

2. Materials and Methods

2.1 Survey area and data collection

This study was carried out in the rice growing areas within Paro dzongkhagduring the paddy harvesting season in October 2017 using questionnaire through random sampling technique. Five rice varieties (*No. 11, Yusi Ray Maap-2, Yusi Raykaap-2, Khangma Maap and Dum Ja (local)* commonly cultivated by the farmers in the study area was selected for data collection and field experiment.

Paddy plots were divided into quadrants (1 m × 1 m) and the harvesting losses were determined from each quadrant. Samples from same plot were used for losses for in-field drying, in-field transportation and threshing losses.

2.2 Determination of post-harvest losses

The post-harvest loss of rice in the study area was determined by collecting the data from different post-harvest handling operations of the rice viz. harvesting, in-field drying, in-field transportation and threshing.

2.3 Determining moisture content (MC) of paddy

The moisture content of the paddy grains was measured one day prior to harvesting. Five hills of paddy harvested from a plot were randomly picked and moisture content was measured using moisture tester (Riceter f506, Kett Electric Laboratory, Tokyo, Japan).

2.4 Determining maturity of the paddy

The matured paddy grains were calculated using the total number of grains and total immature grains. Maturity of the paddy grains at the time of harvesting in the study area was determined using following formula:

$$\%\,Maturity = Total\ no.\ of matured\ grains \div Total\ no.\ of grains \times 100$$

2.5 Determination of harvesting losses (HL)

Harvesting losses (HL) is loss of paddy grains from/or during the time of harvesting. Harvesting losses were determined according to the method adapted from Badawi (2003). Farmers were allowed to harvest the paddy using harvesting sickles. Leftover grains from each quadrant (on the ground and from standing plants) were collected, cleaned, dried and weighed and harvesting loss percent was calculated as below:

$$\%\,HL = Left\ over\ grains \div Total\ harvested\ paddy \times 100$$

2.6 Determination of infield drying losses (DL)

Farmers normally dry their paddy in the fields after harvesting for two-three days before stacking and threshing. The fallen grains from the sampling plots were collected, cleaned, dried and weighed. The percentage of in-filed drying loss was calculated using the following equation:

$$\%\,SL = Weight\ of fallen\ grains \div Total\ weight\ of\ harvested\ grains \times 100$$

2.7 Determination of in field transportation losses (TrL)

The dried paddy hills are then stacked in place before threshing in the field. To determine the infield transportation loss, the weight of the grains before transporting and the weight of the grains after reaching the stacking point were recorded and then expressed as:

$$\% \text{ TrL} = \text{Weight of the grains after transportation} \div \text{Initial weight of the grains to be transported} \times 100$$

2.8 Determination of threshing losses (TL)

The stacked paddy was threshed after two-three days in favorable sunny weather. Two kinds of threshing methods are currently used by Bhutanese farmers viz. pedal/power thresher and beating of rice stalk on wood or stone with tarpaulin sheet beneath. Threshing of the grains at the chosen study site was done with the latter technique. The amount of rice grains that fell outside the threshing area as well as those left on the rice stalks were weighed and accounted into threshing loss. The percent threshing loss was then determined as below:

$$\% \text{ TL} = \text{Weight of left over grains} \div \text{Total weight of collected grains} \times 100$$

2.9 Total post-harvest loss (TPL)

The total post harvest losses of rice grains in this study are the cumulative of losses from harvesting, in field drying, and in field transportation and threshing. A sum of losses at these post-harvest handling operations gave the total post harvest losses as given in the following equation:

$$\text{TPL} = \text{Harvesting losses} + \text{in field drying losses} + \text{in field transportation losses} + \text{Threshing losses}$$

3. Results and Discussion

3.1 Moisture content

The moisture content of the paddy grains before harvesting and at the time of threshing was measured to understand the maturity and the dryness of the grains for further processing. The moisture content of the grains varied among the varieties at the time of harvesting (Table 1). Among the varieties, Bajo No. 11 recorded the highest moisture content while Dum Ja (Local variety) recorded the lowest at 14.19%.

Table 1.Moisture content of the rice grains at the time of harvesting and threshing

Variety	Moisture content at harvest (%)	Moisture content at threshing (%)
No. 11	22.85	15.30
Dum Ja (Local)	14.19	11.35
Khangma Maap	23.20	14.25
Yusi Ray Maap -2	18.71	14.20
Yusi Ray kaap - 2	17.50	15.60
Mean	20.55	14.29

At the time of harvesting, the moisture content in all the varieties reduced significantly, indicating that the paddy grains were threshed at the optimum moisture level. The optimum moisture content for the rice grain storage is between 10 to 15% (Juliano, 1985). This indicates the farmers were not only harvesting the paddy grains at the right stage in terms of moisture content, but also the paddy grains were dried sufficiently in the field before threshing and storage.

3.2 Maturity of the grains

Maturity of the paddy grains was also measured day before harvesting according to the method previously discussed. The maturity was measured in terms of percentage. At the time of harvesting, the maturity percentage was slightly different among few of the rice varieties as shown in Table 2. Harvesting rice immediately after the cessation of biological maturity ensures maximum yield and better milling characteristics (Sarkar, Datta & Chattopadhyay, 2013). Too early harvest results in more chaff and ill-filled grains while delayed harvest results in low yield as the crop suffers various pre-harvest losses and milling quality is impaired.

Table 2.Maturity percent of the rice varieties at the time of harvesting

Rice Varieties	Maturity (%)
No. 11	87.17 ± 10.37^{ab}
Dum Ja (Local)	100.00 ± 0.00^{a}
Khangma Maap	74.63 ± 20.81^{b}
Yusi Ray Kaap 2	88.02 ± 1.87^{ab}
Yusi Ray Maap 2	91.88 ± 8.10^{ab}

The lowest maturity percentage was determined in the variety Khangma Maap while 100% maturity was recorded from another local variety (Dum Ja). This indicates that the former was slightly prematurely harvested while the latter was harvested at full maturity. Harvesting the grains at the right maturity will have impact on the shelf life and quality of the grains during post-harvest handling operations (Yang & Zhang, 2010). If grain were harvested prior to its physiological maturity, it would have low drymatter, poor quality grains and will shrivel upon drying (Kester et al., 1963).

3.3 Harvesting losses

Between the rice varieties no significant difference in losses was observed during harvesting operation. Harvesting losses was observed below one percent for all the varieties as shown in Table 3. Harvesting loss was highest in Yusi Ray Kaap 2 at 0.74% of the total harvested grains while Yusi Ray Maap 2 recorded lowest harvesting loss at 0.15% (Table 3).

3.4 In-field drying losses

Drying losses among the varieties was in the range of 0.246 to 1.084 with Yusi Ray Maap 2 recording lowest drying loss (0.024%) with statistically significant difference from other varieties ($p < 0.05$).

3.5 In-field transportation losses

Yusi Ray Kaap 2 recorded highest in-field transportation losses (1.124%) while No. 11 had minimum losses (0.145%) with significant differences ($p < 0.05$).

3.6 Threshing losses

Threshing losses was highest in the Dum Ja variety (9.31%) with statistically significant difference compared to all other varieties. The remaining varieties of rice recorded threshing losses in the range of 0.38% to 2.82%. A significant portion of losses occurs during the threshing operations compared to other post-harvest operations as per this study. Interventions with improvised methods of threshing in this stage of post-harvest handling operation could result in lowering the threshing loss and thus reduce the total post-harvest loss.

The percentage losses of rice in different stages of post-harvest operations were also determined for each variety of rice (five varieties) as shown in table 4. For No. 11, there was no significant difference in the percent losses of rice at different stages (0.14% - 0.70%).

For the other rice varieties (Dum Ja, Khangma Maap, Yusi Ray Kaap 2, Yusi Ray Maap 2), there was no significant difference in losses between harvesting, drying and in-field transportation stages. The percent losses during threshing of rice were statistically higher for all these varieties (Dum Ja, KhangmaMaap, Yusi Ray Kaap 2, Yusi Ray Maap 2) compared to harvesting, drying and in-field transportation operations. The mean of all the rice varieties included in the study also showed significantly higher threshing losses (5.69%) compared to harvesting (0.47%), drying (0.66%) and in-field transportation (0.72%).

Table 3.Percent post-harvest losses of rice at different stages of post-harvest operations between different varieties of rice in Paro

Rice Varieties	Harvesting loss (%)	Drying loss (%)	In-field transportation loss (%)	Threshing loss (%)
No. 11	0.706±0.934[a]	0.089±0.090[b]	0.145±0.070[c]	0.389±0.231[b]
Dum Ja (Local)	0.464±0.101[a]	0.904±0.218[a]	0.927±0.208[ab]	9.313±4.003[a]
Khnagma Maap	0.303±0.174[a]	1.000±0.337[a]	0.738±0.146[b]	2.237±0.825[b]
Yusi Ray Kaap 2	0.741±0.101[a]	1.084±0.258[a]	1.134±0.139[a]	2.823±0.585[b]
Yusi Ray Maap 2	0.159±0.056[a]	0.246±0.064[b]	0.672±0.225[b]	2.097±1.169[b]

Mean values within the column with different superscript are significantly different between the varieties at $p < 0.05$ by Tukey's test (Mean ± SD, n=5)

Table 4.Comparison of losses of rice at different stages of post-harvest operations among the varieties

Rice Varieties	Harvesting loss (%)	Drying loss (%)	In-field transportation loss (%)	Threshing loss (%)
No. 11	0.706±0.938[A]	0.089±0.090[A]	0.145±0.070[A]	0.389±0.231[A]
Dum Ja	0.464±0.101[B]	0.904±0.218[B]	0.927±0.208[B]	9.313±4.003[A]
Khangma Maap	0.303±0.174[B]	1.000±0.337[B]	0.738±0.146[B]	2.237±0.825[A]
Yusi Ray Kaap 2	0.741±0.101[B]	1.084±0.258[B]	1.134±0.139[B]	2.823±0.585[A]
Yusi Ray Maap 2	0.159±0.056[B]	0.247±0.246[B]	0.672±0.225[B]	2.097±1.169[A]

Mean values within the rows with different capital superscript are significantly different between harvesting operations for each variety at $p < 0.05$ by Tukey's test (Mean ± SD, n=5)

3.6 Total post-harvest loss

The total post-harvest loss of rice grains for all the varieties were calculated as the sum of losses from the post-harvest handling operations. From the five common varieties cultivated by the farmers, only No. 11 showed significantly low percent of total post-harvest loss at 1.33% while Dum Ja (local variety) showed significantly high total post-harvest losses at 11.60%. The total post-harvestloss in remaining varieties ranges from 4.27% in Khangma Maap to 5.23% in Yusi Ray Maap 2 as shown in Table 5.

Table 5.Total post-harvest losses of rice grains

Sl No	Variety	Total post-harvestlosses (%)
1.	No. 11	1.33
2.	Dum Ja (Local)	11.60
3.	Khangma Maap	4.27
4.	Yusi Ray Kaap 2	5.05
5.	Yusi Ray Maap 2	5.23
	Average	5.50

According to FAO (2015) report, the post-harvest losses of rice grains in Southeast Asia is 8% while the post-harvest loss of rice in neighboring Bangladesh is 10% (Nath et al., 2015). Thus, comparing the losses of rice grains in neighboring countries, the findings of this study indicates that 5.50% (Table 4) post-harvest losses of rice in Bhutan (Paro valley) is not significantly high though interventions can be made to further reduce the losses by putting in place proper post-harvest management practices.

4. Conclusion

This study conducted concludes that the post-harvest losses of rice in Paro dzongkhag occurs across all the post-harvest operations involved, irrespective of the varieties. Maximum grain loss occurred during threshing process which contributed the most to the total pot-harvest losses in all the varieties. While some matured grains stay attached to the panicle in the straw during threshing, some grains scatter on the ground, accounting for the total threshing loss. The total post-harvest loss, irrespective of the different varieties accounts to 5.50% of the total production. Dum-Ja variety showed the maximum total post-harvest losses with 11.60% of the total production, while the No. 11 variety exhibited the minimum post-harvest loss of 1.33% of the total production from this study.

