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EFFECT OF DROUGHT STRESS ON DIFFERENT PHYSIOLOGICAL TRAITS IN BREAD WHEAT

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ABSTRACT

The present study was designed to evaluate the changes in different physiological traits such as proline content, cell membrane stability, relative water content and chlorophyll content under drought stress in sixteen wheat genotypes. Wheat genotypes (99FJ-03, Marvi-2000, WC-13, WC-24, WC-19, Faisalabad-85, Kaghan, Bahawalpur, Zarlashta, Punjab-96, Shafaq, Maxi-pak, WC-20, Chenab-70, AUR-0809, Chakwal) were sown during rabi season of 2013-14 following randomized complete block design with three replications. Drought stress was induced by withholding water for 30 days at heading and anthesis stage. Genotypes were significant for different physiological traits like relative water content, proline content, cell membrane stability and chlorophyll content under drought stress which indicated that some genotypes were more tolerant against drought stress than others. Among tested wheat genotypes, Maxi-Pak was found to be potential variety for relative water content, cell membrane stability, chlorophyll content and yield. Hence, it can be used in future wheat breeding programme for developing drought tolerant genotypes.

Keywords: Wheat, cell membrane stability, chlorophyll content, drought, proline content, relative water content.

INTRODUCTION

Wheat, the world’s third important cereal, is cultivated in Pakistan both in irrigated and rainfed areas. One of the major constraints of wheat production in rainfed area is drought that needs to be addressed. Although many genotypes were released that were tolerant to drought stress by improving different physiological traits to boost wheat productivity. Improvement in wheat yield came through dwarfing genes which was first used by Japanese wheat breeders.

* Corresponding author email: mum96@hotmail.com

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Water stress is a worldwide issue which predicts the sustainable agricultural production (Jaleel et al., 2007). Drought leads to stomata closure and reduction of water content, turgor loss. Sometime it leads to death of plant by disturbing metabolism (Jaleel et al., 2008). Drought affects the physiological traits of wheat crop. Efforts have been made to improve the yield of wheat crop under rainfed condition by improving the traits which are affected by drought. Water stress not only affects the morphological traits but also affects the physiological traits. Severity of stress depends on cultivar, growth stage, duration and intensity of stress. Different stages respond differently to water stress; some stages can cope with the stress by maintaining its water potential or turgor pressure or efficient utilization of water. Water stress can reduce the biomass, tillering ability, grain size etc. Photosynthesis as well as various factors that contribute to photosynthesis like chlorophyll content, relative water content and various pigments is affected by drought. Drought causes leaf senescence in various wheat genotypes, thus causing the chlorophyll degradation. Proline is an amino acid, which accumulates during various stresses as an osmo-regulatory protein. Genotypes that accumulate more proline shows tolerance against stress by maintaining the plant water potential. However, literature regarding physiological traits in wheat genotypes affected by drought stress is very limited. Hence, the present experiment was conducted to evaluate the changes in physiological traits in wheat genotypes under drought stress condition.

MATERIALS AND METHODS
A field experiment was undertaken during *rabi* season of 2013-14 at PMAS Arid Agriculture University Rawalpindi, Pakistan on loamy soil having moderate fertility. A set of sixteen genotypes of bread wheat (99FJ-03, Marvi-2000, WC-13, WC-24, WC-19, Faisalabad-85, Kaghan, Bahawalpur, Zarlashta, Punjab-96, Shafaq, Maxi-Pak, WC-20, Chenab-70, AUR-0809, Chakwal) were planted in the rain-out-shelter following Randomized Complete Block Design with three replications. Seeds were sown on November 03, 2013 with the help of dibbler having two rows of each genotype. Fertilizer was applied at the rate of 42-34-25 NPK kg ha\(^{-1}\) at the time of sowing. Drought stress was induced by withholding water for 30 days at the heading and anthesis stages. At the end of stress periods, irrigation was given up to field capacity. The control plants were irrigated during the stress period, and all plants were left to grow until grain maturation under normal irrigation. Data for physiological traits like cell membrane stability, relative water content, chlorophyll content and proline content were determined following the published protocols of other researchers. Chlorophyll content was determined as chlorophyll index using Chlorophyll Meter (SPAD-502). Proline content was determined by the method of Bates et al. (1973). Analysis of variance was worked out following Steel et al. (1997) to determine differences among different physiological traits under drought stress in wheat. Correlation coefficients were carried out following the method used by Kwon and Torrie (1964).
RESULTS AND DISCUSSION

Relative water content
Highly significant variation was present among the genotypes sown in the tunnel for this trait (Table 1). Genotype WC-20 had maximum value of 81.92 % which was statistically identical with Marvi-2000, Zarlashta and Maxi-Pak while the minimum value (64.88 %) for this trait was recorded in genotype WC-24 (Table 3).

Difference between GV value (18.924) and PV value (29.623) indicated that environmental influence was present. Little difference between GCoV and PCoV values showed that the phenotype was representative of genotypic factor and influence of environmental factor was less on the trait. Broad sense heritability was high for this trait showed that greater proportion of variability was due to genetic factor (Table 2). These findings are in accordance to those of Ahmed et al. (2014).

Proline content
Proline content was highly significant for the genotypes and the range for this trait was 0.02 mg g\(^{-1}\) - 0.15 mg g\(^{-1}\) (Table 1). Maxi-Pak had minimum proline content while Chenab-70 and Punjab-96 had maximum proline content which was similar to 99FJ-03, Kaghan, WC-20, AUR-0809 and Chakwal-50 (Table 3). Genotypes having maximum proline content would be able to survive under drought stress conditions as it protects the membranes from damage under stress and can be used for the development of varieties for rainfed areas.

Table 1. Analysis of variance for physiological traits of different wheat genotypes under rainfed conditions

<table>
<thead>
<tr>
<th></th>
<th>RWC</th>
<th>PC</th>
<th>CMS</th>
<th>CC</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS( VAR / TR )</td>
<td>67.470</td>
<td>0.006</td>
<td>429.752</td>
<td>3.339</td>
<td>455315</td>
</tr>
<tr>
<td>MS (REPLICATES)</td>
<td>47.016</td>
<td>0.000</td>
<td>0.641</td>
<td>2.914</td>
<td>670800</td>
</tr>
<tr>
<td>MS (ERROR)</td>
<td>10.699</td>
<td>0.001</td>
<td>11.014</td>
<td>1.486</td>
<td>411858</td>
</tr>
<tr>
<td>F.RATIO (V)</td>
<td>6.306 **</td>
<td>10.363 **</td>
<td>39.017 **</td>
<td>2.246 *</td>
<td>1.106 NS</td>
</tr>
<tr>
<td>F.RATIO (R)</td>
<td>4.395 *</td>
<td>0.680NS</td>
<td>0.058NS</td>
<td>1.960NS</td>
<td>1.629 NS</td>
</tr>
</tbody>
</table>

** =Highly significant, * =Significant, NS =Non-significant, RWC =Relative water content, PC =Proline content, CMS =Cell membrane stability, CC =Chlorophyll content, Yield =Yield per hectare
Table 2. Components of variation for physiological traits of different wheat genotypes under rainfed conditions

<table>
<thead>
<tr>
<th></th>
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<th>PC</th>
<th>CMS</th>
<th>CC</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>GV</td>
<td>18.924</td>
<td>0.002</td>
<td>139.579</td>
<td>0.617</td>
<td>14485.5</td>
</tr>
<tr>
<td>PV</td>
<td>29.623</td>
<td>0.002</td>
<td>150.594</td>
<td>2.104</td>
<td>426343.8</td>
</tr>
<tr>
<td>GCoV</td>
<td>6.012</td>
<td>46.778</td>
<td>17.088</td>
<td>1.944</td>
<td>6.273</td>
</tr>
<tr>
<td>PCoV</td>
<td>7.522</td>
<td>53.753</td>
<td>17.75</td>
<td>3.589</td>
<td>34.03</td>
</tr>
<tr>
<td>CoH</td>
<td>0.639</td>
<td>0.757</td>
<td>0.927</td>
<td>0.293</td>
<td>0.034</td>
</tr>
</tbody>
</table>

GV = Genotypic variance, PV = Phenotypic variance, GCoV = Genotypic Coefficient of Variation, PCoV = Phenotypic Coefficient of Variation, CoH = Coefficient of Heritability, RWC = Relative Water Content, PC = Proline Content, CMS = Cell Membrane Stability, CC = Chlorophyll Content, Yield = Yield per hectare

Table 3. Mean values of physiological traits for different wheat genotypes under rainfed conditions

<table>
<thead>
<tr>
<th>Genotype</th>
<th>RWC (%)</th>
<th>PC (mg g⁻¹)</th>
<th>CMS</th>
<th>CC</th>
<th>Yield (kg ha⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>99FJ-03</td>
<td>70.76cd</td>
<td>0.12ab</td>
<td>62.42f</td>
<td>38.00e</td>
<td>1720</td>
</tr>
<tr>
<td>Marvi-2000</td>
<td>80.71a</td>
<td>0.04de</td>
<td>73.12df</td>
<td>39.67bcde</td>
<td>1750</td>
</tr>
<tr>
<td>WC-13</td>
<td>67.72de</td>
<td>0.05de</td>
<td>63.51f</td>
<td>40.19abcd</td>
<td>1780</td>
</tr>
<tr>
<td>WC-24</td>
<td>64.88e</td>
<td>0.07d</td>
<td>50.17g</td>
<td>41.11abc</td>
<td>2090</td>
</tr>
<tr>
<td>WC-19</td>
<td>69.52cde</td>
<td>0.07cd</td>
<td>79.62bc</td>
<td>41.29abc</td>
<td>1610</td>
</tr>
<tr>
<td>Faisalabad-85</td>
<td>70.36cd</td>
<td>0.11bc</td>
<td>64.91f</td>
<td>39.91bcde</td>
<td>1770</td>
</tr>
<tr>
<td>Kaghan</td>
<td>70.98cd</td>
<td>0.12ab</td>
<td>72.00e</td>
<td>39.28cde</td>
<td>1450</td>
</tr>
<tr>
<td>Bahawalpur</td>
<td>70.83cd</td>
<td>0.05de</td>
<td>54.38g</td>
<td>40.55abcd</td>
<td>1660</td>
</tr>
<tr>
<td>Zarlashta</td>
<td>77.79ab</td>
<td>0.07cd</td>
<td>55.67g</td>
<td>40.98abcd</td>
<td>1350</td>
</tr>
<tr>
<td>Punjab-96</td>
<td>71.31cd</td>
<td>0.15a</td>
<td>78.57cd</td>
<td>40.22abcd</td>
<td>2140</td>
</tr>
<tr>
<td>Shafaq</td>
<td>69.20 cde</td>
<td>0.03de</td>
<td>83.6abc</td>
<td>41.09abc</td>
<td>1570</td>
</tr>
<tr>
<td>Maxi-pak</td>
<td>77.45ab</td>
<td>0.02e</td>
<td>85.62a</td>
<td>42.14a</td>
<td>2310</td>
</tr>
<tr>
<td>WC-20</td>
<td>81.92a</td>
<td>0.12ab</td>
<td>84.72ab</td>
<td>40.91abcd</td>
<td>2040</td>
</tr>
<tr>
<td>Chenab-70</td>
<td>68.89cde</td>
<td>0.15a</td>
<td>55.56g</td>
<td>41.52ab</td>
<td>2290</td>
</tr>
<tr>
<td>AUR-0809</td>
<td>71.92cd</td>
<td>0.12ab</td>
<td>80.00 bc</td>
<td>40.81abcd</td>
<td>2400</td>
</tr>
<tr>
<td>Chakwal-50</td>
<td>73.49bc</td>
<td>0.13ab</td>
<td>62.32f</td>
<td>39.00de</td>
<td>2770</td>
</tr>
</tbody>
</table>

RWC = Relative water content, PC = Proline content, CMS = Cell membrane stability, CC = Chlorophyll content, Yield = Yield per hectare. Same letter(s) in a column indicate statistically identical at 0.05 level of probability according to DMRT test.
Value for GV was not different from the PV value (Table 2). Little difference between GCoV value (46.778%) and PCoV value (53.753%) showed that environmental effect was less on this trait. High heritability value (75%) predicted that influence of environmental variation was less on this trait (Table 2). These results were similar to the findings of Rad et al. (2013) who reported 97% heritability for proline content. Proline content would be helpful for selecting drought tolerant genotypes.

**Cell membrane stability**

Variation among genotypes was highly significant for this trait under drought stress suggested that selection of drought tolerant genotypes could be done on the basis of this trait (Table 1). Range for this trait was 50.17-85.62. Genotype WC-24 showed minimum value while Maxi-Pak showed maximum value for this trait which was at par with Shafaq and WC-20 (Table 3). Genotypes that would be able to maintain their cell membrane stability under drought stress shows more tolerance against drought.

GV and PV value was 139.579 and 150.594 respectively, which showed that environmental influence was present on this trait under drought condition. GCoV and PCoV values showed little difference between them. Under drought conditions, different genotypes behaved differently. Those genotypes which showed more cell membrane stability could be selected for further breeding programs aimed at improving drought tolerance. High broad sense heritability was observed for this trait, which indicated that selection would be effective (Table 2). Similar results were also discussed by Bayoumi et al. (2008) and Naeem et al. (2015).

**Chlorophyll content**

Significant variation in genotypes for this trait depicted that selection could be done to obtain drought tolerant genotypes (Table 1). Range for this trait was 38.00-42.14. 99FJ-03 and Maxi-Pak were the genotypes with minimum and maximum value for chlorophyll content, respectively (Table 3).

GV and PV value for this trait was 0.617 and 2.104 respectively. GCoV value (1.944 %) and PCoV value (3.589 %) depicted that more variation in this trait was due to environmental factor. Based on this trait selection of genotypes should be done with great care for development of drought tolerant genotypes. Broad sense heritability for this trait was low (29 %) and low heritability for this trait revealed that character has low genetic potential (Table 2). Similar findings were also reported by Keyvan (2010).

**CONCLUSION**

High heritability for relative water content, proline content and cell membrane stability indicated that selection of genotype would be effective for these traits as they are heritable to next generation.
Variation in physiological traits like proline content, relative water content and cell membrane stability in wheat genotypes studied under drought stress revealed that some genotypes could be selected for future breeding programme to develop drought tolerant, high yielding wheat genotypes. Among tested wheat genotypes, Maxi-Pak was found to be potential variety considering relative water content, cell membrane stability, chlorophyll content and yield. Hence, it can be used in future wheat breeding programme for developing drought tolerant varieties.

REFERENCES


FACTORS AFFECTING EXTENT OF RURAL LIVELIHOOD DIVERSIFICATION IN SELECTED AREAS OF BANGLADESH


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ABSTRACT

The study was carried out to investigate the patterns and extent of livelihood diversification in rural Bangladesh. It also identified the major factors affecting extent of livelihood diversification. The study drew a random sample of 500 rural farm households in Bangladesh through a multi-stage sampling technique. The primary data were collected using semi-structured questionnaires, and analyzed using descriptive statistics and statistical techniques. The results showed that remittance contributed the highest to the household income followed by petty business and rice farming. The estimated values of Simpson Index of Diversification (SID) showed that majority of the rural households had “medium” and “high” level diversified livelihood activities. Tobit regression analysis found that gender of the household head, household size and amount of credit had positive and significant effects; and number of migrant household member, dependency ratio, household assets, education of the household head and amount of savings had negative but significant effects on the extent of livelihood diversification. The small and medium landholding households were more likely to diversify their livelihoods compared to the functionally landless and large landholding households. The study recommended that non-farm employment opportunities should be expanded to combat poor households’ vulnerability to shocks and income fluctuations. Functionally landless households should be given more attention to increase and diversify their incomes.

Keywords: Livelihood diversification, simpson index, Tobit regression, rural Bangladesh.

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INTRODUCTION

The economy of Bangladesh is typically agriculture driven. More than 45% of the country’s population live in rural areas (World Bank, 2016). Agriculture has remained the main source of livelihoods of rural people since many years. But, in recent years rural livelihoods are rapidly transforming. The importance of agriculture in rural livelihoods is declining, while the importance of the non-agricultural sources, such as business, services, remittance and non-farm labourer is increasing (Hossain and Bayes, 2010). The contribution of agriculture to rural household income dropped from 60% in 1988 to 45% in 2013. Land owned per household has declined from 0.60 ha in 1988 to only 0.30 ha in 2013 (Hossain and Bayes, 2014). On the other hand, agriculture is a risky investment due to the volatility in price and weather. The impact of “Risk” and “Seasonality” in agriculture triggered the diversification process in rural occupations and income. On the other hand, non-farm occupations reduce the risk by combining activities that have different risk profiles, while they can also ameliorate labor and consumption smoothing problems associated with seasonality (Ellis, 2005).

Rural livelihood diversification can be defined as the process by which rural households construct an increasingly diverse portfolio of activities and assets in order to survive and to improve their standard of living (Ellis, 2000). It also refers to a continuous adaptive process whereby households add new activities, maintain existing ones or drop others, thereby maintaining diverse and changing livelihood portfolios. People diversify their livelihoods by adopting a range of activities and income sources. Thus, income sources may include ‘farm income’, ‘non-farm income’ (non-agricultural income sources, such as non-farm wages and business income), and ‘off-farm income’ (wages of exchange labor on other farms, i.e. within agriculture, including payment in kind) (Ellis, 2000).

Bangladesh is one of the densely populated countries in the world, with 1252 people living per square kilometre (World Bank, 2016). The average arable land per capita declined from 0.13 ha in 1971 to 0.05 ha in 2013 (FAO, 2015). Due to limited land per capita and scarcity of resources, people are now shifting their livelihoods from agriculture to non-agricultural sectors. Large numbers of people are migrating from rural areas to urban areas and also to abroad as overseas foreign workers (OFW). Moreover, climate change has made agriculture more vulnerable and risky. The youth are more interested to non-agricultural jobs as it give higher income compare to the job in agricultural sector. It has been seen that there are significant changes happening in terms of earning income from different sources as well as livelihood patterns of the people living in rural areas of Bangladesh (Hossain and Bayes, 2010). But the process and extent of rural livelihood diversification is not same through all the regions of Bangladesh.

The ramifications of livelihood diversification on rural development are colossal. But, the literatures on rural livelihood diversification in Bangladesh are fragmented
and scanty. Some studies deal with the income variation and determinants of non-farm and off-farm income diversification in Bangladesh (Malek and Usami, 2009; Rahman, 2013). However, no literature is available on the level of livelihood diversification and its determinants. Therefore, it is very essential and useful to measure the extent of livelihood diversification in rural areas of Bangladesh and determine the factors affecting the extent of livelihood diversification. The specific objectives of this study are to (a) find out the extent of livelihood diversification; and (b) determine the factors affecting extent of livelihood diversification. Based on the primary data, it will provide empirical evidences regarding the factors contributing to the transformation of rural livelihoods in Bangladesh. This study will assist the policy makers and donor agencies who frame policies and finance to different projects for the development of rural economy of Bangladesh.