The total post-harvest loss of 5.50% in rice is not so alarming. However, considering the country's increase in rice import every year, effective measures have to be put in place to minimize the post-harvest losses in rice. Effective post-harvest handling and management of rice crop includes use of appropriate tools and techniques during harvesting, in-field drying, in-field transportation and threshing. Approaches like awareness campaign and training programs on proper post-harvest handling and management of rice to the growers in the country can also have significant impact. These can not only help minimize losses incurred during the various post-harvest operations but also in reducing rice imports and in achieving rice self sufficiency in the country.

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Effect of Harvesting Stages of Maize on Quality and Consumption Preferences of *Tengma*

Kinley Wangmo[w]

ABSTRACT

Local cornflake or beaten maize known in the eastern Bhutan as Tengma is one of the most popular processed maize products in Bhutan. The colour, taste and texture are important quality attributes of Tengma, which are influenced by harvesting stage of maize. This study on colour, taste, texture and the general quality acceptability of Tengma was conducted to identify optimal harvesting stage of maize for processing Tengma. The study was conducted at Thridangbi Chewog under Saling geog, Mongar Dzongkhag. Maize harvested at milk, dough, dent and physiologically matured as well as maize from previous season were collected from Agriculture Research sub-center, Lingmethang and were processed using farmer's cornflake machine following local processing method. Colour, taste, texture and overall acceptability were done by 30 panelists representing various sectors while total soluble solids (TSS), moisture content (MC) and weight were recorded using refractometer, moisture meter and weighing scale, respectively. The results indicate MC and TSS were significantly higher ($p \leq 0.05$) in Tengma processed from maize grains harvested at milk stage (MS). Tengma processed with maize grains harvested at dent stage scored the highest "extremely like" of 47% in terms of color. In terms of taste and texture, Tengma processed using maize grains harvested at milk and dough stage scored the highest "extremely like" and "like" rating of 90% to 97% each. In overall acceptability category, Tengma processed using maize grains harvested at MS and from the previous season had the highest "extremely like" or "like" score of 100% and "extremely dislike" score of 25%, respectively.

Keywords: *Colour, Moisture content, Physiologically matured, Taste, Texture and total soluble solid*

1. Introduction

Maize (*Zea mays*) is the most widely cultivated cereal crop globally. The average yields of traditional varieties grown by small-scale farmers is around 0.8 tha^{-1}, compared with 2 to 5 tha^{-1} for improved varieties (Hoopen & Abdou, 2012). The Total area under maize cultivation in Bhutan in 2010 was 61,676 acres with a total production of 57,666 t with a national average yield of 2.38 t ha-1 (DOA, 2013).

Maize in several countries is both a staple food and a cash crop for small holder farmers. As a food it can be prepared in many different ways (fried, grilled, salad or soup). Processing maize

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can also produce a wide range of products such as corn flour, corn flakes and corn meal. In Bhutan, maize products are consumed in different forms, such as *Tengma* (roasted and pounded maize), *Kharang* (maize grit), popcorn, roasted and flour. It is also used for brewing beverages such as *Bangchhang* (local ale) and *Ara* (local alcohol). Maize is also used as a feed for livestock.

In Bhutan maize is grown all over the country, but is more popular in the eastern region. About 45% of the total maize production comes from the six eastern districts of Trashigang, Samdrup Jongkhar, Pemagatshel, Trashiyangtse, Monggar and Lhuntse (FAO, 2014). Over 70% of the households cultivate maize mainly for subsistence (NBC, 2008) and therefore, it plays a crucial role in achieving household food security. Maize constituted 43.7% of the national food composition in 2011. The area under maize cultivation is 70,171 acres (constituting 44.2% of the cultivated area) with a total production of 79,667 t (DoA, 2013). The national average yield is 1.135 t ha^{-1}, while the average yield of maize in eastern region is 1.310 t ha^{-1}(DoA, 2013).There are 81 varieties or landraces of maize cultivated in Bhutan (NBC, 2008) covering an altitudinal range of 300 masl to 2,800 masl (Katwal et al., 2013).

The rural households sell about 6% of the total maize production (Katwal, 2013). Maize is either sold to the Food Corporation of Bhutan or to feed companies or to dealers across the borders. *Kharang* and *Tengma* are the most popular processed maize products in the country, and sold mostly in the local markets. Katwal et al. (2007) reported that there were about 73 effective *Tengma* processors in the country with most of them concentrated in the eastern region.

To process *Tengma* maize has tobe roasted in a pan and pounded either in a machine or in a traditional wooden pound. The maize at milk, dough, dent, and physiologically matured can be used for processing *Tengma* immediately after harvesting whereas maize grains harvested in previous season have to be boiled and soaked in hot water overnight before processing. In absence of scientifically recommended harvesting maturity index for maize meant for processing *Tengma*, farmers depend on visual and other clues to judge whether maize are good for processing. Too matured or immature maize grain meant for processing *Tengma* could compromise eating and other quality attributes such as texture, sugar content and keeping quality of the processed *Tengma*.

Therefore this study is designed to determine the optimal harvesting stage(s) of maize meant for processing good quality *Tengma*in terms of colour, taste and texture and overall acceptability.

2. Materials and Methods

The experiment was conducted in Thridangbe Chewog under Saling Geog, Monggar dzongkhag. Maize is one of the major cereals grown in this geog and it is cultivated twice a year.The 347 households in the geog cultivate maize. The total area under maize cultivation is 504 acres with production of 617 t and an average yield of 1,224kg Ac^{-1} (DoA, 2013).

The study had five treatments corresponding to five different development stages of maize grain (T 1 – dough, T 2- milk, T3- dent, T4- physiologically matured and T5-previous season maize). Each treatment was replicated five times and each replication weighed 4 kg of maize grains.

Maize (Yangtsepa[1] variety) was harvested from field of ARDC sub-center, Lingmethang at five different stages (milk, dough, dent and physiologically matured), and the cobs were de-husked and shelled. The maize harvested in previous season was collected from the store of the ARDC sub-center based in Lingmethang. Maize cobs harvested at milk stage were blanched in boiling water for about five minutes and kept overnight before shelling to ease shelling. After shelling the grains were spread on bamboo mat and plastic sheets to dry the surface water.

For each replication the shelled maize grains were weighed using an electrical weighing balance (Model # DS-252). The maize grains were then roasted for 20 to 30 minutes in traditional roasting pan at a temperature of 110^{0}C – 120 ^{0}C. A laser gun thermometer was pointed at the grains to take the temperature reading of the grains. For each replication three readings were taken and averaged.

The grains were kept overnight in room temperature and roasted again for a second time before pounding into tengma. The temperature for second roasting ranged from 130 ^{0}C – 140 ^{0}C. For the maize from previous season the grains were first boiled for 30 minutes and soaked overnight as traditionally practiced by the farmers in the locality before roasting and pounding into *Tengma*,

Moisture content (MC) of maize grains was measured before and after roasting using a moisture meter (G-7, Grain Moisture meter, DELMHORST INSTRUMENT Co.). About 10-14 grains were randomly selected for each replication and were placed on the measuring plate of moisture meter to read the moisture content.

The final processed product *Tengma* was tested for qualities such as colour, taste, texture, overall acceptability and TSS content (sugar content). For determining TSS, the processed *Tengma* was first powdered using a mixture grinder and 5 g of this powder from each replication stirred in 45 ml of distilled water in a breaker and heated for five minutes. The solution was filtered and a few drops of filtered solution were placed on the lens of a refractometer to obtain the reading for a particular sample *Tengma*.

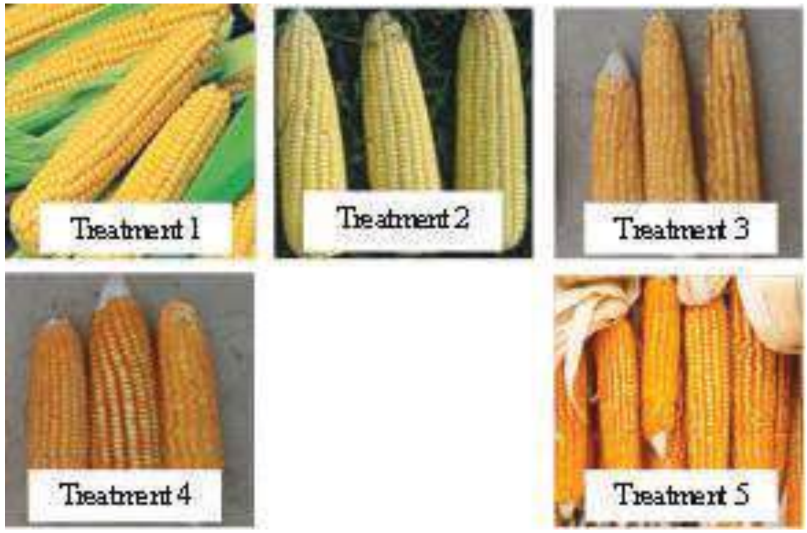

Figure1.Different stages of maize used for *Tengma* processing

The weight of fresh grains and the final processed *Tengma* for each replication was measured using electric digital weighing balance.

Sensory evaluation of colour, taste, texture and overall acceptability was done using a 5-point hedonic scale with 5 being extremely like and 1 being extremely dislike. A 30-member panelist comprising farmers and agricultural officers evaluated the above qualities. The panelist tasted as well made visual and sensory observation of individual samples and their individual observations were recorded in the evaluation form.

The data generated from experiment were analyzed using the Statistical Package for Social Science (SPSS) 16.0. Quantitative data such as weight, sugar content and MC were analyzed using One Way ANOVA and Microsoft Excel 2008. *P* values ≤ 0.05 were considered significant in all the analyses. For the qualitative data on sensory evaluation, the results were interpreted in proportions/percentages because statistical analysis of such data is still mired in controversies.

3. Results and Discussion

3.1. Moisture content of maize grains before and after roasting and processing

Moisture content (MC) of maize grains before roasting was significantly different between all the stages of harvest (Table 1). The highest MC was observed in maize grains harvested at milk stage (35 ±.13%) followed by those grains harvested at dough stage (29.06 ± .08%). The lowest MC (12.50± .05%) was in maize grains of previous season harvest.

The MC (25.18 ± .40%) after roasting was significantly higher in maize grains harvested at dough stage compared to maize grains harvested at all other stages. While the MC after roasting was not significantly different between the maize grains harvested in milk stage and physiologically matured ones, the former had significantly higher than maize grains harvested at dent stage and from the previous season maize. The latter and dent stage maize grains had significantly higher MC than the maize grains harvested at previous season. The MC of *Tengma* was significantly higher in maize grains harvested at milk stage (13.14± 1) compared with the maize grains harvested at all other stages except the dough stage. There was no significant difference in MC of maize grains harvested in other stages.

According to Reyneri and Mairano (2010) developing maize kernels accumulate more water than reserves early on the development stage, reaching a 90% or higher moisture content. The kernel moisture content then declines progressively as kernel continuous to mature. During early grain filling stage, moisture declines from around 70% to around 18% at harvest. As per the Indian standard, moisture content of corn flakes should be more than 7.5% and all brands of corn flakes in the Indian markets are found within the required limit (Consumer voice, 2012). Lhendup (2009) pointed out that while roasting maize in open flame, the moisture from maize dries up, thereby reducing the MC in *Tengma*.

Table 1.MC of maize grain before and after roasting and processing, TSS and weight of *Tengma* (Mean ± Standard Deviation)

Treatments	**MC before roasting (%)**	**MC after roasting (%)**	**MC of tengma (%)**	**TSS(^{0}B)**	**Weight (Kg)**
Dough stage	29.06 ± 0.08^{b}	25.18 ± 0.40^{a}	12.00 ± 1.40^{ab}	10.00 ± 1.22^{ab}	1.81 ± 0.02^{a}
Milk stage	35 ± 0.13^{a}	20.22 ± 1.10^{b}	13.14 ± 1.00^{b}	12.04 ± 0.08^{b}	2.83 ± 0.14^{b}
Dent stage	27.32 ± 1.26^{c}	18.82 ± 0.64^{c}	10.58 ± 1.04^{a}	9.00 ± 2.44^{ac}	2.94 ± 0.09^{bc}
Physiological maturity stage	24.76 ± 0.43^{d}	19.35 ± 0.15^{bc}	10.92 ± 1.21^{a}	6.20 ± 2.28^{d}	3.07 ± 0.10^{c}
Previous season maize	12.50 ± 0.05^{e}	14.36 ± 0.59^{d}	10.82 ± 0.61^{a}	5.60 ± 2.60^{d}	2.91 ± 0.10^{bc}

Means within a column with different superscripts differ significantly ($p \leq 0$ .05)

3.2. Total Soluble Solids

The total soluble solids (TSS) in *Tengma* processed from maize grains harvested at milk stage was significantly higher compared to *Tengma* processed from maize grains harvested at all other stages except the *Tengma* processed from maize grains harvested at dough stage (Table 1). The TSS in the latter and *Tengma* processed from maize grains harvested at dent stages were similar, but they were significantly higher from the TSS of *Tengma* processed from maize grains harvested at physiologically matured and in previous season. The TSS of the latter two stages was not significantly different from each other. The findings of this study are in line with the study of Pajic et al. (2004), which states that maize when consumed at milk stage is sweeter because it contains around 15-35% sugar and 20-30% starch. However, Sygenta® (2011) reports that maize variety Winter Sweet contains about 15-20% sugar and variety Sugar 75 about 16% sugar, which is higher than sugar content found in *Tengma*.

Huang (2013) stated that MC at the time of harvesting affects the composition and nutritive value of the maize. Similarly the high sugar content in milk stage of the corn could be due to proper nutrient management practices, which results in higher protein and sugar content (Shinde, Patange & Dhage, 2014).

According to Office of the Gene Technology Regulator (2008), sugar content in maize kernels will be high at the milk and early dough stage of the grain development.

Plessis (2003) reported that at soft dough stage maize grain mass increases and the sugar get converted into starch and in the hard dough stage sugar in the maize grains decreases rapidly, while starch accumulation increases.

A black layer cell formation at the base of kernels indicates the stoppage of flow of sugars from leaves to the kernels (Brewbaker, 2003). A black or brown layer cell formation on the kernel indicates the physiological maturity of corn (Lee, 2011**)**.

3.3. Weight of *Tengma*

Tengma recovery was significantly influenced by the stage at which maize was harvested ($p \leq 0.05$). Tengma made from maize grains harvested at physiological maturity had the highest weight at 3.07 kg. This was followed by *Tengma* processed from maize grains harvested at milk stage and dough stage at 2.83 kg and 1.8 kg, respectively (Table 1). It was however, not significantly different from weight of *Tengma* processed from maize harvested at dent stage and previous season. The latter two were not significantly different from each other, but differed significantly with the weight of *Tengma* processed from maize grains harvested at dough stage.

The highest recovery (or final weight) obtained in *Tengma* processed from maize grains harvested at physiologically matured could be because of lower MC and high starch accumulation in the grains compared to *Tengma* processed from maize grains harvested at milk and dough stages.

3.4. Sensory Evaluation

3.4.1. Colour of Tengma

Visual appearance such as color is an important quality aspect that could, to some extent, influence consumer's decision to purchase. The color of *Tengma* processed using grains harvested at different stages was varied. *Tengma* processed using maize grains harvested at dent stage scored the highest "extremely like" rating at 47% followed by *Tengma* processed using maize grains harvested at physiologically matured at 27% (Figure 2). The least color rating was for *Tengma* processed using maize grains harvested at dough and milk stage at 10% each. This could be because dough and milk stage maize grains contain more moisture and sugar than protein, which is responsible for golden yellow color. Milk sugar being whitish does not give attractive coloration in physiologically advanced maize grains.

The color of *Tengma* processed using maize grains harvested at dough, milk, dent and physiologically matured did not have "extremely dislike" rating, while at least 3% of the respondents "extremely disliked" the color of *Tengma* processed using maize grains harvested at previous season.

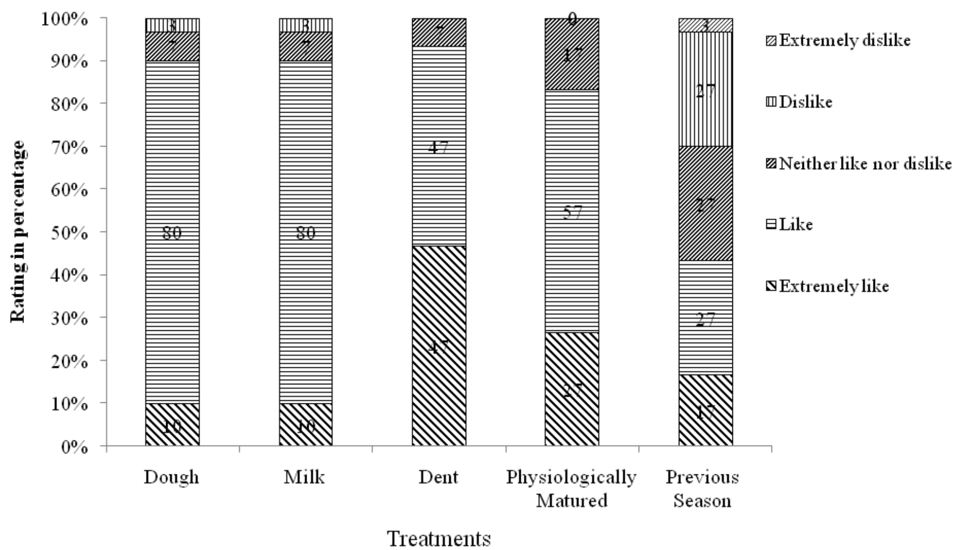

Figure 2.Analysis of colour of *Tengma* processed from maize harvested at different stages

3.4.2. Taste of Tengma

The taste of *Tengma* processed using maize grains harvested at different stages showed different ratings with *Tengma* processed using maize grains harvested at milk and dough stage scoring the highest "extremely like" or" like" ratings of 90% followed by *Tengma* processed using maize grains harvested at dent stage at 80% (Figure 3). The least "extremely like" or "like" taste rating was for *Tengma* processed using maize grains harvested from the previous season at 26%. This could be because *Tengma* harvested at dough and milk stages contain higher level of sugar unlike maize harvested at other stages.

While no respondents extremely disliked the taste of *Tengma* processed using maize grains harvested at dough, milk, dent and physiologically matured, at least 10% of the respondents extremely disliked *Tengma* processed using maize grains harvested from previous season.

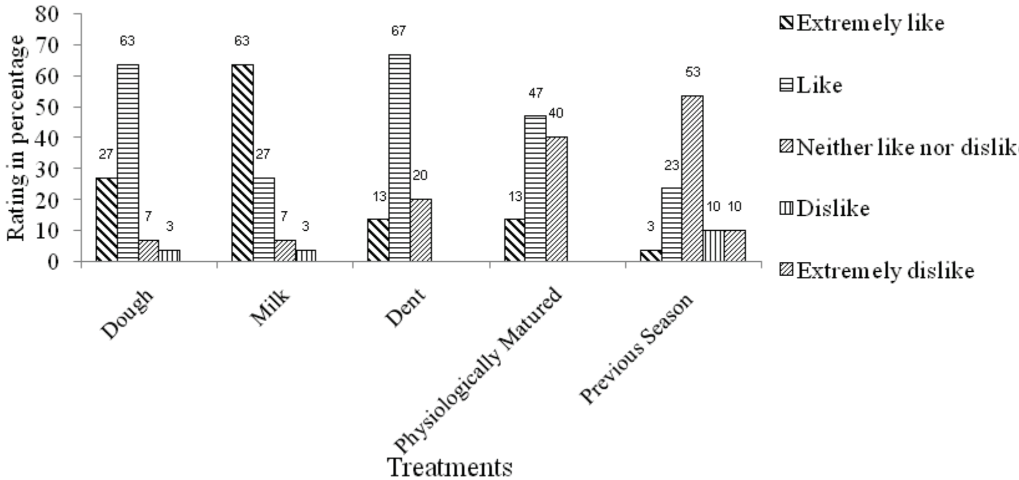

Figure 3.Analysis of taste of *Tengma* processed from maize harvested at different stages

3.4.3. Texture of Tengma

Tengma can be hard, mushy or crunchy and such texture can define the quality and likeability of *Tengma*. The texture of *Tengma* can be influenced by moisture content as well as the way of processing, including roasting method. Between 90% to 97% “liked” or “extremely liked” the texture of *Tengma* processed using maize harvested at milk and dough stage, respectively compared with only about 47% and 70% for *Tengma* processed using maize grains harvested from previous season and at physiologically matured, respectively (Figure 4).

While no respondents “extremely disliked” the texture of *Tengma* processed using maize grains harvested at dough, milk, dent and physiologically matured, at least 3% of the respondents “extremely disliked” the texture of *Tengma* processed using maize grains harvested from previous season. This could be because of low moisture and sugar content in maize grains harvested in previous season compared to maize grains harvested in other stages, especially dough and milk stage

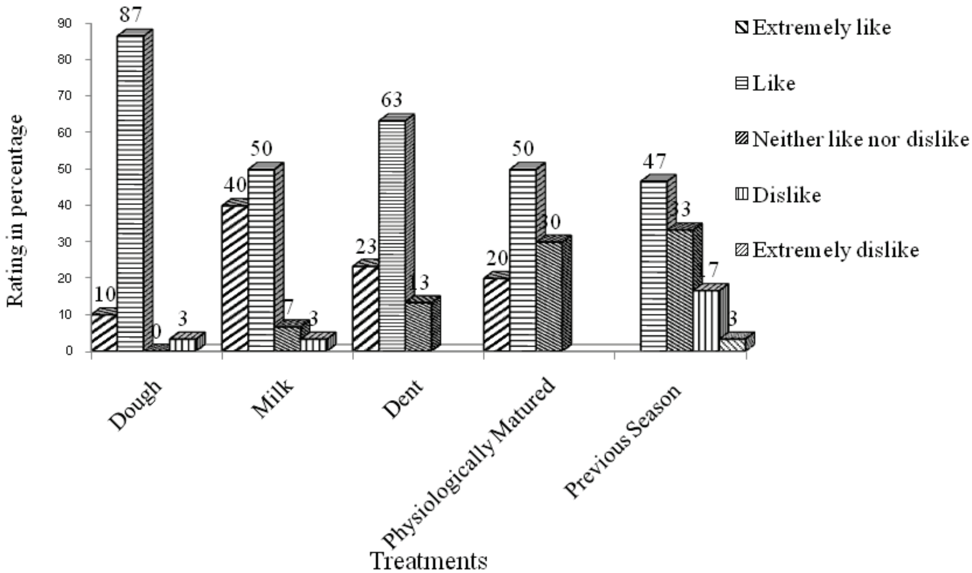

Figure 4.Analysis of texture of Tengma processed from maize harvested at different stages

3.5. Overall acceptability

In the overall acceptability rating, *Tengma* processed using maize grains harvested at milk stage and dough stage had the highest “extremely like” and “like” scores of 100% and 94%, respectively, while those processed using maize grains harvested at dent stage and previous season had the least at 67% and 0%, respectively (Figure 5). This could be presumably because *Tengma* processed from maize grains harvested at milk and dough stages contain relatively higher sugar which could have influenced both sweetness and attractive golden yellow coloration.