**METHODOLOGY**

**Data source and sampling design**

This study was conducted in 12 villages representing major agro-ecologies and diverse livelihoods of Bangladesh. Eleven districts were selected purposively to represent large geographical area and diverse livelihoods of the country. Those districts are Narsingdi, Madaripur, Mymensingh, Bogra, Comilla, Chandpur, Chuadanga, Jhenaidah, Patuakhali, Kurigram and Thakurgaon. Multi-stage random sampling technique was followed to select sample villages. In 10 districts, one sub-district from each district, one union from each sub-district and one village from each union were selected randomly. In Mymensingh district, which is the 5th largest district in the country (Wikipedia, 2018), two sub-districts, one union from each sub-district, and one village from each union were selected randomly. Thus, 12 villages were randomly selected from 11 districts and four geographical regions (e.g. northern region, middle region, south-eastern region and south-western region) of the country. Finally, 45 rural households were randomly chosen from each selected village making a total sample of 540 households. Only 500 households were included in the analysis as some households’ data were incomplete.

The study used primary data collected through face to face interview using pre-tested semi-structured questionnaires during 2012–2013. The collected information included demography, land ownership, primary and secondary occupations of household members, migrations and remittances, assets ownership, labor force, on-farm activities, off-farm activities, non-farm activities, credit and savings, agricultural prices, income from different sources and living conditions to name major ones.

The most important determinant of livelihood for any society is income (Gebreyesus, 2016). In this study, household income refers to net income generated by deducting total cost from total return. The share of income from different sources was the basis
to assess their livelihood diversification. Extra attention was paid during data
collection and analysis to estimate household’s income accurately because farmers do
not keep record about their crop production related data and often they tend to under-
report their income. Sometimes they do not consider their own production and the in-
kind received as income.

Household income was grouped into nine sources.
1) Rice crop (net income from all rice crops in a year);
2) Non-rice crops (net income from all non-rice crops in a year);
3) Non-crop agriculture (income from livestock, fishery and forestry);
4) Agricultural labourer (labour employed in agricultural sectors);
5) Non-agricultural labourer (included both formal and informal types of
employment);
6) Petty business;
7) Salaried job and services;
8) Remittance income (received from family members presently living outside the
family: both domestic and abroad); and
9) Transfer payment

For analysing purpose sampled households were also classified in four groups based
on their landholding.
(1) Functionally landless (>= 0.2 ha),
(2) Small (0.21-0.80 ha),
(3) Medium (0.81-1.50 ha) and
(4) Large (>1.50 ha).

Analytical tools
Simple descriptive analysis (average, mean, median, percentage, etc.) was carried out
to determine the household income from different sources. Tabular analysis was done
to find the share of various income sources and the extent of livelihood
diversification. Tobit multiplicative heteroscedasticity regression was employed to
determine the factors affecting the extent of livelihood diversification. The Microsoft
Excel and STATA-12 was used to analysis the data.

Part I: Extent of livelihood diversification
The most common measure of livelihood diversification is the vector of income share
associated with different income sources (Khatun et al., 2012; Datta et al., 2011).
Livelihood diversification can be measured using different indicators and indices,
such as Simpson index, Herfindahl index, Ogive index, Entropy index, Modified
Entropy index and Composite Entropy index (Khatun et al., 2012; Datta et al., 2011;
Shaha et al., 2011; Shiyani and Pandya, 1998). Several studies have used the Simpson index to measure livelihood diversification (Shaha et al., 2010; Babatunde et al., 2009; Joshi et al., 2003 and Hill, 1973). This study followed the suite because of its computational simplicity, robustness and wider applicability. The formula for Simpson index (SID) is:

$$SID = 1 - \sum_{i=1}^{n} P_i^2$$

Where, $n$ is the total number of income sources and $P_i$ is the income proportion of $i$-th income source. The value of SID falls between 0 and 1. The index’s value is zero if there is just one source of income. As the number of sources increase, the shares ($P_i$) decline, as does the sum of the squared shares, so that SID approaches to 1. Households with most diversified income sources have the largest SID value, and the least diversified income sources have the smallest SID value. The higher the number of income sources as well as more evenly distributed the income shares, the higher the value of SID. The Simpson index of diversity is affected both by the number of income sources as well as by the distribution of income among different sources. Based on the SID values, the level of livelihood diversification was defined as:

1. No diversification ($SID \leq 0.01$)
2. Low level of diversification ($SID = 0.01 - 0.25$)
3. Medium level of diversification ($SID = 0.26 - 0.50$)
4. High level of diversification ($SID = 0.51 - 0.75$)
5. Very high level of diversification ($SID > 0.75$)

**Part II: Determinants of livelihood diversification**

The value of livelihood diversification index ranges between zero and 1. An Ordinary Least Square (OLS) estimate is not appropriate to find the parameters because OLS cannot censor the variables. Tobit model is more suitable to find the parameter estimates if latent or censored sample presents in the dependent variable (Gujarati, 2003). A sample in which information of the dependent variable is not available for some observation is known as censored or latent sample (Gujarati, 2003).

The following Tobit model was employed:

$$SID* = \beta_0 + \beta_1\text{Gender} + \beta_2\text{Household size} + \beta_3\text{Farm size} + \beta_4\text{Member_org} + \beta_5\text{Migrants} + \beta_6\text{Dev_prog_part} + \beta_7\text{HH_assets} + \beta_8\text{Primary_Occupation} + \beta_9\text{Dependency_ratio} + \beta_{10}\text{Age_HH_head} + \beta_{11}\text{Edu-HH_head} + \beta_{12}\text{Amount_credit} + \beta_{13}\text{Amount_savings} + \beta_{14}\text{Distance_district_town} + \beta_{15}\text{Distance_market} + \beta_{16}\text{Region_D1} + \beta_{17}\text{Region_D2} + \beta_{18}\text{Region_D3} + \beta_{19}\text{Land_D1} + \beta_{20}\text{Land_D2} + \beta_{21}\text{Land_D3} + \mu_i$$

$$SID = \begin{cases} 
SID* & \text{if } SID* > 0 \\
0 & \text{Otherwise}
\end{cases}$$
Where,

SID* = Livelihood diversification index

$\beta_0$ = Intercept

Gender = Gender of Household Head (1 = Man, 0 = Women)

Household size = Household size (Number)

Farm size = Total amount farm size (ha)

Member org = Member of any organization (1 = yes, 0 = No)

Migrants = Number of household members staying outside of house (considering both domestic and international migration) (1 = yes, 0 = No)

Dev prog part = Households’ participation in any govt. development program (1 = yes, 0 = No)

HHassets = Household Assets (Estimated value of all physical assets owned by a household, except the value of cultivable land in BDT)

Primary Occupation = Primary occupation of the household head (1 = farming, 0 = otherwise)

Dependency ratio = Dependency ratio of the household (ratio of economically inactive persons (younger than 18 and older than 59) over the active persons (ages 18-59 years) expressed in percentage

Age_HH_Head = Age of household head (years)

Edu-HH_Head = Education of household head (Year of schooling)

Amount_credit = Amount of credit (Received credit from any sources in a year expressed in BDT)

Amount_savings = Amount of savings (Money saved in any account in a year expressed in BDT)

Distance_district_town = Distance of district town (Distance of household from the district town in Kilometer)

Distance market = Distance of market place (Distance of household from the nearest market place in Kilometer)

Region_D1 = Regional Dummy1 (1 = Northern region, 0 = otherwise)

Region_D2 = Regional Dummy2 (1 = Middle region, 0 = otherwise)

Region_D3 = Regional Dummy3 (1 = South-Eastern region, 0 = otherwise)

Land_D1 = Land class dummy1 (1 = Landless, 0 = otherwise)

Land_D2 = Land class dummy2 (1 = Small land class, 0 = otherwise)

Land_D3 = Land class dummy3 (1 = Medium land less, 0 = otherwise)

$\mu_i$ = Error term, which is normally distributed with mean zero and constant variance
The method of Maximum Likelihood Estimates (MLE) is employed to Tobit model as it gives consistence parameter estimates and makes error term asymptotically normal.

**Test of multicollinearity**

A test for multicollinearity was done to establish and examine the cross-correlations among explanatory variables. The result of correlation analysis showed that the correlation coefficient between farm size and land-man ratio was 0.84 which suggests the present of serious multicollinearity problem. The most practical remedial measure of multicollinearity is to drop one of the correlated variables from the regression model (Gujarati, 2003). Here, land-man ratio was dropped from the model. Another diagnostic test of multicollinearity, variance inflation factor (VIF), was also conducted and found no serious multicollinearity problem anymore.

**Test of heteroscedasticity**

One of the assumptions of linear regression model is that the disturbance terms ($\mu_i$) are homoscedastic, which means that they have same variance. If the variance of $\mu_i$ is not same ($\text{Var} \mu_i \neq \sigma^2$), then the heteroscedasticity problem can be raised. To test for heteroscedasticity, this study applied the Cook-Wiesberg test (1983) and the “hettest” test in STATA and both the tests confirmed the presence of heteroscedasticity problem in the model. This study used Tobit multiplicative heteroscedasticity regression by executing “tobithetm” command in STATA-12 to find the estimates by omitting the effects of heteroscedasticity problem. It deals with multiplicative heteroscedasticity and produces coefficients that can be used to test a formal hypothesis.

**RESULT AND DISCUSSION**

**Sources of household income**

Income share from different sources indicates the level of livelihood diversification. The statistically significant F-value of ANOVA indicates that income share differ significantly across various sources in rural Bangladesh. The average annual total income of the sample household was found to be about USD 2,400 (Table 1). According to the Household Income and Expenditure Survey (HIES) of 2010, Average yearly household income of Bangladesh is USD 1722 (BBS, 2015). The result also shows that remittance contributed highest share (29%) to the household’s total income, followed by Petty business (20%) and rice crop (16%). The other sources also had considerable contribution to the household income.
Table 1. Distribution of household yearly income by sources

<table>
<thead>
<tr>
<th>Source of income</th>
<th>Amount of income (USD/year)</th>
<th>Share of income (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice crop</td>
<td>380</td>
<td>16</td>
</tr>
<tr>
<td>Non-rice crops</td>
<td>202</td>
<td>8</td>
</tr>
<tr>
<td>Non-crop agriculture</td>
<td>221</td>
<td>9</td>
</tr>
<tr>
<td>Agricultural laborer</td>
<td>61</td>
<td>3</td>
</tr>
<tr>
<td>Non-agricultural laborer</td>
<td>171</td>
<td>7</td>
</tr>
<tr>
<td>Petty business</td>
<td>487</td>
<td>20</td>
</tr>
<tr>
<td>Salaried job and services</td>
<td>155</td>
<td>6</td>
</tr>
<tr>
<td>Remittances</td>
<td>699</td>
<td>29</td>
</tr>
<tr>
<td>Transfer payment</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>2,393</td>
<td>100</td>
</tr>
</tbody>
</table>

F-value of ANOVA: 14.27 (P = 0.000)

Source: Survey result, 2012-13, Note: 1 USD = 80 BDT

Household livelihood diversification

Majority of the rural households diversified their livelihoods into several activities and earned significant amount of income from multiple sources. As depict in the Table 2, 94% of the total sampled households pursued some extent of diversification in their livelihoods. Only 6% of households had zero Simpson index, meaning they earned income from just one source for their livelihoods. Of the sampled households, 20% had low, 32% had medium, 38% had high and 4% had very high level of livelihood diversification. The result implies that majority of the households are diversifying their livelihoods at medium and high level.

Table 2. Distribution of sampled household as per the level of livelihood diversification

<table>
<thead>
<tr>
<th>Sid range</th>
<th>Percentage</th>
<th>Level of diversification</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;=0.01</td>
<td>6.0</td>
<td>No</td>
</tr>
<tr>
<td>0.01 – 0.250</td>
<td>19.6</td>
<td>Low</td>
</tr>
<tr>
<td>0.260– 0.500</td>
<td>31.8</td>
<td>Medium</td>
</tr>
<tr>
<td>0.510– 0.750</td>
<td>38.4</td>
<td>High</td>
</tr>
<tr>
<td>&gt;= 0.760</td>
<td>4.2</td>
<td>Very high</td>
</tr>
</tbody>
</table>

Source: Survey result and author’s computation, 2012-13
Factors affecting livelihood diversification

From the value of Simpson index of diversification (SID), it is clear that most of the households in rural areas of Bangladesh participated at different level of livelihood diversification. The Tobit multiplicative heteroscedasticity regression was estimated, based on the cross-sectional data gathered from the sample households, to determine the factors affecting the extent of livelihood diversification among the rural households of Bangladesh. The limited chi-squared distribution was found insignificant at 5% level of significance and thus the null hypothesis of homoscedasticity ($\alpha = 0$) was accepted. This confirms no more heteroscedasticity problem in the model. The estimated results of the Tobit regression and the marginal effects are presented in table 3 and table 4, respectively.

The Tobit regression results (Table 3) indicated that gender of household head, household size, number of migrants, household’s participation in development program, household assets, dependency ratio, education of household head, amount of credit, amount of savings, regional dummy and land classification dummy were the factors which had significant contribution in determining livelihood diversification in rural areas of Bangladesh. The results showed that man headed household had a positive and significant effect on the extent of livelihood diversification among the rural households (at 1% level of significance). This is probably because in the rural areas of Bangladesh a man has more access and social acceptance to have more employment opportunities both inside and outside of the house than a woman. On the other hand, women have less access to work outside of the home and thus less scope to diversify their sources of income. This results are consistent with some other studies on income diversification in Nigeria (Alaba and Kayode; Babatunde and M. Qaium, 2009). The marginal effect of gender explains that if the household is headed by a man then the extent of livelihood diversification is likely to increase by 13.7%.

Household size was also found important and significant in determining the livelihood diversification. It had positive contribution to the extent of livelihood diversification and found highly significant at 1% level of significance. The positive contribution of household size is as expected because having more members in a household means more scope to access different income sources and earn higher amount of income. The result is consistence with the work of Oluwatayo (2009). The marginal effect of household size revealed that the extent of livelihood diversification is likely to be increased by 2% for an additional member in the household (Table 3).

The results also showed that number of migrants and household assets had negative and significant contribution to the level of livelihood diversification. Both the number of migrants and household assets are significant at 1% level of significance. The households which have more number of migrant members both in-country and abroad, are receiving regular and higher amount of income as remittance. Thus, these households do not seek for alternative sources of income which result low level of
livelihoods diversification. The same type of result was found by Malek and K. Usami (2009) in their study on non-farm income diversification in Bangladesh. They found negatively significant coefficient of out-country migration capital on non-farm income diversification.

In rural farm areas of Bangladesh, household assets constituted by mostly farm and agricultural assets which are an investment for increasing farm production. Having higher amount of household assets influence the household to act that they are more secured in context of vulnerability and thus, they hardly go for non-farm income. Therefore, those households that had more assets are less diversified in their income sources and probably more involved in farm activities. The marginal effect of number of migrants explains that for an additional out-migrant member from the household the level of diversification is likely to goes down by 2.7%.

The dummy variable of participating in some development program was found significant at 1% level of significance and had positive impact on the level of livelihood diversification. This implies that, if the households are members or beneficiaries of some developmental programs or projects then they are more likely to be diversified in their livelihoods. Because, being a member or beneficiary of some developmental projects or programs they can have more access to information and scope to intensify their income sources. Moreover these types of developmental programs itself provide some amount of income to its participants. The marginal effect showed that, if a household is a member of any developmental program then the likelihood of livelihood diversification increases by 10.5% compared to the household that is not involve in some development programs.

Dependency ratio is also found significant at 1% level of significance and it had negative sign over the level of livelihood diversification. Which means if dependency ratio increases then the level of livelihood diversification will decreases and vice versa. The marginal effect of dependency ratio explains that for one percent increase in dependency ratio the level of livelihood diversification will goes down by 0.06% and vice versa.

Higher level of education among the household heads had a negative and significant (1% level of significance) effect on livelihood diversification in the study areas of rural Bangladesh. This is probably because school education increases the human capital level and provides necessary skills to an individual to get a decent and more permanent type work which leads him or her to get income from a single source. Moreover, educated persons also hardly look for farm and non-farm employment types of job. Marginal effect of education of household head implies that the level of livelihood diversification is likely to be decreased by 0.7% for every additional year of schooling of household head. This result contradicts with the findings of Asmah (2011), Shaha (2010) and Babatunde (2009).

The results found that amount of credit had positive impact on the level of livelihood diversification. It was significant at 1% level of significance. This implies that
households having more amount of credit are likely to be more diversified in their livelihood activates. The probable reason of this is the credit money helps the household to invest for both farm and non-farm types of activities and boast up their income. This result is supported by some other similar types of study e.g. Asmah (2011), Saha et al. (2010), Oluwatayo (2009) and Babatunde (2009).

On the other hand, amount of savings had a negative and highly significant (at 1% level of significance) effects on livelihood diversification. This implies that households having some saving money are likely to be less diversified in their livelihood activities. It is because; more savings means less investment which eventually results less income as well as less number of income source.

The dummy variable for South-Eastern region was found positive and significant at 5% level of significance which means that households in South-Eastern region had significantly higher level of livelihood diversification compared to Western region. The marginal effect of this dummy variable can be explained as if the households are in the South-Eastern region then the likelihood of livelihood diversification increases by 7.1% compared to the households from Western region. While the dummy variable for Northern and Middle region was insignificant.

Similarly, all three land classification dummy variables were found significant at 5% level of significance. The functionally landless households had negative coefficient while the small and medium land holding households had positive coefficient compared to the large land holding households. This means that the functionally landless households are likely to be less diversified in their livelihoods compared to large land holding households. On the other hand, small and medium land holding households are likely to be more diversified in their livelihoods compared to the large land holding households.

Table 3. Tobit regression results to determine factors affecting the livelihood diversification in rice-based areas of Bangladesh

<table>
<thead>
<tr>
<th>Variables</th>
<th>Coefficients</th>
<th>Std. Err.</th>
<th>Z-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>0.1441</td>
<td>0.0687</td>
<td>2.10</td>
<td>0.036</td>
</tr>
<tr>
<td>Gender of household head</td>
<td>0.1366***</td>
<td>0.0349</td>
<td>3.91</td>
<td>0.000</td>
</tr>
<tr>
<td>Household size</td>
<td>0.0209***</td>
<td>0.0038</td>
<td>5.53</td>
<td>0.000</td>
</tr>
<tr>
<td>Farm size</td>
<td>0.0140</td>
<td>0.0114</td>
<td>1.23</td>
<td>0.219</td>
</tr>
<tr>
<td>Member of any organization</td>
<td>0.0148</td>
<td>0.0189</td>
<td>0.78</td>
<td>0.433</td>
</tr>
<tr>
<td>Migrants</td>
<td>-0.0270***</td>
<td>0.0061</td>
<td>-4.43</td>
<td>0.000</td>
</tr>
<tr>
<td>Development program participation</td>
<td>0.1054***</td>
<td>0.0247</td>
<td>4.27</td>
<td>0.000</td>
</tr>
<tr>
<td>Household assets</td>
<td>-8.73e-08***</td>
<td>1.97e-08</td>
<td>-4.42</td>
<td>0.000</td>
</tr>
<tr>
<td>Primary occupation</td>
<td>-0.0128</td>
<td>0.0199</td>
<td>-0.65</td>
<td>0.518</td>
</tr>
</tbody>
</table>
### Table 4. Marginal effect of the variables on livelihood diversification after running the Tobit regression