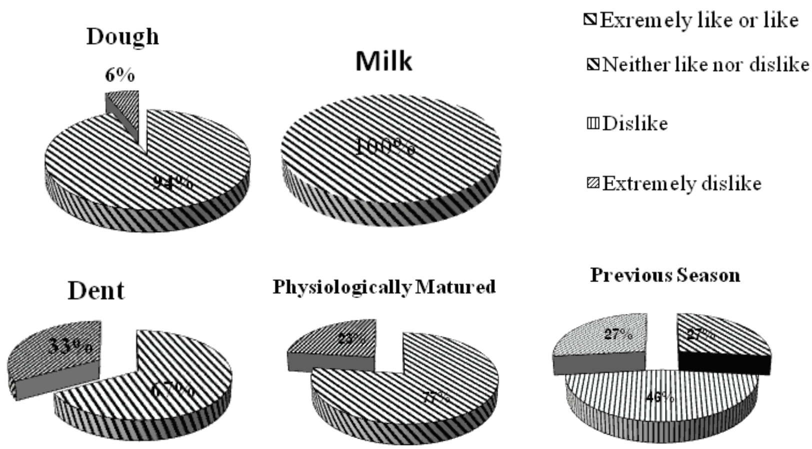

Figure 5.Analysis of overall acceptability of *Tengma* processed from maize harvested at different stages

4. Conclusion

Maize is cultivated in all part of the country either as sole or intercrop. To add value as well as extend shelf life, maize is processed into various products, including *Tengma*, the roasted and pounded maize, which is gaining popularity in the country. *Tengma* processing method is still very traditional because of which its quality is often compromised. This study assessed the quality of *Tengma*, particularly colour, taste and texture by processing *Tengma* using maize grains harvested at different stages namely milk, dough, dent and physiologically matured. In addition, maize grains harvested from previous season was also processed.

So *Tengma* processed using grains harvested at dough and milk stages may have good taste and better acceptability in terms of taste, but the final weight (or recovery) would be low, which could impact returns. Therefore, to make up for low return prices for *Tengma* processed using grains harvested at dough and milk stage will have to be increased, particularly because these *Tengma* taste good.

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Inventory of Important Insect Pests, Diseases and the Beneficial Insects in Fruits and Vegetables in West Central Bhutan

Ugyen Dorji[x], Kinley Dorji[x] and Azusa Fujiie[y]

ABSTRACT

Bhutan, an agrarian country gearing towards food self-sufficiency and food security is challenged by pests and diseases in crops. New pests emerge on old crops or known pests become adapted to new crops as a result of climatic change. These shifting in crop insectpests, pathogens and their hosts range result in outbreaks and crop losses which results in food insecurity. Pest outbreaks and crop losses can be addressed through integrated insect pests and diseases management specific to local environment. However, the comprehensive knowledge on occurrence and habitat range of beneficial and harmful organisms required for development of integrated pests and disease management is limited in the country. Therefore, we conducted the survey to monitor and inventorize the occurrence of major insect pests and diseases in fruit trees and vegetables, and their natural enemies in the west central Bhutan.

The Chinese citrus fruit fly (Bactrocera minax Enderlein*), trunk borer (Anoplophora versteegi* Rits.) *and citrus leaf miner (Phyllocnistis citrella* Stainton) *were the three important insect pests of citrus while Huanglongbing was the important disease of citrus crops in the west central Bhutan. Mango fruits in the region were infested by two insect pests i.e., trunk borer (Batocera rufomaculata* Dejan*) and Oriental fruit fly (Bactrocera dorsalis* Hendel). *Grapes were infested by the leaf beetle (Scelodonta strigicollis). Insect pests of chilli were solanum fruit fly (Bactrocera latifrons* Hendel) *and armyworm (Mythimnia separate* Walker.*) while phytopthora blight (Phytopthora capcisi) was its major disease. The bean pod borer (Muruca vitrata* Fabricius*) and armyworm were the two major insect pests of beans in west central Bhutan. The important natural enemies observed during the study period were ladybird beetles (Coccinellidae), assassin bugs (Family: Reduviidae), parasitic wasps (Family: Braconidae), dragon flies (Order: Odonate), spiders (Order: Araneae), mantis (Family: Mantidae), hoverflies (Family: Syrphidae) and big-eyed bug (Family: Geocoridae). The first hand information from this study confirmed the occurrence of the insect pests and diseases of major fruit and vegetable crops grown in the west central Bhutan.*

Keywords: *Chinese citrus fruit fly, Oriental fruit fly, Trunk borers, Bean pod borer, Natural enemy*

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1. Introduction

Bhutan comprises an agrarian society, with majority relying on agriculture for their livelihood. The major horticultural crops in the west central Bhutan are mandarin (*Citrus reticulate* Blanco), areca nut (*Areca catechu* L.), banana (*Musa spp.*), chilli (*Capsicum annum* L.), kidney bean (*Phaseolus vulgaris* L.), and radish (*Raphanus sativus* L.) (DoA, 2016). With climatic change, new pests emerge on old crops or known pests get adapted to new crops (Oso & Borisade, 2017). These shift in crop insect pests, pathogens and their hosts range result in outbreaks and crop losses (Coakley, Scherm & Chakraborty, 1999) leading to food insecurity. Farmers in the west central Bhutan reported insect pests and diseases as a major challenge in horticultural crop cultivation (Dorji, Sasaki, Jigme & Chofil, 2017). Similarly, pests and diseases were reported as a major challenge for food security and food self-sufficiency in Bhutan (CIAT; World Bank, 2017). In the past, Bhutan relied on plant protection chemicals to contain or control the major pests and diseases in crops.

However, starting 1992, managing these pests and diseases in the country geared towards safe guarding the environment with centralization of pesticide distribution (FAO, 2012), banning import of toxic chemicals (NPPC, 2015a), and declaring agriculture to become organic (Tashi, 2015). The Bhutanese government promotes organic agriculture in the country by discouraging the use of chemical inputs and intends to phase out the use of harmful plant protection chemicals (RGoB, 2010). However, in the absence of effective control options for pests and diseases in organic agriculture, the government's policy to achieve food self-sufficiency through higher crop yields remains a primary challenge.

The "Pesticide Act of Bhutan" enacted in 2000 has emphasized Integrated Pest Management (IPM). IPM is the preferred method for pest management that foster conservation and protection of ecosystems given the current scenario where agriculture development still focus on crop promotion, increasing production and productivity using chemicals (NPPC, 2015a). IPM, an ecosystem based strategy that focuses on the long term prevention of pests or their damage (FAO, 2017) uses a combination of techniques: cultural, biological, physical and chemical. For successful IPM, one basis is the pest identification; distinguish between pest, beneficial organisms through monitoring the insect pest and natural enemies. It is important to know the occurrence of various insect pests, their natural enemies, diseases and their vectors (Fake, 2011). IPM requires information on potential loss, pathogen biology, ecology, epidemiology, and basic concepts of plant disease management (Razdan & Sabitha, 2009).

The knowledge and understanding of the occurrence of various insect pests and diseases in Bhutan is limited as there is very limited information on level of crop damage or losses caused by insect pest and diseases (NPPC, 2015b). Therefore this survey was conducted to inventorize various insect pests and their natural enemies, diseases and their vectors in fruits orchards and vegetable farms in west central Bhutan. The first hand information from the study should serve as a basis for determination of effective IPM control measures under the current climatic conditions in the region and as a step towards organic agriculture and in achieving food self-sufficiency and food security.

2. Materials and Methods

2.1. Survey site

The study sites were selected in consultation with the district agriculture officers of the region and research officers of the Agricultural Research and Development Center - Bajo (ARDC-Bajo). The study sites include a total of 35 farms and/or orchards in the elevation of 300 masl to 2,150 masl in west central Bhutan. The crops surveyed at different farms and orchards are tabulated against the farm or orchard name (Table 1).

Table 1.Investigation points of the survey

Elevation (m)	Point	Fruit plants	Vegetable
1,220	Bajo, WangduePhodrang	Citrus, Grape, Mango, Papaya, Pear, Persimmon	Beans
1,220	Bajo, WangduePhodrang	Apple, Citrus, Mango	Beans, Chilli, Indian mustard, Radish, Tomato
1,220	Bajo, WangduePhodrang	Apple, Citrus, Grape, Mango, Papaya, Pear	Beans, Chilli, Indian mustard, Radish, Tomato
1,250	Phuntsho, Pelri, Punakha	Mango	-
1,250	Phuntsho, Pelri, Punakha	Avocado	Indian mustard, Radish
1,920	Noobgang, Talo, Punakha	Peach, Persimmon, Plum	-
1,840	Laptsakha, Talo, Punakha	Pear	Beans, Broccoli, Chilli
1,520	Wolakha, Talo, Punakha	Citrus, Mango, Peach, Pear	Chilli
1,340	Damchoe, Kabjisa, Punakha	Citrus	Beans
1,370	Rimchu, Goenshari, Punakha	Citrus	-
1,740	Jazikha, Shangana, Punakha	Persimmon	-
1,610	Silna, Toepisa, Punakha	Avocado, Citrus, Peach, Pear, Persimmon	-
1,280	Chimipang, Baap, Punakha	Avocado, Citrus	-
610	Gewog, Daga, WangduePhodrang	Avocado, Citrus, Guava	-
640	Dohamchey, Athang, WangduePhodrang	-	Chilli, Eggplant, Indian mustard
1,510	Talidoho, Nahi, WangdiPhodrang	Citrus	Broccoli, Indian mustard

1,630	Doltochen, Nahi, WangdiPhodrang	Walnut	-
2,070	Tshokothanglca, Nahi, WangdiPhodrang	Apple, Citrus, Pear, Persimmon,	Beans, Cabbage, Chilli, Eggplant, Indian mustard, Radish

Table 1 contd…

Elevation (m)	Point	Fruit tree	Vegetable
2,190	Bjaktey, Kazhi, WangdiPhodrang	-	Cabbage, Chilli, Eggplant, Beans, Radish
2,150	Bjaktey, Kazhi, WangdiPhodrang	-	Beans, Chilli, Eggplant, Radish
1,840	Jagatokha, Kazhi, WangdiPhodrang	Citrus, Persimmon	-
1,190	Pangthang, Beteni, Tsirang	Citrus	Beans, millet
1,180	Damphu, Kilkorthang, Tsirang	Citrus	Radish
1,180	Damphu, Kilkorthang, Tsirang	Citrus	-
440	Southern area, Tsirang	Citrus	-
300	Southern area, Tsirang	Citrus	-
850	Noorbuthang, Phuentenchhu, Tsirang	Citrus	Beans, Chilli
690	SergithangMaeg, Tsirangtoe, Tsirang	-	Beans, Chilli
580	GaiceyKharka, Tsirangtoe, Tsirang	-	Beans
760	Trashiding, Dagana	-	Beans, Chilli, Indian mustard
980	Khagochen, KalidzingKha, Dagana	Citrus	-
870	Baleygang, Gozhi, Dagana	Guava	Beans, Indian mustard, Millet
880	Baleygang, Gozhi, Dagana	-	Broccoli
924	Baleygang, Gozhi, Dagana	Citrus	-
1,030	Lower Gozhi, Gozhi, Dagana	-	Beans, Chilli
1,280	Middle Gozhi, Gozhi, Dagana	Citrus	Chilli
836	Lower Tsendagang, Tsendagang, Dagana	-	Beans, Chilli
845	Lower Gesarling, Gesarling, Dagana	Citrus	Chilli

2.2. Sampling method

The simple random sampling method (Mead, Curnow & Hasated, 2002) was used to select the trees in an orchard for survey followed by stratified random sampling to inspect the leaves for presence of insect pests or disease symptoms. In an orchard, 5 random trees were selected from which 5 old and 5 new leaves were randomly inspected for the presence of insect pests, beneficial insects, and the disease symptoms. For vegetable farms, simple random sampling method was used where 25 plants were inspected for insect pests, beneficial insects and disease symptoms.

2.3. Data collection

In the months of September to October 2017, a total of 35 farms and/or orchards were surveyed (Table 1). Insect pests, disease incidence and their severity were visually rated in the farms. The disease incidence in an orchard or farm was calculated as below:

$$Disease\ incidence = \frac{\text{Number of disease plantsdenc}}{\text{Total number of plants}}$$

The percent infestation of different pests was calculated based on number of insects per leaf or fruit or stem or the whole plant. To analyze and rank the incidence of a pest across the region, frequency distribution was used. The insect pests and diseases for important fruit and vegetable crops were categorized in ranks according to the frequency of occurrence across the region.

To investigate the fruit fly damage in tomato, citrus, eggplant, pear, chilli, pear, guava, and persimmon; 20 numbers of fruits were sliced and investigated for fruit fly larvae. The fruits were collected from the local markets in Punakha and Wangdue districts and from the survey farms and orchards of the region and stored at room temperature for a week or two before investigating the presence of larvae.

To investigate the vectors of citrus greening disease, careful visual inspection on the leaves of the citrus plants and alternate hosts was carried out. Insect pests and natural enemies were also investigated by a sweeping method. Using the sweep net, 20 sweepings were carried out in an investigation on weeds that were growing near the different crops. Yellow sticky traps were also used for trapping insect pests. The yellow sticky traps were appropriately changed and data on different insects trapped were recorded for analysis.

2.4. Tools and equipment

A sweep net (Shiga Konchu Fukyusya, 35 cm in diameter and stick with 120 cm in length) was used to catch the insects. Insect samples were collected in vials (17*27*55mm) and preserved in 70% ethanol for identification with the help of microscope (MIZAR-TEC.SW-20). Yellow sticky traps (Arysta Life Science, 257*100mm) were used to trap insect pests and natural enemies.

3. Results and discussion

3.1. Major insect pests and diseases of important fruit crops of the west central Bhutan

Mandarin, mango, and pear are the top three fruit crops in west central Bhutan. Grapes, persimmon, subtropical apple, papaya, kiwi, walnut, pecan, peach and apricot are other fruit crops cultivated in the region (DoA, 2016). Pests and diseases of these crops are ranked in descending order (Figure 1).These ranks are based on the frequencies observed under each crop.

3.2. Incidence/occurrence of citrus insect pests and diseases in the region

Citrus leaf miner, HLB and trunk borer were the top three frequently observed pests or disease in the region during this study. Of the 20 citrus orchards surveyed, we found leaf miner in 11 orchards; HLB symptoms in nine orchards and trunk borer in six orchards.

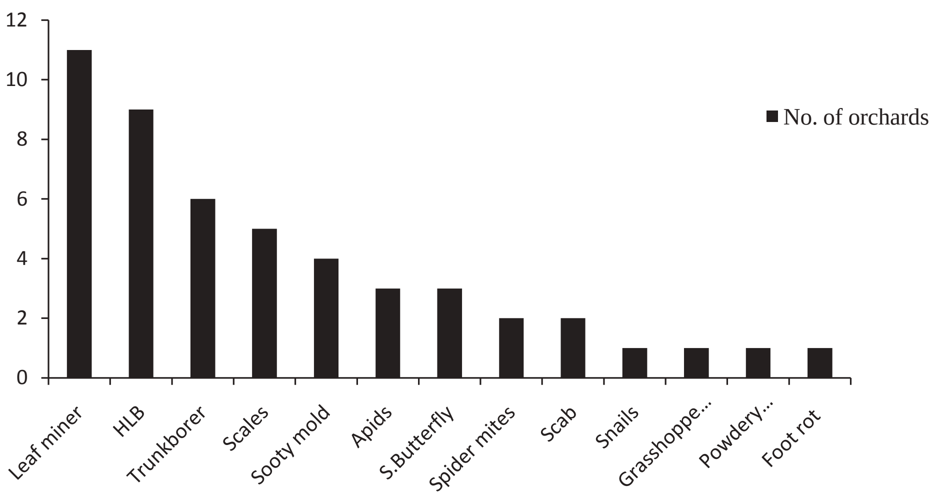

Figure 1.Incidence of citrus insect pests and diseases in the west central Bhutan (September-October, 2017)

3.2.1. Citrus leaf miner

We observed citrus leaf miner in 11 of the 16 citrus orchards surveyed. Citrus leaf miner percent infestation on citrus leaves ranged from 6% to 60% in Punakha, 12% to 59% in Wangdue and 0.4% to 56% in Tsirang. NPPC reported that citrus leaf miner is present in all mandarin orchards in the country (NPPC, 2017a). Citrus leaf miner is potentially a serious pest of citrus and its related Rutaceae family species, and some related ornamental plants (Beattie, 1989; Clausen, 1993). Citrus leaf miner favours spread of citrus canker (Ando et al., 1985; Hill, 1918) because of leaf damage from the miner. The information on spread of diseases by this pest in Bhutan is limited.

3.2.2. *Citrus greening disease and its vector*

Of the 22 citrus orchards surveyed, nine showed HLB symptoms. Ranging from 90% to 100% of the mandarin trees investigated in orchards in Dagana district was showing symptoms of HLB while it ranged from 22% to 100% in Tsirang district and 40% to 67% at Wangdue. NPPC reports that HLB disease is present in almost all citrus growing districts of Bhutan (NPPC, 2017b). *Diaphorina citri i*s the vector of the citrus greening disease. We did not find *D. citri* on citrus trees and collateral hosts. However, *Diaphorina communis*, a relative psyllid was observed on curry leaf trees at Dagana and Wangdue. Donovan et al. (2012) also reported *Diaphorina communis* inhabitation on curry leaf.

3.2.3. *Citrus trunk borer*

The percent infestation of citrus trunk borer ranged from 3.1% to 24% per trees in our study. It ranged from 8.3% to 24% per tree in Tsirang district and 3.1% to 16% in Dagana district. Citrus trunk borer is present in all the citrus growing regions and mainly problematic in poorly managed or neglected orchards (NPPC, 2017c). The orchard sanitation was poor across the region.

3.2.4. *Fruit fly*

Mandarin (*Citrus reticulate* Blanco) is the most important citrus crop in Bhutan. No fruits were available during the survey period. In Tsirang, 100% of the lime fruits investigated was infested by Chinese citrus fruit fly (*Bactrocera minax)*. NPPC reports Chinese citrus fruit fly as the most serious insect pest of citrus causing losses up to 70% through late fruit drop (NPPC , 2017d). Chinese citrus fly causes more than 50% fruit drop in mandarin (Dorji, et al., 2006). However, during this study, fruit dropcaused by fruit fly in mandarin was not studied.

3.2.5. *Other pests and diseases of citrus*

Other pests observed during the study were scales (*Aonidillae auranntii*), swallowtail butterflies (*Papilio aegeus*), cutworm (*Spodoptera litura)*, whiteflies (*Dialeurodes citri* Ashmead)), black citrus aphid (*Toxoptera aurantii***)**, and snails. The percent infestation of these pests ranged from 0.02% to 6% on few separate incidences. Sooty mold was observed in most of the citrus orchards. The most severe sooty mold with 100% infestation on leaves was observed in Damphu, Tsirang. The sooty mold infestations on leaves in other orchard trees ranged from 14% to 30%. One orchard in Tsirang was infested with 36% powdery mildew, while 20% fruits investigated in Toepisa, Punakha had scab disease**.**

3.3. Incidence of mango insect pests and diseases in west central Bhutan

The incidence of mango insect pests and diseases is shown in Figure 2. Trunk borer was observed in two orchards out of four orchards surveyed. In Punakha, an unidentified weevils and black spot disease were observed in two different orchards, respectively.

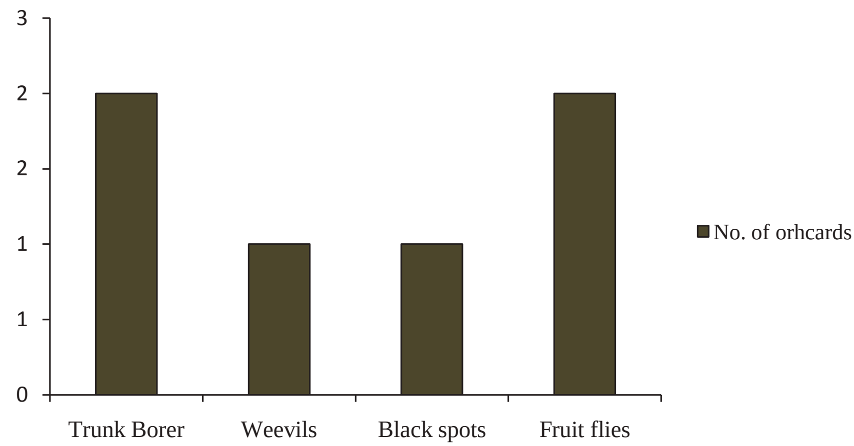

Figure 2.Incidence of mango insect pests and diseases inthewest central Bhutan (September-October, 2017)

3.3.1. Mango trunk borer

Of the four orchards surveyed, trunk borer (*Batocera rufomaculata* DeGeer) infestation was observed only at ARDC-Bajo.About 4% of the mango trees were observed infested by the pest.

3.3.2. Fruit fly (Bactrocera dorsalis)

Although no fruits were available during the study period, there were adult flies trapped on the yellow sticky traps. An average of 4 flies was trapped in a week during the survey period. Two studies reported that fruit fly (*Bactrocera dorsalis*) densities are very high in Bhutan (Ghalley, Lham & Wangdi, 2014; Moriya, Phuntsho, Gyeltshen & Penjor, 2014).

3.3.3. Black spots

The mango trees at Phuntsho Pelri, Punakha was infested by black spots. The disease incidence on leaves was about 68%.

3.4. Pear pests and diseases in west central Bhutan

No major pest was detected during the study period. Minor pests such as green apple aphid (*Aphis pomi* DeGeer), red spider mite (*Tetranychus urticae*) and oriental moth (*Grapholita molesta*) were observed. The highest aphid density or percent infestation was 5.4% per leaf at Talo, Punakha. The percent infestation for rest of the pests was less than one.

3.5. Grape pests in west central Bhutan

Grapes are relatively a new crop cultivated in Bhutan and information on its pests and diseases is limited. At ARDC-Bajo, the leaf beetle (*Scelodonta strigicollis*) had infected about 50% of the leaves. There is no previous report on this pest in Bhutan. The adults of the *Scelodonta strigicollis* feed on the foliage and sprouting buds while the larvae feed on roots of the vine (Jeyaseelan & Mikunthan, 2004). The damage on foliage initially began on the small netted veins and then to veins and midrib. The adult feeds first on the leaf veins from the lower side and later may feed on the other parts of the leaves. The females lay eggs in the soil or underneath the split bark. ARDC-Bajo with the current Japan International Cooperation Agency (JICA) funded Integrated Horticulture Promotion Project (IHPP) distribute many grape seedlings as out-reach programs in the region. Chances of eggs dispersal/transportation of the pest (eggs) in the split barks of grape seedlings is high, although the seedlings distributed are pruned before distribution. With the percent hatchability of 95.0±2.4 (Jeyaseelan & Mikunthan, 2004), *S. strigicollis* pose a threat to the relatively new crop in the country if not controlled at the earliest.

3.6. Important vegetable insect pests and diseases in the west central Bhutan

Chilli, beans and radish are the top three vegetable crops grown in west central Bhutan. For the study, 14 chilli farms, 17 bean farms and seven radish farms were surveyed. Other vegetable crops grown in the region are broccoli, cabbage, mustard green, eggplant, tomato, etc.

3.7. Incidence of chilli insect pests and diseases in west central Bhutan

The incidence of chilli insect pests and diseases is shown in Figure 3.The most frequently observed disease of chilli was blight with seven farms out of 14 farms surveyed. Although aphid infestation was observed only in one farm, unknown disease symptoms were observed in four farms of the 14 farms.