<table>
<thead>
<tr>
<th>Variables</th>
<th>dy/dx</th>
<th>Std. err.</th>
<th>z-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender of household head</td>
<td>0.1366</td>
<td>0.0350</td>
<td>3.91</td>
<td>0.000</td>
</tr>
<tr>
<td>Household size</td>
<td>0.0209***</td>
<td>0.0038</td>
<td>5.53</td>
<td>0.000</td>
</tr>
<tr>
<td>Farm size</td>
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<td>-0.0128</td>
<td>0.0199</td>
<td>-0.65</td>
<td>0.518</td>
</tr>
<tr>
<td>Dependency ratio</td>
<td>-0.0007***</td>
<td>0.0002</td>
<td>-3.83</td>
<td>0.000</td>
</tr>
</tbody>
</table>

---

**Source:** Authors’ computation, 2013

*** Significant at 1% level; ** Significant at 5% level; *Significant at 10% level
### Variables Affecting Extent of Rural Livelihood

<table>
<thead>
<tr>
<th>Variables</th>
<th>dy/dx</th>
<th>Std. err.</th>
<th>z- value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of household head</td>
<td>0.00002</td>
<td>0.0008</td>
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Source: Authors’ computation, 2013

*** Significant at 1% level; ** Significant at 5% level; *Significant at 10% level

### CONCLUSION

Majority of the rural households in Bangladesh have diversified their livelihoods from agriculture to different activities. Most of them have diversified their livelihoods at medium and high level. Several factors either positively or negatively impacted extent of livelihood diversification. Gender of the household head, household size, households’ participation in development program and amount of credit have positive and significant effect on the extent of livelihood diversification. On the other hand, number of migrants, household assets, education of household head, dependency ratio and amount of savings have significant but negative effects on livelihood diversification. It was also found that south-eastern region was significantly more diversified in their livelihood activities as compared to western region. The significant and negative coefficient of dummy variable for functionally landless households indicated that they pursued lower level of livelihood diversification compared to the large households. While, the significant and positive coefficient of dummy variable for small and medium land holding households indicated that they pursued higher level of livelihood diversification compared to large land holding households. The declining farm size limits the household ability to earn adequate livelihoods from agriculture. The diversification of agriculture-based rural livelihoods is necessary to improve rural livelihoods. The government should craft and implement policies and programs to promote diversification of
livelihoods through increasing and creating the opportunities of more income generating activities especially for the poor households to combat the risk of income fluctuation. The policies and programs should focus on factors that have large impact on livelihood diversification.

REFERENCE


Developing Country Studies, ISSN 2224-607X (Paper), ISSN 2225-0565 (Online), 6(4), 10-18.


Gebreyesus, B. (2016). The most important determinant of livelihood for any society is income.


EFFECT OF SYNTHETIC OR HERBAL PRESERVATIVES ON THE QUALITY OF BEEF MEATBALLS AT DIFFERENT SHELF LIFE PERIODS

Department of Animal Science, Bangladesh Agricultural University, Mymensingh, Bangladesh

ABSTRACT
The experiment was conducted to compare the effect of Moringa oleifera leaf extract with synthetic antioxidant on beef meatball. Five types of beef meatballs were formulated for this purpose. Meatballs were made with control (0%), 0.1% Beta Hydroxy Anisole (BHA), 0.1, 0.2 and 0.3% Moringa oleifera leaf extract, respectively. Quality and safety evaluation of meatballs were determined by sensory, physiochemical, biochemical and microbiological tests. After preparation meatballs were preserved at -20°C. The analyses were conducted at 0, 15, 30 and 60 days of interval. An ANOVA of a 5x4 factorial experiment in completely randomized design having three replications per treatment was used for the analyses of data. Considering CP, tenderness, juiciness, overall acceptability, cooking loss, FFA, POV and TBARS value it can be concluded that Moringa oleifera leaf extract up to a level of 0.3% may replace BHA for meatball preservation without deteriorating its quality. In case of sensory evaluation 0.2% Moringa leaf extract shows better results. But on the basis of nutrient quality, physicochemical properties, biochemical analysis and microbial analysis 0.3% Moringa leaf extract group is more satisfactory than other treatment groups.

Keywords: Antioxidant, BHA, beef meatball, Moringa oleifera leaf, shelf life.

INTRODUCTION
Lipid per-oxidation causes meat spoilage. It occurs during processing and storage when meatballs are exposed to oxygen, heat, and light (Fasseas et al., 2007). Antioxidants have an ability to prevent or reduce the oxidative damage of a tissue indirectly by enhancing natural defense of cell and/or directly by scavenging free radical species (Verma et al., 2009). Over the years, synthetic antioxidants such as, beta hydroxyl anisole, butyrate hydroxyl toluene and tertiary butyl hydroquinone

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have been widely used to preserve meat and meat products (Fasseas et al., 2007). The use of these antioxidants is questionable since they have been discovered to have toxic, mutagenic and/or carcinogenic effects on human and animals (Hayes et al., 2010). There has been a growing interest in the use of natural antioxidants as alternatives to synthetic antioxidants. In addition, consumers’ interests to natural antioxidants have been growing, considering them safer than the synthetics (Jung et al., 2010). It has also been reported that natural antioxidants, especially of plant source, have greater application potential for consumer’s acceptability, palatability, stability and supporting longer shelf-life of meat products (Jung et al., 2010). *Moringa oleifera* is the most widely cultivated species of the Moringaceae family in Bangladesh. As a nutritious tree, it has various function including pharmacological and antioxidant properties (Verma et al., 2009). Phenolics and flavonoids are the authentic antioxidants found in moringa leaf that have been reported to be safe and bioactive (Sreelatha and Padma, 2009). No investigation, so far been done, on the effect of different levels of Moringa leaf extracts on beef meatball, in terms of their quality at different shelf-life. The present study was, thus, undertaken to investigate the effect of using Moringa (*Moringa oleifera*) leaf extract on the quality of beef meatballs at different storage periods compared to using Beta Hydroxylanisole (BHA).

**MATERIALS AND METHODS**

The study was conducted during the period of January, 2014 to December, 2014 in the Department of Animal science, Bangladesh Agricultural University, Mymensingh. The beef sample was collected from the local market of Mymensingh. Meatballs were prepared using fresh beef, garlic pest, onion pest, ginger pest, meat spices, garam masala (spices), egg, biscuit crumbs, soybean oil, ice flakes, refined vegetable oil, refined wheat flower, *Moringa oleifera* leaf extract, BHA, salt and sauces. There were five treatment groups i.e. control (To), meatball with 0.1% BHA (T1), 0.1, 0.2 or 0.3% *Moringa oleifera* leaf extract as T2, T3, T4 respectively. Sensory qualities (Color, flavor, tenderness, juiciness and overall acceptability) were evaluated by a trained 6-member panel. Samples were evaluated after cooking. When internal temperature of meat reached at 71\(^0\)C then cooking was finished and it was checked by a food grade thermometer. After that meat sample was used for sensory evaluation using a 5-point scoring method that ranks the panelist’s sense of qualities. Sensory scores were 5 for excellent, 4 for very good, 3 for good, 2 for fair and 1 for poor. All samples were served in Petri dishes. Sensory evaluation was accomplished at 0 day and repeated at 15, 30 and 60 day. The Dry Matter, Ether Extract, Crude Protein and Ash of meatballs were determined by the method described by AOAC, (1995). The pH of raw and cooked meatball was determined using a pH meter. The cooking loss of meatballs was also determined by a weighing balance and a hot water bath. FFA value, POV value and TBARS value were determined by Sharma et al. (2012). TVC, TCC and TYMC were determined according to Ikhlas et al. (2011). All determination was done in triplicate and a mean value was reported.
QUALITY OF BEEF MEATBALLS AT DIFFERENT SHELF LIFE PERIODS 25

Statistical analysis
Data were analyzed using SAS Statistical package by 5x4 factorial experiments in CRD having three replications per treatment. Five factors were five treatment groups like control (To) and 0.1% BHA (T1), 0.1, 0.2 or 0.3% Moringa oleifera leaf extract as T2, T3, T4. Four days of intervals as 0, 15, 30 and 60 days as level. Altogether there were 40 treatment combinations in 5x4 factorial experiment. DMRT test was used to determine the significance of differences among treatment means.

RESULTS AND DISCUSSION
Sensory evaluation
It was found that sensory quality after fortification with Maringa leaf extracts was improved. The range of overall observed color, odor, tenderness, juiciness and overall acceptability score was 3.55 to 4.50, 3.55 to 4.42, 3.73 to 4.75, 3.73 to 4.75 and 3.55 to 4.17, respectively (Table 1). From the data it shows that Maringa leaf extracts level significantly (p<0.05) increase the overall acceptability. The most preferable color observed at 0 day and less preferable color at 60 day. The range of color, odor, tenderness, juiciness and overall acceptability among different days of interval was 3.00 to 4.71, 3.00 to 4.71, 3.60 to 4.79, 3.60 to 4.79 and 2.87 to 4.64 respectively. Among these five treatments most preferable color, flavor, and overall acceptability was observed at T3 (0.2%) and most preferable tenderness and juiciness was found at T4 (0.3%) Moringa leaf extract group, respectively. Present findings is in agreement with Gonzalez et al. (2008) where he stated that dried plum ingredients in raw and precooked pork sausage positively effect the sensory attributes viz. color, texture, odor, and flavor as well as nutritional quality of the product.