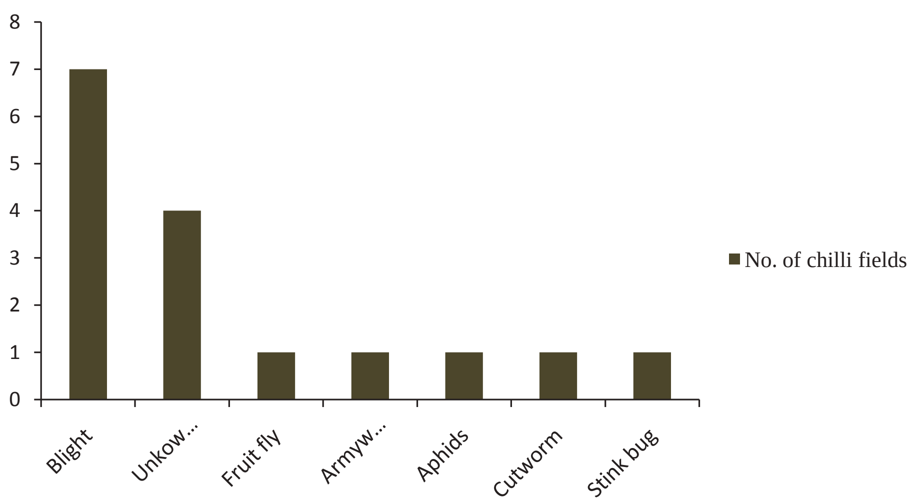

Figure 3.Incidence of chilli insect pests and diseases inthewest central Bhutan (September-October, 2017)

3.7.1. Chilli blight, damping off and foot rot symptoms

Chilli plants in the region were showing damping off and foot rot symptoms. The disease incidence ranged from 1% to 96% in the west central Bhutan. At Kazhi, the disease incidence on the small Indian chilli cultivar was 90% while the local chilli cultivar had about 24% infestation. NPPC reports that chilli blight is the most serious disease of chilli in Bhutan (NPPC, 2017e).

3.7.2. *Solanum f ruit fly* **(***Bactrocera latifrons* Hendel)

The fruit fly percent infestation was highest inWangdue district with 20% (field condition) followed by Punakha (9%). We found solanum fruit fly (*Bactrocera latifrons*) infesting tomato at ARDC Bajo in September, 2017. Moriya et al. (2014) reported that chilli is infected by solanum fruit fly in Bhutan.

3.7.3. Other pests of chilli

The chilli crop at Sergithang, Tsirang was affected by *Mythimna spp.*The percent infestation was 13%.Other pests such as aphids, termites, ants and cutworms (*Agrotis segetum*) were observed during the study. The density of aphids, cutworms and termite in Dagana was 1.5% per leaf, 2% per plant and 1% per plant, respectively.

3.8. Incidence of bean insect pests and diseases in the west central Bhutan

The incidence of bean insect pests and diseases is shown in Figure 4. A total of 16 bean farms were surveyed for the study. Armyworm (*Mythimna spp.*) was observed only at Sergithang under Tsirang district. Bean pod borer (*Maruca vitrata*) was observed in two farms under Dagana district. There were several pests and diseases observed but only in one or two spots of the region with percent infestation and disease incidence less than 1%.

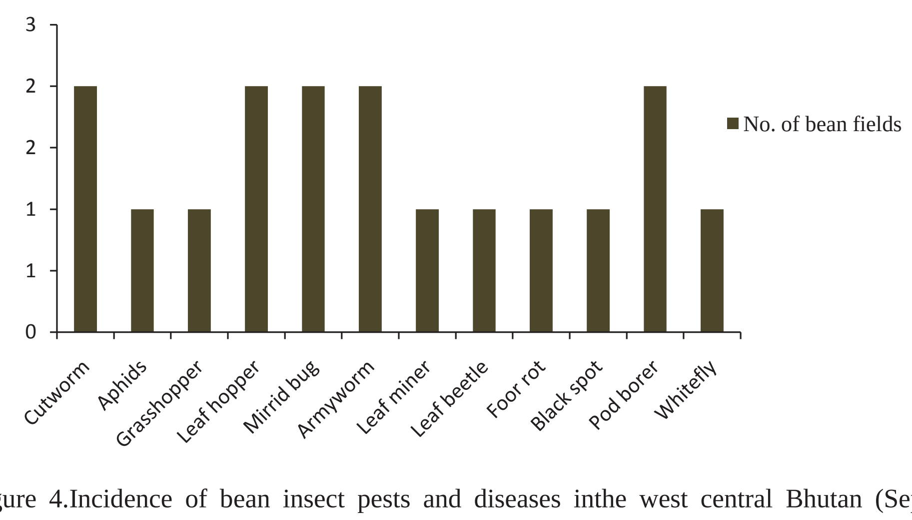

Figure 4.Incidence of bean insect pests and diseases inthe west central Bhutan (September-October, 2017)

3.8.1. Armyworms (Mythimna spp.)

The beans at Sergithang were affected (10% of the pods) by armyworms. The beans at other places were not affected by armyworm.

3.8.2. Bean pod borer (Maruca vitrata)

The bean pod borer was observed only in two villages under Dagana district. The percent infestation of the pest was 8% of the pods surveyed at Tashidhing and 6.7% at lower Gozhi.

3.8.3. Unidentified diseases in beans

Beans at Bjaktey, Wangdue were affected by a kind of black spot disease while the beans at Tashidhing, Dagana were showing foot rot symptoms. In Tashidhing, 2% of the beans were showing foot rot symptoms. The causal organism of the foot rot and black spots were unknown.

3.9. Incidence of insect pests and diseases in radish in the region

The incidence of radish insect pests and diseases is shown in Figure 5. The most commonly observed insect pests were aphids and armyworms. However, the percent infestation of these insect pests was minimal.

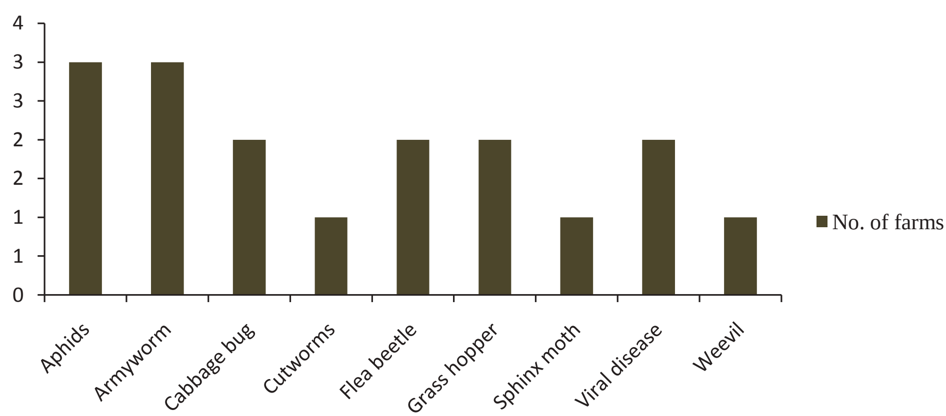

Figure 5.Incidence of radish insect pests and diseases in the west central Bhutan (September-October, 2017)

At ARDC-Bajo, 3% of the transplanted radish seedlings were affected by cutworms (*Agrotis segetum*). At Kazhi, the radish crop was affected by flea beetles of about 0.52% per leaf while 16% of plants were affected by an unknown viral disease. The percent infestation of other pests was less than 1% in all the farms surveyed.

3.10. Other vegetables pests

Other minor vegetable crops such as tomato and eggplants were also infested by several pests. In Tsirang and Dagana, 100% of mung beans were infested by armyworm (*Mythimna spp.*)

3.11. Major native natural enemies collected from fruit trees, vegetables and weeds in the cultivated fields

Natural enemies against their preys observed during the survey are shown below (Table 2). In this study, ladybird beetles belonging to subfamily Epilachninae and family Coccinellidae (i.e. *Henosepilachna indica* and *Henosepilachna vigintioctopunctata)* were observed along with the beneficial lady beetles. Dorji, Loday and Vorst (2017) reports that as high as 33 species of lady beetles were observed in the western region with 21 species in Wangdue dzongkhag alone. Ladybird beetles are well known for their predation for soft bodied arthropod pests (especially aphids and scales which are agriculture pests) and considered beneficial. However, these Epilachnae beetles are rather leaf feeding herbivores than predators. *Henosepilachna vigintioctopunctata,* commonly known as 'Hadda beetle' cause damage to solanaceous crops.

Table 2.Natural enemies in the west central Bhutan (September-October, 2017)

Major target insect pests	Natural enemy
Aphids	Lady beetles, Parasitic wasps, Predacious godflies, Spiders, Hoeverflies
Armyworms	Lady beetles, Brachonidae wasps
Spider mites	Predacious mites
Cabbage bugs and weevils	Big eyed bugs
Tussock moths and Grasshoppers	Spiders
Longhorn beetles	Spiders
Bagworm moths	Spiders
Stink bugs	Assassin bugs

In our study, parasitoid wasps belonging to subfamily Braconinae, family Braconidae, and several others wasps were found in the west central Bhutan. The parasitoid wasps in Braconinae attack nearly the entire range of lepidopteran insects, and are used for biological control of agriculture lepidopteron pests (Whitefield, 2002). Om et al. (2017) reported that a kind of a wasp called *Tamarixia drukyulensis*, the parasitoid nymphs of *Diaphorina communis* was observed in Wangdue dzongkhag. In Reunion Island, it is reported that parasites such as *Tamarixia dryi* and *T. radiata* Waterson significantly reduced the psyllid populations and lessened the damage of HLB (Gottwald, da Graca & Bassanezi, 2007). However, the wasp *Tamarixia drukyulensis* was not observed in this study and the information on the effects of this wasp on psyllid populations is limited.

Other beneficial insects recorded were assassin bugs, dragon flies, earwigs, mantis, hoverflies and big eyed bugs. These bugs show predatory behavior as reported by several authors worldwide. The assassin bugs and mantises feed on a wide variety of arthropods (Hurd, 1999; Ambrose, 2003). The dragonflies' diet mainly constitutes small dipterans (Pritchard, 1964). Hoverflies and earwigs feed on aphids while the big eyed bugs feed on more than 60 preys in three classes of arthropods (Sadeghi & Gilbert, 2000). These beneficial organisms are probably of great importance in preventing pests as bio-control agents in agriculture.

4. Conclusion

The survey result produced an extensive inventory of the occurrences of insect pests, their natural enemies, diseases, and their vectors in fruits orchards and vegetables farms in the west central region of Bhutan. The major pests observed in fruit crops such as citrus, mango and pear were fruit flies and trunk borers. HLB and its vector, citrus psyllid (*Diaphorina citri*) were found to be the major pests on citrus. Armyworm was the major insect pest in vegetable crops such as chilli, beans and radish. *Phytopthora* blight was reported as the major disease on chilli. Important beneficial insects observed during the survey were ladybird beetles, assassin bugs, parasitic wasps, dragon flies, spiders, earwigs, mantis, hoverflies and big-eyed bugs.

Acknowledgement

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Post- harvest Damage and Loss of Apples in Bhutan (Thimphu and Paro)

Dorji Rinchen[z], Sonam Tobgay[z], Dechen Tshering[z], Sonam Dorji[z]

ABSTRACT

Post-harvest loss of apple (Malus domestica) poses major challenge to agricultural marketing in Bhutan. Though various post-harvest interventions were made, it is still a challenge to tackle post-harvest losses due to unavailability of data on losses of apple in the different stages of supply chain. This study was conducted in the two major apple growing districts of Paro and Thimphu to determine the post-harvest loss in apple during the harvest and post-harvest operations and also to identify different factors contributing to these post-harvest damages and losses. A major portion of production is lost during harvesting and post-harvest handling stages, which includes transportation to depot, sorting, grading, packaging, storage and further transportation to the foreign markets in India and Bangladesh. The total post-harvest loss of apples in Bhutan is estimated at 73.10% of the total production. Of the total damages and losses, 12.78% of the fruits are completely damaged and are unacceptable for both consumption and marketing, while 60.32% of the fruits are partially damaged and can still be consumed and marketed. Only 26.90% of the total production reaches the market without any post-harvest damages or defects. Natural causes such as diseases, insect and bird damages and physiological disorders also significantly contributed to the total loss of apple (30.16%) during and after harvesting.

Keywords: *Apple, Post-harvest damages and losses*

1. Introduction

Food crops are living entity and they begin to deteriorate the instant they are separated from their parent plant. Post-harvest management techniques can largely determine the final quality of the produce and help in minimizing post-harvest losses (Wills & Golding, 2016). The post-harvest system should encompass the delivery of a crop from the time and place of harvest to the time and place of consumption, with minimum loss, maximum efficiency and maximum return for all involved in the supply chain (Masood, 2011). Post-harvest losses of horticultural crops pose a major challenge to agricultural marketing. It has been estimated that over 40% of fresh fruits and vegetables in Bhutan are lost due to poor post-harvest handling and management (Tobgay, 2005).

Most of the post-harvest losses of apple in Bhutan can be related to poor harvesting and handling operations. According to Thompson (2008), mechanical injuries from improper harvesting

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techniques and use of inappropriate tools result in loss of moisture from the fruits, causes physical damage and also increase the susceptibility of the fruits to decay causing microorganisms. In post-harvest handling, the initial process of storage right after harvest is critical. Every crop has its own storage temperature and optimum humidity requirement, and it is recommended to store produces of different nature separately to avoid unwanted chemical reactions (Kader, 2002). In general, the basic post-harvest handling principles are same for all crops; careful handling after harvest to avoid physical damages, culling to remove the damaged parts or damaged crops, pre-cooling and maintaining the desired cooling conditions (Kader, 2002; Thompson, 2008).

There is scope to significantly reduce the post-harvest damage and losses of fruits right from harvesting to end users or final market. Interventions can be made in the post-harvest operation activities as the fruits transit along the supply/value chain (Kader, 2002). Understanding the maturity indices of apples, selection of appropriate harvesting schedule and use of appropriate tools and techniques significantly help in minimizing post-harvest damages and losses (Kader, 2002).

The main objective of this study is to assess the post-harvest damage and losses of apple from harvesting till transportation (before being exported to international markets). This study is aimed to provide information on the portion of the produce damaged and lost during different stages of post-harvest handling and also determine the major causes and factors that contribute to post-harvest loss of apples in Thimphu and Paro.

Farmers, local and regional traders are involved in post-harvest practices such as harvesting, sorting, grading, packaging, transporting and storing. There are significant losses in terms of quality and quantity in each stage of post-harvest practice. For instance, in Afghanistan, the total post harvest loss of apples was estimated at 31% at the farmers' level while the loss was estimated at 40.7% when the fruit reaches the local traders (Masood, 2011). The level of damage and losses (quality and quantity/physical) is different among chain actors at different stages of the chain (Ilyas et al., 2007). Proper interventions at the different post harvest operations can help reduce post harvest losses which will ultimately contribute to increasing supply of apples without corresponding increase in the acreages under cultivation (Kader, 2002).

2. Materials and Methods

2.1 Study area

The survey data was collected from the apple orchards of eleven major apple-growing villages; Gebjana, Jephu, Drugyel, Pangbisa, Woochu, Shari, Dawakha, Hongtsho, Yusipang, Gemina, Tsaphuunder Paroand Thimphu districts of Bhutan through random sampling technique**.** The altitude of these selected orchards range from 2200 – 2400 m thus making these places highly suitable for growing apple (Meisami-asl, Rafiee, Keyhani, & Tabatabaeefar, 2009).

2.2 Data collection

The study was done quantitatively through field survey and data collections. Field staffs of NPHC collected the data from the various apple orchards, collection depots and marketing places in the eleven apple growing villages during the apple-harvesting season.

2.3 Primary data

The primary data was collected through field survey. Survey of 18 apple orchard owners and exporters/suppliers were done through pre-structured sampling sheet. Two random samples from each selected site (orchards/exporters and suppliers) were taken with three replicates for each sample. The data was collected from these samples to assess the damages and losses incurred in the four different operations: (i) harvesting, (ii) field to depot transportation, (iii) sorting, grading and packaging and (iv) depot to market transportation. Further damages and losses caused by natural factors such as physiological disorder, bird and insect damages and disease were also assessed.

2.4 Data analysis

Data was analyzed using Web Agri Stat Package (WASP 2.0), and the results are presented in charts, graphs/figures and tables.

3. Results and Discussion

3.1 Post-harvest handling damage and loss of apples

Harvesting damages account to 18.5% of the total damages whereas 30.54% of the apples get damaged while transporting from the field to the depot (Figure 1).Bruises and punctures can be noticed on most of these fruits damaged during harvesting and transportation. The damage during harvesting can be attributed mainly to the use of inappropriate harvesting tools and poor harvesting technique (Kader, 2002). A significant part of the loss during transportation can be attributed to poor transportation modes and the poor road conditions (Kader, 2002).

The post-harvest quality and composition of fruits can be significantly affected by the time of harvesting and the methods of harvesting. Mechanical injuries from improper harvesting techniques and inappropriate tools will accelerate moisture loss from the fruits (Thompson, 2008). Selection of an appropriate time of harvesting is also very important. Harvesting during extreme heat or during rainfall can adversely affect the post-harvest quality of fruits (Thompson, 2008). Harvesting early may result in shriveling of fruits and formation of bitter pit during storage and may also result in lack of flavor on ripening, while late harvesting can result in flesh breakdown and increased susceptibility to rot (Wills & Golding, 2016). Proper harvesting management includes selection of a favorable harvesting time in relation to climatic condition and harvest maturity, use of appropriate harvesting tools and techniques and sound implementation of effective quality control (Kader, 2002; Thompson, 2008). Post-harvest management techniques can largely determine the final quality of the produce and can significantly help in minimizing post-harvest losses (Wills & Golding, 2016), thus it is important to understand the proper harvesting methods and use of appropriate harvesting tools and equipment.

Damage during sorting, grading and packaging accounted to 37.15%, and final depot to market transportation contributed to 42.94% (Figure 1). Rough handling during sorting, grading and packaging, use of inappropriate containers and improper storage are the main factors contributing to these damages.

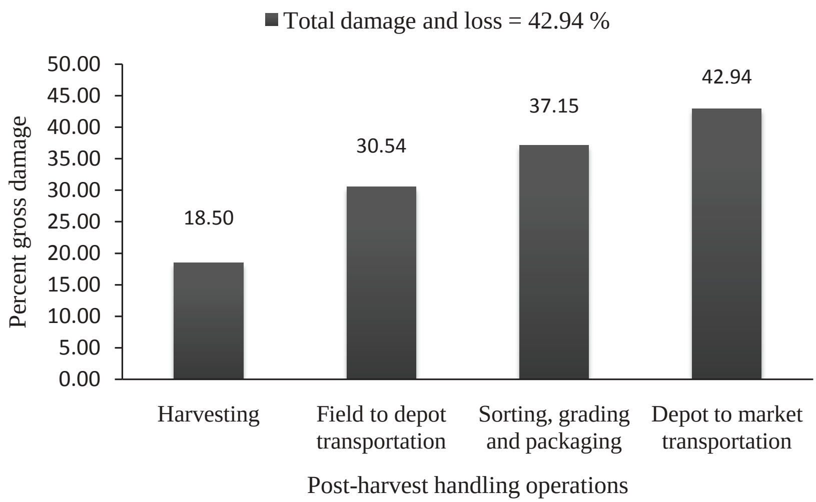

Figure1. Post-harvest damage of apple during harvesting, handling and transportation

It is clear that the damages in the fruits keep on increasing as the fruits travel through different post-harvest operations. With this data, it can be concluded that the estimated gross post-harvest damage in apple during the different post-harvest handling operations is 42.94%.

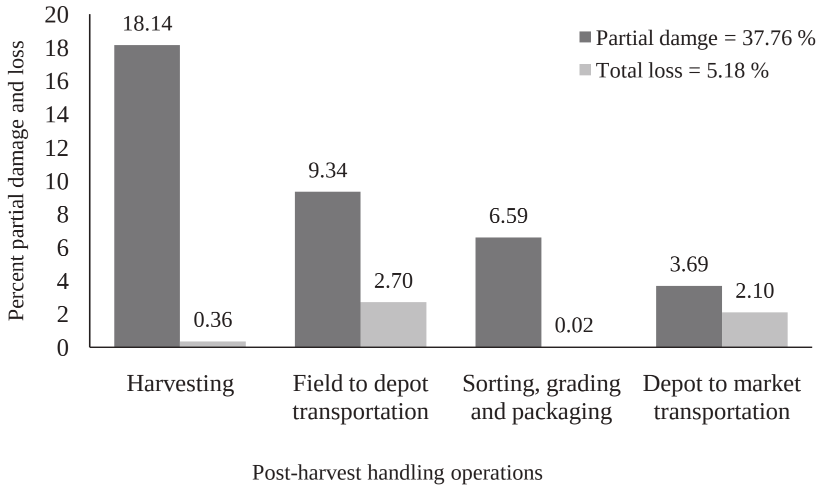

Figure 2.Partial damage and total loss during harvesting, handling and transportation

The gross damage of the apples after the post-harvest handling operations accounted to 42.94% (Figure 1), which includes partial damage (37.76%) and total loss (5.18%) (Figure 2).

3.2 Post-harvest damage and loss of apples due to natural causes

Post-harvest damage of apples due to diseases, physiological disorders and insect and bird damages accounted to 30.16% of the total post-harvest damage. Post-harvest loss of apple due to birds and insects stands at the highest (39.79% of the total damages due to natural causes) followed by physiological disorders (37.33%), and diseased at 22.88% (Figure 3).

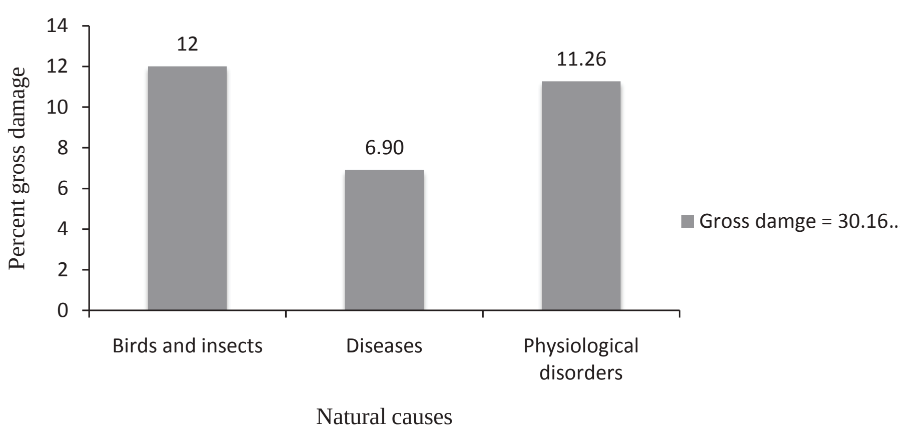

Figure 3.Post-harvest damage of apples due to natural causes

The gross damage of the apples due to natural causes stands at 30.16% (Figure 3), which includes partial damage (22.56%) and total loss (7.60%) as reflected in Figure 4. Only 26.90% of the apples reach the market without any defects.

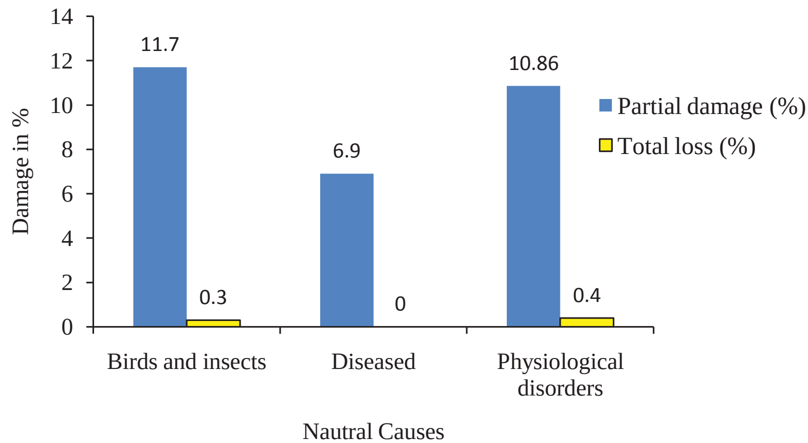

Figure 4.Partial damage and total loss due to natural causes

3.3 Economic impact of the post harvest damage and loss of apples

Revenue of Nu 69.17 Million was generated in 2016-2017 from the export of 2659.21 Mt of apples (DRC, 2017; MoAF, 2017). Taking this into account, if the partially damaged fruits (4849.12 Mt) are also considered exported a total loss of Nu. 26.72 Million (Table 2) was incurred from the fruits (1027.38 Mt) which could not reach the market due to post harvest losses.