Proximate analysis
From table 2 overall DM content at different treatment was 58.13 to 54.11%. The highest and the lowest DM content observed at 0 day and 60 day, respectively. The highest DM content indicates this product is less preferable. DM content increased with increased storage period because moisture loss decreased with storage period. Similar results were reported for Indonesian traditional meatballs with a moisture content ranged from 69.52 to 71.17% (Purnomo and Rahardiyan, 2008). CP content at different treatments was 21.89 to 23.00%. The maximum CP content was observed at 0.3% Moringa leaf extract group. CP content at different days of interval was 23.25 to 22.10%. Gradual loss of DM and CP over the days of intervals was due to loss of moisture during storage. EE content at different treatments was 11.38 to 12.06%. Synthetic antioxidant group contain lower amount of EE than control group. The most preferable EE content was observed at T4 (0.3%) Moringa leaf extract group. The range of EE content at different days of interval was 11.83 to 11.84%. The most preferable EE content was observed at 0 day and less preferable EE content at 60 day (Table 2). Serdaroglu et al. (2005) reported a similar fat content ranged from 7.9 to 8.8% in low-fat traditional Turkey koefte beef meatballs. Overall ash content at different treatments was 3.63 to 3.74%. Synthetic antioxidant group contain lower amount of ash than control group.
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<td>3.74±0.01</td>
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</table>
Physicochemical properties

The range of overall observed raw pH at different treatments was 5.68 to 6.04%. Among five treatments most preferable raw pH was observed at T4 (0.3%) Moringa leaf extract group (Table 3). The highest amount of raw meat pH indicates this product is most preferable for consumers’ health. Raw pH among these treatments was decreased with increased storage period (p<0.01). Raw pH at different days of interval was 6.05 to 5.72%. The most preferable raw pH was observed at 0 day and less preferable was observed at 60 day. Overall cooked pH at different treatments was 5.87 to 6.15. The most preferable cooked pH was observed at T5 (0.3%) Moringa leaf extract group. Irrespective of treatments pH had significantly increased (p<0.01) with the increase of storage period and with the increase of days of intervals pH had decreased significantly (p<0.01) in all treatment groups. Similar results have also been found in the study of antioxidant treatments during storage time using a mixture of BHA and BHT in precooked pork patties (Biswas et al., 2004). The overall cooking loss at different treatments was 24.21 to 23.96%. The highest cooking loss was observed at T1 (0%) Moringa leaf extract group. The overall cooking loss at different days of interval was 26.19 to 21.78%. The lowest cooking loss was observed at 0 day and the highest cooking loss was observed at 60 day of observation (p<0.01). Cooking loss refers to the reduction of weight of meatballs during cooking process (Jama et al., 2008) is the similar trend with this experiment. From the study it reveals that treated Moringa leaf extracts had significantly reduced (p<0.01) cooking loss compared to control group.

Biochemical properties

From table 4 the overall FFA, POV and TBARS value at different treatment was 0.45 to 0.35, 4.54 to 4.17 and 0.58 to 0.46, respectively. The overall FFA, POV and TBARS at different days of interval were 0.34 to 0.44, 3.96 to 4.59 and 0.39 to 0.64, respectively. The most preferable FFA value observed at 0 day and less preferable FFA observed at 60 day observation. The FFA value (0.45) in the control group was significantly (p< 0.01) higher than the values of the samples treated with BHA, 0.1, 0.2, and 0.3% Moringa oleifera leaf extract. Lee and Kunz (2005) found that fermented sausages showed an increasing FFA content over time. It has been reported that these natural antioxidants, especially of plant source, have greater application potential for consumer’s acceptability, palatability, stability and shelf-life of meat products (Jung et al., 2010). Throughout the storage time, POV were generally higher in control sample than in others. The most preferable POV was observed at T5 (0.3%) Moringa leaf extract group. The control sample, without any added antioxidants, showed a higher level of TBARS than samples treated with 0.1, 0.2, and 0.3% Moringa leaf extract or BHA. The TBARS level of samples treated with 0.1, 0.2, and 0.3% Moringa leaf extract was also lower than those treated with BHA; this difference was especially significant (p < 0.05) after 60 days of storage.
time. Natural antioxidants, in particular polyphenols, are the major plant compounds which have the ability to attenuate the oxidative damage of a tissue indirectly by enhancing natural defenses of cell and/or directly by scavenging the free radical species combat pathological disorders generated by physicochemical Reactive Oxygen Species (ROS) (Du et al., 2010). Antioxidants have an ability to prevent the oxidative damage of tissue indirectly by enhancing natural defenses of cell and directly by scavenging the free radical species (Verma et al., 2009). It has also been reported that these natural antioxidants, especially of plant source, have greater application potential for consumer’s acceptability, palatability, stability and shelf-life of meat products (Jung et al., 2010). One such plant with a potential to be used as an antioxidant is Moringa leaf extracts. It has pharmacological activities and antioxidant properties. Sankhalkar and Vernekar (2016) reported that there are higher phenolics and flavanoid content in Moringa leaf and flower. From the present findings it shows that FFA, POV and TBARs value had significantly (p<0.01) reduced with the addition of Morigna leaf extracts compared to unsupplemented group. It had happened due to the presence of higher phenolics and flavanoid content in Moringa leaf.

**Microbiological assessment**

From table 5 TVC value of fresh beef was 5.12 logs CFUg\(^{-1}\) beef, indicates good quality beef. The overall aerobic plate count, TCC value and TYMC of beef meatball was 5.06–4.36 (log10 CFUg\(^{-1}\)), 1.10–0.91 (log CFUg\(^{-1}\)), and1.50 to 1.25 (log CFUg\(^{-1}\)), respectively at different treatment levels. Among the five treatments, the plate count in control sample (5.06 log CFUg\(^{-1}\)) were significantly (p<0.01) higher than in the samples treated with BHA, 0.1, 0.2 and 0.3% of Moringa leaf extracts. The range of TVC value, TCC value and TYMC values at different days of interval was 4.45 to 4.86, 1.12 to 0.87 and1.79 to 1.04, respectively. All the microbial values had linearly decreased (p<0.01) in the Moringa leaf extract treated groups compared to control groups. The TVC value of fresh beef was 1.25 logs CFUg\(^{-1}\) beef. Among these five treatments, the TCC in the control sample (1.10 logs CFUg\(^{-1}\)) was significantly (p<0.01) higher than in the samples treated with BHA, 0.1, 0.2, and 0.3% of Moringa leaf extracts. The different superscript was observed from different treatment indicates there were significant differences of TYMC values among these five treatment groups. Among five treatments, the total yeast-mold count in the control sample (1.50 log CFUg\(^{1}\)) were significantly (p<0.05) higher than in the samples treated with BHA, 0.1, 0.2, and 0.3% of Moringa leaf extracts. Some bacteria may be present in the product, but their growth is controlled under storage conditions (Fernandez Lopez et al., 2005).
Table 3. Effect of *Moringa oleifera* leaf extract and BHA on physicochemical properties in beef meatball

<table>
<thead>
<tr>
<th>Parameter</th>
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<th>Treatments</th>
<th>Mean</th>
<th>Level of Significance</th>
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<td>T₁</td>
<td>T₂</td>
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Table 4. Effect of *Maringa oleifera* leaf extract and BHA on biochemical parameters in beef meatball
Table 5. Effect of *Moringa oleifera* leaf extract and BHA on different microbe’s population in beef meatball

<table>
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<th>Level of Significance</th>
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</thead>
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<td><strong>T</strong>2</td>
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<td>1.50±0.01</td>
<td>1.43±0.01</td>
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</table>
CONCLUSIONS

The study may be concluded that 0.3% of Moringa leaf extract as natural antioxidant can be used in beef meatballs preparation instead of synthetic antioxidant (BHA). In case of cooking loss at 0.2% Moringa leaf extract is more preferable than that of other treatment groups. On the basis of sensory evaluation, physicochemical properties, biochemical analysis and microbial assessment indicates that 0.3% Moringa leaf extract groups shows better results compare to synthetic antioxidant.

ACKNOWLEDGEMENTS

We would like to express our appreciation to the Bangladesh Agricultural University Research System (BAURES), Mymensingh for funding this research and their continuous encouragement to complete the study successfully.

REFERENCES


AVAILABLE STATUS AND CHANGING TREND OF MICRONUTRIENTS IN FLOODPLAIN SOILS OF BANGLADESH

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ABSTRACT

Soil micronutrient deficiency has arisen in Bangladesh as a consequence of soil fertility depletion with time. Increasing cropping intensity and cultivation of modern varieties with high yield potential are the major reasons of nutrient depletion from soil, which results in decline of crop yield. With an objective of studying delineation of soil micronutrient status, their depleting trend over time, relationship with other soil variables and interrelationship among micronutrients, a study was carried out in Old Meghna Estuarine Floodplain (AEZ 19) soils of the country. In the present study, soil analysis of top soils (0-15 cm soil depth) shows that availability of Zn and B declined after a decade of time. However for other micronutrients, viz. Cu, Mn and Fe, still prevailing high status. Micronutrient levels of subsoil (15-30 cm soil depth) were in general lower than those of top soils. The soil Zn had significant positive correlation with clay content (r=0.712**) and its availability was found influenced more by clay fraction in case of higher Zn concentration (>1.35 µg g⁻¹) than lower concentration (<1.35 µg g⁻¹). The Cu content in soil was positively influenced by soil clay content (r=0.267*), organic matter content (r=0.279*), N content (r=0.579**) and Mg content (r=0.364**), and was negatively influenced by soil pH (r= -0.347**) and P content (r= -0.340*). Concerning interrelationship among soil micronutrients, Cu content showed positive interaction with Zn content (r=0.244) and negative interaction with B content (r= -0.255). In sub soil, except soil B content, all other micronutrient contents were negatively correlated with soil pH. Only Cu content was significantly correlated (r=0.362**) with clay content. There exists positive relationship between soil organic matter and Zn (r=0.269*), Cu (r=0.357**) and Fe content (r=0.362**). The Zn, Cu and Fe content in soil was positively correlated with soil N content, r values being 0.455**, 0.526** and 0.659**, respectively.