Table 1.Total postharvest loss of apples

Sl No.	Factors	Loss (%)	Partial damaged (%)	Total damaged (%)
1	Handling Operations	5.18	37.76	42.94
2	Natural causes	7.60	22.56	30.16
	Total	12.78	60.32	73.10

Table 2.Estimate of economic loss due to post-harvest losses of apples (Bhutan, 2016-17)

Sl. No	Apples produced (Mt)	Postharvestpartial damage (Mt)	Economic losses (Million Nu.)	Postharvest losses (Mt)	Economic losses (Million Nu.)
1	8,039.00	4,849.12 (60.32%)	NA	1,027.38 (12.78%)	26.72

Note: Cost estimation was done on the basis of Nu. 26.01 per kg of apple exported**.**

5. Conclusion

The total post-harvest damage of apple in the two districts of Thimphu and Paro (Table 1) stands at 73.10% of the total production. While 42.94% of the post-harvest damage is incurred during harvest and post-harvest handling operations, 30.16% of the fruits are damaged due to natural causes like diseases, physiological disorders and damages caused by birds and insects. Of the total losses and damages, 12.78% of the fruits are completely damaged and are unacceptable for both consumption and marketing, while 60.32% of the fruits which are partially damaged can still be consumed and marketed. Only about 26.90% of the total production reaches the market without any post-harvest damages or defects.

Wastages of food through post-harvest losses not just translate into human hunger but also result in lesser revenue generation for the growers (FAO, 2013). A major portion of the produce is lost due to poor harvest handling-management of apples during harvestand after-harvest handling. Rough handling by the operators involved throughout the supply chain contributes to almost half of the post-harvest damages and loss in apples. All the handlers along the supply chain need to be trained on proper methods of harvesting and handling practices through awareness and capacity building programs.Post-harvest losses due to natural causes and physiological disorders canalso be minimized through introduction of superior varieties, proper pest and disease management of fruit crops, including that of birds attack, and through good orchard management practices. Pre-harvest interventions such as selection of improved varieties (disease and pest resistant), pest and disease treatments and Integrated Pest Management strategies could significantly help in reducing these post-harvest damage and losses in apple.

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Guidelines for Bhutanese Journal of Agriculture, Department of Agriculture; MoAF

Content

The **Bhutanese Journal of** Agriculture focuses on original and innovative scientific research relevant to sustainable development of agriculture sector in Bhutan.

Target audience

Papers should address a scientific community interested in agriculture, and cross-cutting themes including; markets, biodiversity, irrigation/water, soils, farming systems and, climate change aspects of sustainable agriculture development in Bhutan.

Types of contribution

Research articles: (5000 – 7000 words) the articles should not have been previously published elsewhere.

Papers should

- Present original and innovative research insights;
- Be well researched and documented
- Clearly describe the research methodology including design and statistical analysis used where appropriate;
- Results should be presented based on sound facts, scientific procedures and well founded arguments;
- Be embedded in the relevant local, national or international debate;
- Refer to the most recent academic literature on the issues discussed;
- Be presented in a clearly structured and comprehensible manner; in the interest of disciplinary and interdisciplinary communication.

Short communication (2000-4000 words): A concise and complete with description of an investigation. The bulk of the text should be organized in a continuous form with separate sections such as Introduction, Materials and Method, Results and Discussion. It should, however, include a short Abstract and a list of keywords at the beginning of the communication, and Acknowledgements (if any) and References at the end. These components are to be prepared in the same format as used for full-length research papers.

Preparing your submission

Manuscripts submitted to the Bhutanese Journal of Agriculture should strictly conform to the following instructions and technical guidelines:

Total length of the article:

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The first page of each manuscript starts with the title of the paper which should be typed in bold-faced print using both upper and lower case letters and set in the centre of the page. The title should be as concise and catchy to reflect what the paper is all about? Abbreviations are not permitted in the title. The title should be in Times New Roman with **14** point bold.

Author(s) and agency

The names of the author (s) should be written in full. Indications of titles, professorial ranks or other professional titles should not be used. The address of the agency to which the author belongs to shall be written as footnote. Please provide the email address of the first corresponding author only in italic, **8** Times New Roman.

Abstract and key words:

The abstract consists of **150 – 300** words in one paragraph. The abstract should summarize pertinent results in a brief but understandable form. The abstract should start with a clear statement of rationale, brief objectives of the experiment/research/study and methods, results and must conclude with one or two sentences that highlight important conclusions. References are never cited in the abstract. Abstract should be indented by **0.2** inch on left and right and centre justified with **12** point italic, Times New Roman. Below the abstract, list three to five key words that best describe the nature of the research.

The term "**Keywords**" is typed in bold-faced print followed by a semicolon. The first letter of each key word is capitalized and key words are separated by semicolon. Keywords should include the main attributes of your papers; should be between 3-5 keywords; font **12** point italic, Times New Roman.

Introduction

The introduction starts on a new page following the abstract. The introduction briefly justifies the research and specifies the hypotheses to be tested. Discussion of relevant literature should be cited to support/justify your research in the introduction. Introduction should be divided in to concise paragraph, each paragraph dealing with a topic of your research theme. Mention of objectives of the study or research will be not done under separate heading. If at all necessary, it can be included in the introduction. Objectives should be clear, concise and realistic.

Materials and Method

Materials and Method should be clearly described in a step-by-step guide for others to understand and follow.

General:

Study area, location and their description must be given with illustration if possible. Authors may supply latitude and longitude coordinates for the study area/location referred to. For any equations used in the papers, standard equation format should be followed. Equations must be numbered and placed directly in the text with serial number. Figures, tables, and boxes must be referred to in the text, in numerical order.

Statistics: The data should be analysed using standard statistical model. The use of incorrect or inadequate statistical models to analyze and interpret data is not acceptable. The statistical model, classes, blocks, and experimental unit must be described. Statistical test (S) used should be clearly stated. Both confirmatory inferential statistics such as LSD, *r*-value, 95% CI, etc should be at P-value of 95% confidence level and descriptive statistics such as standard error, standard deviation, CV%, etc should be given where appropriate.

Results and Discussion

Results should be presented in tabular or graphical form with description of key results in the text. The text should explain or elaborate on the tabular/graphical data, but numbers should not be repeated extensively within the text. Sufficient data with index of variation should be presented to allow the reader to interpret the results of the experiment.

Discussion should interpret the results clearly and concisely and should integrate similar literature results with the research findings to provide the reader with a broad base on which to accept or reject the hypotheses tested.

Conclusion

This section should consist of **300-500** words. Conclusion section should highlight key findings and their implications to relevant users of the information. It should explain in lay terms, without abbreviations, acronyms, or citations, what the findings of research/study are. Do not repeat statements made in the result and discussion sections.

Acknowledgement

Acknowledgement should be made to key persons other than authors and co-authors including the anonymous reviewers and funding agencies. However, it should not be lengthy.

References

Tables and Boxes

Tables are used to present numerical data in a self-explanatory manner. They should be intelligible without consulting the text and should not duplicate data already given in the text or in illustrations. Any abbreviation used in a table must be defined in that table. All tables should be cited in the text. Arabic numerals are used to number tables. The table number (i.e. Table 1) is typed followed by a period. The title of the table should be given just above the table with only the first letter capitalized; font 12 Times New Roman. Do not use a period at the end of the title. Column headings should have the first letter of each word capitalized while the names of variables are typed with only the first letter capitalized (i.e. Average growth rate). For numerals less than 1, insert a zero to the left of the decimal point (columns should be set up so that decimal points are aligned). If there are no data for a particular entry, insert a dash. If an explanation is necessary, use an abbreviation in the body of the table (e.g. NA) and explain clearly what the abbreviation means.

- In boxes, include caption in a title bar (topmost line across entire box)
- Your submission may have no more than 5 tables or boxes in total
- Very large tables and long lists should be avoided.
- Tables should be inserted as enhanced metafiles
- No border lines, only boundary lines will be used, 10 point, Times New Roman and no colors
- Text in tables must always be horizontal; no bold.

Example

Table 1.Mean growth rate of chilli plants

Treatments	Plant height (cm/plant)	No. of leaves per plant	Stem girth (mm/pant)	Yield (kg/plant)
Poultry manure	145	78	10.5	144
NPK	132	76	9.2	128
Cow manure	128	64	8.9	121
None	93	21	9.8	108

Figures

- Possible file formats: .JPG, PDF, .XLS, .GRF.
- The figures should be inserted as enhanced metafiles
- Figures should be black and white print
- The submission should not have more than 5 figures (including photos, diagrams, maps)
- Put captions below the figures; 12 point Times New Roman
- No color, but choose different shade(s) that is appropriate for black/white printing
- Photos, illustrations, flow charts can be used if necessary

Example (Figure 1)

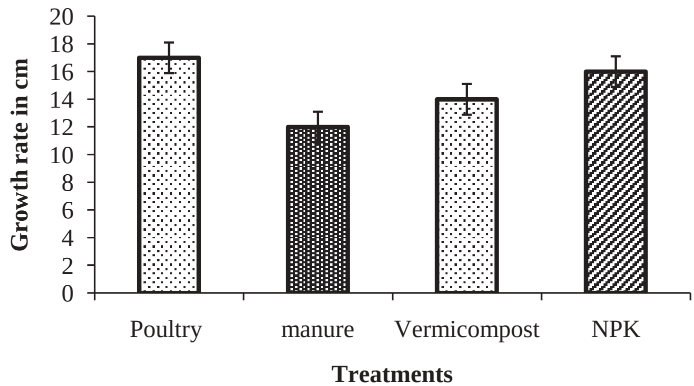

Figure1. Effect of treatments on growth rate of broccoli plant

Note: For editorial convenience, authors are also required to submit charts (figures) and tables as separate files with appropriate corresponding file references as they appear in their manuscripts.

Referencing

All literature cited in any part of your paper should be listed at the end of the body text file in a section entitled "Reference," without numbering. The references should be arranged in alphabetically by author and then chronologically, giving the complete unabbreviated source citation.

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For convenience and ease of use for our contributing colleagues in the research centres and our field personnel, BJA has adopted the APA (6^{th}) style of referencing.

In-text references

Use author–year style in chronological, then alphabetical, order. Use "et al" with three or more authors. Use colon and number to indicate page reference.

Examples:

Karma (1993, 1995a, 1995b)

- Yuden and Dorji (2004)
- (Sonam et al., 1975)
- (Stremlow 1998; Antrop 1999; Tress and Tress 2001; Backhaus et al., 2007a, 2007b)
- (see figures 4 and 5 in Keen et al., 1971)
- Wangchuk et al. (2014)

Campbell (1993: 55);

- **Three or four authors**: Where there are three or four authors, all surnames should be used the first time the in-text reference appears in the document. For all subsequent citations, include only the surname of the first author followed by **"et al."**

First mention:

- Smith, Grierson, Malthus, and Nicholson (2015) found . . .
- According to evidence . . . (Smith, Grierson, Malthus, & Nicholson, 2015).

Subsequent mention:

- Smith et al. (2015) suggest . . .
- The study concluded . . . (Smith **et al.**, 2015).

- **Five, six or seven authors:** Where there are five, six or seven authors, use the first surname only followed by et al.
  - According to Abercrombe et al. (2008) . . .
  - It was shown that . . . (Abercrombe et al., 2008).
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- For a group, or work authored by organizations or agencies, proper abbreviation or acronym should be used in in-text citation. However, the names of the group or organizations should appear in full with their abbreviations in parenthesis in the reference (end-text) section.

In-text:

- (NBC, 2014); NSB (2014)
- MoAF, 2017); MoAF (2017)

End-text:

NBC. (2014). *Biodiversity Action Plan* Thimphu: National Biodiversity Centre (NBC), Ministry of Agriculture & Forests.

MoAF. (2017). *Agriculture Statistics 2017*. Thimphu: Ministry of Agriculture and Forests (MoAF), Royal Government of Bhutan.

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For books published within the United States, use the name of the city with the two official US postal service abbreviations.

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Sample References

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