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**INTRODUCTION**

Bangladesh has an agro-based economy blessed with its fertile soil resources. In the recent years, huge pressure has been exerted on soil resources of the country to ensure food security for its ever increasing population. Intensification of agricultural land use has been increased remarkably, along with increasing use of modern crop varieties, which in turn has resulted in deterioration of soil fertility with emergence of new nutrient deficiencies. In 1983-84, the cropping intensity of the country was 171% whereas it was 183% in 2009-10 (BBS, 2012). Accordingly, coverage of HYVs and hybrid varieties of only rice increased from 6499 thousand acres in 1983-84 to 23,461 thousand acres in 2011-12 (BBS, 2012). Thus, with advancement of time, soil fertility has declined (Islam, 2008; SRDI, 2010a; SRDI, 2010b); chronologically N, P, K, S, Zn and B deficiency have arisen in the country’s soils (Jahiruddin and Sattar, 2010). Declining productivity in Bangladesh due to the decrease of soil fertility has been cited by many authors (Islam, 1990; Ali, 1991).

Thirty agro-ecological zones (AEZs) have been identified in Bangladesh on the basis of certain information of physical environment which are relevant for land use and assessing agricultural potential. Among the AEZs, Old Meghna Estuarine Floodplain (AEZ 19) is one of the intensively cropped agricultural zones covering considerably larger land area. The total land area under AEZ 19 is 774026 ha having major coverage of 14 districts of the country (FRG, 2012).

Among the micronutrients, zinc deficiency is widely reported. In early 1980s, the Zn deficiencies in rice were observed. In early 1990’s, the B deficiency of some crops is reported. There is sporadic information of Cu, Mo and Mn deficiencies in crops (Bhuiyan et al., 1998). Deficiencies of Fe and Cl are not yet reported in Bangladesh. Considering the above facts, this study was conducted in the intensively cropped area covering AEZ 19 to search out the present status of micronutrients, their depleting trend over time, relationship of micronutrients with other soil variables and interrelationship among micronutrients.

**MATERIALS AND METHODS**

The bench mark information of fertility status in soils of the study area has generated and published by the Soil Resource Development Institute in respective Upazila Nirdeshikas (Adorsho Sadar and Burichong of Comilla district). For delineating the present fertility status, soil sampling was done from the representative sites of the study area in 2011-2012. The sampling sites were selected based on the land type and soil series. The corresponding previous sampling spots cited in respective Upazila Nirdeshika were also in consideration. Highest efforts were given for selecting closer spots to the previous sampling spots maintaining the other above mentioned criteria. GPS (Geological Positioning System) reading was recorded for each site. Fifty five
sampling sites were selected, and from each site two samples were collected at two soil depths (0-15 cm and 15-30 cm). Maps showing the locations of selected soil sampling sites have included in figures 1 & 2.

The collected soil samples were spread on a brown paper in the laboratory for air-drying. After removing the plant roots and other debris, the air-dried soil was ground and passed through a 2-mm sieve for lab analysis. The processed samples were kept in polyethylene bags. Subsequently, the soil samples have been analyzed for basic soil properties (pH, organic matter and texture), macronutrients and micronutrients (Cu, Fe, Mn, Zn and B) status.

Soil pH was measured by glass electrode pH meter (McLean, 1982), texture by hydrometer method (Gee and Bauder, 1986), organic carbon by wet oxidation method (Nelson and Sommers, 1996), total N by micro-Kjeldahl method (Bremner and Mulvaney, 1982), available P for neutral and alkaline soil by Olsen method (Olsen and Sommers, 1982), available P for acidic soil by Bray and Kurtz method (Bray and Kurtz, 1945), exchangeable K, Ca & Mg by 1N CH₃COONH₄ extraction (Knudsen et al., 1982), available S by 0.15% CaCl₂ extraction (Tabatabai, 1996), available Zn, Fe, Mn & Cu by DTPA extraction (Lindsay and Norvell, 1978) and available B by hot water-0.02M CaCl₂ solution (1:2) extraction followed by determination using azomethine-H method (Keren, 1982).

Based on the analytical results of each micronutrient, soil samples were categorized into very low, low, medium, high and very high status (FRG, 2012). Relationship of each micronutrient with basic soil characteristics (clay content, pH and organic
matter content) and macronutrients, and also interrelationship among different micronutrients were examined by correlation statistics (Gomez and Gomez, 1984). A comparison was also made between present soil test value and previous soil test values obtained from Upazila Nirdeshika. The analytical results derived from collected soil samples and Upazila Nirdeshika is denoted in this manuscript as present and previous status, respectively. The soil samples for preparing the respective Upazila Nirdeshikas were collected by SRDI in 1997-2002. Standard statistical tools were used in comparing the data.

RESULTS

Present status of soil micronutrients and comparison with respective previous status

A summary statistics of soil data with the information of maximum, minimum, mean and standard deviation for each micronutrient is presented in Table 1. For easy comparison, previous status of the micronutrients in top soil only is also provided in the same table. Present status of each micronutrient was compared with their previous status, as depicted in Upazila Nirdeshika, 1996-2003 (SRDI, 1999; SRDI, 2000; SRDI, 2006). Thus there was almost a decade of time gap between present and previous results. The comparative features between present and previous status of different micronutrients are shown in figures 3-7.

Table 1. Summary statistics of zinc, boron, copper, iron and manganese levels (mg kg\(^{-1}\)) of soils at two depths (n = 55)

<table>
<thead>
<tr>
<th>Micronutrients</th>
<th>0-15 cm depth</th>
<th>15-30 cm depth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min.</td>
<td>Max.</td>
</tr>
<tr>
<td>Zn</td>
<td>0.62</td>
<td>4.41</td>
</tr>
<tr>
<td>B</td>
<td>0.10</td>
<td>0.57</td>
</tr>
<tr>
<td>Cu</td>
<td>1.12</td>
<td>7.62</td>
</tr>
<tr>
<td>Fe</td>
<td>44.0</td>
<td>428</td>
</tr>
<tr>
<td>Mn</td>
<td>3.00</td>
<td>141</td>
</tr>
</tbody>
</table>
b) Previous status

<table>
<thead>
<tr>
<th>Micronutrients</th>
<th>0-15 cm soil depth</th>
<th>0-15 cm soil depth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min.</td>
<td>Max.</td>
</tr>
<tr>
<td>Zn</td>
<td>0.80</td>
<td>6.20</td>
</tr>
<tr>
<td>B</td>
<td>0.04</td>
<td>1.33</td>
</tr>
<tr>
<td>Cu</td>
<td>0.85</td>
<td>4.70</td>
</tr>
<tr>
<td>Fe</td>
<td>35.0</td>
<td>470</td>
</tr>
<tr>
<td>Mn</td>
<td>4.00</td>
<td>148</td>
</tr>
</tbody>
</table>

Min.- Minimum, Max.-Maximum, s.d- Standard deviation

**Zinc status**

The available Zn (DTPA extractable) content of soil samples at 0-15 cm depth varied from 0.62-4.41 mg kg\(^{-1}\) with a mean value of 1.48 mg kg\(^{-1}\) (Table 1). The previous result of top soil Zn in this AEZ ranged from 0.80 to 6.20 mg kg\(^{-1}\) having mean value of 1.83 mg kg\(^{-1}\). Based on the critical limit of Zn as 0.60 mg kg\(^{-1}\), the soil samples were classified into 20% low, 38% medium, 22% optimum, 5% high and 15% very high categories (FRG, 2012) (Figure 3). The corresponding previous status was 15, 29, 31, 4 and 22%, respectively. Concerning subsoil Zn status, it ranged from 0.37-2.45 mg kg\(^{-1}\) having mean result of 0.82 mg kg\(^{-1}\).

![Figure 3. Changing trend of soil available Zn status over time in AEZ 19](image)

**Boron status**

The available B status of top soil ranged from 0.10 to 0.57 mg kg\(^{-1}\) and 0.04 to 1.33 mg kg\(^{-1}\) having mean values of 0.29 and 0.37 mg kg\(^{-1}\) in the present and previous time, respectively (Table 1). The present B status of top soil can be grouped into 2% very low, 58% low, 35% medium and 5% optimum against previous status of 5% very low, 40% low, 29% medium, 11% optimum, 2% high and 13% very high, respectively (FRG, 2012) (Figure 4). The present subsoil B status was found to vary from 0.07-0.32 mg kg\(^{-1}\) where the average value being 0.19 mg kg\(^{-1}\).
The available Cu content of top soil varied from 1.12–7.62 mg kg\(^{-1}\) with a mean value of 2.56 mg kg\(^{-1}\) (Table 1). Previously this Cu value was 0.85 mg kg\(^{-1}\) as the minimum, 4.70 mg kg\(^{-1}\) as the maximum and 2.25 mg kg\(^{-1}\) as the mean. The soil Cu status was of 100% very high for both present and previous cases (Figure 5). Concerning subsoil Cu status, it ranged from 0.55–9.46 mg kg\(^{-1}\) having mean value of 2.19 mg kg\(^{-1}\).

The soil status of available Fe was 100% very high in both present and previous situations (Figure 6) and it ranged from 44 to 428 and 35 to 470 mg kg\(^{-1}\) with the mean values of 215 and 201 mg kg\(^{-1}\), respectively (Table 1). Concerning subsoil Fe status, the lowest, highest and mean values were 11.0, 375 and 79.0 mg kg\(^{-1}\), respectively.
Manganese status

The available Mn level of soil samples at 0-15 cm depth varied from 3.0-141.2 mg kg\(^{-1}\) with a mean level of 24.8 mg kg\(^{-1}\) (Table 1). According to FRG (2012) 2% soil had optimum Mn status, 2% high status and 96% very high status. Thus, no question of Mn deficiency arises in this AEZ’s soils (Figure 7). On the other hand, previous status of soil Mn was between 4.0 and 148 mg kg\(^{-1}\) having a mean value of 41.2 mg kg\(^{-1}\). These results assumed to be an indication of declining Mn status in the Old Meghna Estuarine Floodplain (AEZ 19) soils. The subsoil status of Mn was lower compared to top soil status. It ranged from 3.95–100 mg kg\(^{-1}\) and the average value being 20 mg kg\(^{-1}\).

Relationship of present micronutrients status with other soil properties and interrelation among the micronutrients

Correlation statistics was performed to examine the relationship of micronutrients with other soil variables and to see the interrelationship among the micronutrients. This statistics was done separately for top soils and sub-soils. These data are presented in tables 2 & 3. The number of soil samples i.e. observations for both cases were 55.
Top soil characteristics
The statistical analysis shows that soil Zn had significant and positive correlation with clay content ($r=0.712^{**}$) meaning that soil available Zn content increases as the soil clay content increases (Table 2). The Zn availability was influenced more by clay fraction in case of higher Zn concentration ($>1.35$ µg g$^{-1}$) than the lower concentration ($<1.35$ µg g$^{-1}$) (Figures 8-9). The Zn content showed no significant relationship with any other soil variables. Among the micronutrients under study, soil B content only exhibited significant positive relationship with soil S content ($r=$...
0.315**), but it did not show significant correlation with other soil characteristics. Negative correlation with soil B was observed with clay content, organic matter, N, K and Ca content. Unlike other micronutrients, the Cu availability in soil was influenced by many soil variables. The Cu content in soil was positively influenced by soil clay content (r=0.267*), organic matter content (r=0.279*), N content (r=0.579**) and Mg content (r=0.364**), and was negatively influenced by soil pH (r= -0.347**) and P content (r= -0.340*). The availability of Fe in soil was slightly affected by soil pH (r= -0.251) and was highly influenced by soil organic matter (r=0.382**) and N content (r=0.356**). Unlike other micronutrients, the soil Mn level did not show significant relationship with any other soil properties. Concerning interrelationship among soil micronutrients, soil Cu content showed positive interaction with soil Zn content (r=0.244) and negative interaction with soil B content (r= -0.255). Soil Fe or Mn content did not show significant interaction with other micronutrients (Zn, Cu and B) in soil (Table 2).

Subsoil characteristics

Availability of micronutrients especially Zn, Cu and Fe in subsoils was markedly affected or influenced by many other soil variables (Table 3). Soil B availability was not at all affected or influenced by any other soil characteristics under study. Except soil B content, all other micronutrient contents were negatively correlated with soil pH indicating that micronutrient availability decreases as soil pH increases and vice-versa. This point is very important for soil fertility concern. Only Cu content was significantly correlated (r=0.362**) with clay content. There exists positive relationship between soil organic matter and soil Zn (r=0.269*), Cu (r=0.357**) and Fe (r=0.362**) content. The Zn, Cu and Fe content in soil was positively correlated with soil N content, r values being 0.455**, 0.526** and 0.659**, respectively. Soil P content was only associated with Zn (r=0.318*) and Fe (r=0.382**) content. The level of basic cations viz. K, Ca & Mg was found positively associated with soil Zn and Cu content in soil. Micronutrient availability in soil did not depend on soil S content. Looking at the interactions of micronutrient availability in sub-soil, there was a positive interaction of Zn-Cu, Zn-Fe, Zn-Mn and Fe-Cu. The other interactions were not significant (Table 3).

Table 2. Correlation matrix of soil variables (soil collection at 0-15 cm depth)
a) Relationship of micronutrients with other soil variables (n=55)

<table>
<thead>
<tr>
<th>Micronutrients</th>
<th>Clay</th>
<th>pH</th>
<th>OM</th>
<th>N</th>
<th>P</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn</td>
<td>0.712**</td>
<td>-0.105ns</td>
<td>-0.002ns</td>
<td>0.032ns</td>
<td>0.227ns</td>
<td>0.212ns</td>
<td>0.206ns</td>
<td>0.014ns</td>
<td>0.188ns</td>
</tr>
<tr>
<td>B</td>
<td>-0.090ns</td>
<td>0.100ns</td>
<td>-0.111</td>
<td>-0.145ns</td>
<td>0.020ns</td>
<td>-0.029ns</td>
<td>-0.110ns</td>
<td>0.044ns</td>
<td>0.315*</td>
</tr>
<tr>
<td>Cu</td>
<td>0.267*</td>
<td>-0.347**</td>
<td>0.279*</td>
<td>0.579**</td>
<td>-0.340*</td>
<td>-0.006ns</td>
<td>0.209ns</td>
<td>0.364**</td>
<td>-0.144ns</td>
</tr>
<tr>
<td>Fe</td>
<td>0.101ns</td>
<td>-0.251ns</td>
<td>0.382**</td>
<td>0.356**</td>
<td>-0.078ns</td>
<td>-0.229ns</td>
<td>0.071ns</td>
<td>0.024ns</td>
<td>0.061ns</td>
</tr>
<tr>
<td>Mn</td>
<td>0.176ns</td>
<td>0.001ns</td>
<td>-0.116ns</td>
<td>-0.024ns</td>
<td>-0.012ns</td>
<td>0.173ns</td>
<td>0.189ns</td>
<td>0.139ns</td>
<td>0.184ns</td>
</tr>
</tbody>
</table>
(b) Interrelationship among micronutrients in soils (n=55)

<table>
<thead>
<tr>
<th>Micronutrients</th>
<th>Zn</th>
<th>B</th>
<th>Cu</th>
<th>Fe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>-0.176ns</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cu</td>
<td>0.244ns</td>
<td>-0.255ns</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fe</td>
<td>-0.063ns</td>
<td>0.112ns</td>
<td>0.211ns</td>
<td>-</td>
</tr>
<tr>
<td>Mn</td>
<td>0.202ns</td>
<td>-0.181ns</td>
<td>0.133ns</td>
<td>-0.136ns</td>
</tr>
</tbody>
</table>

* = Significant at 5% level, ** = Significant at 1% level, ns = Not significant

Table 3. Correlation matrix of soil variables (soil collection at 15-30 cm depth)

(a) Relationship of micronutrients with other soil variables (n=55)

<table>
<thead>
<tr>
<th>Micronutrients</th>
<th>Clay</th>
<th>pH</th>
<th>OM</th>
<th>N</th>
<th>P</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn</td>
<td>-0.070ns</td>
<td>-0.397**</td>
<td>0.269*</td>
<td>0.455**</td>
<td>0.318*</td>
<td>0.422**</td>
<td>0.374*</td>
<td>0.313*</td>
<td>0.115ns</td>
</tr>
<tr>
<td>B</td>
<td>-0.013ns</td>
<td>0.212</td>
<td>-0.025ns</td>
<td>-0.147ns</td>
<td>-0.193ns</td>
<td>0.017ns</td>
<td>0.011ns</td>
<td>0.119ns</td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>0.362**</td>
<td>-0.323*</td>
<td>0.357**</td>
<td>0.526**</td>
<td>0.042ns</td>
<td>0.573**</td>
<td>0.342*</td>
<td>0.484**</td>
<td>-0.005ns</td>
</tr>
<tr>
<td>Fe</td>
<td>-0.096ns</td>
<td>-0.626**</td>
<td>0.362**</td>
<td>0.659**</td>
<td>0.282*</td>
<td>0.214ns</td>
<td>0.144ns</td>
<td>0.065ns</td>
<td>-0.007ns</td>
</tr>
<tr>
<td>Mn</td>
<td>0.009ns</td>
<td>-0.365**</td>
<td>0.147ns</td>
<td>0.182ns</td>
<td>0.219ns</td>
<td>0.195ns</td>
<td>0.080ns</td>
<td>0.107ns</td>
<td>0.098ns</td>
</tr>
</tbody>
</table>

(b) Interrelationship among micronutrients in soils (n=55)

<table>
<thead>
<tr>
<th>Micronutrients</th>
<th>Zn</th>
<th>B</th>
<th>Cu</th>
<th>Fe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>-0.065ns</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cu</td>
<td>0.381**</td>
<td>-0.171ns</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fe</td>
<td>0.402**</td>
<td>0.157ns</td>
<td>0.272*</td>
<td>-</td>
</tr>
<tr>
<td>Mn</td>
<td>0.364**</td>
<td>-0.041ns</td>
<td>0.188ns</td>
<td>0.110ns</td>
</tr>
</tbody>
</table>

* = Significant at 5% level, ** = Significant at 1% level, ns = Not significant

DISCUSSION

Comparison of micronutrients status from its present to past in soils under investigation

In the current study, 80% soil samples (0-15 cm) has been grouped as low to optimum categories considering Zn status and 98% samples of same soil depth lied in low to medium categories on the basis of B content. According to FRG (2012), low to medium Zn status and low to optimum B status prevailed in soils of AEZ 19. Hence, slight negative changes were observed in soil zinc and boron status of the
study area after almost a decade of time. High cropping intensity accompanied with cultivation of modern varieties of crop might be worsening the situation. This result has an agreement with SRDI (2010b) where Comilla was shown as one of the most B deficient areas in Bangladesh. There is a good agricultural practice in that area; the crop residues especially in case of rice, maximum parts of straw are left in the field while crop harvesting. But later on, some other needy people collect those for using as fuel. If those crop residues could be incorporated in soil, there would be a positive amendment of soil. Again, there is a gap of two months in between aus rice and next rabi crops in vegetable-vegetable/maize-aus rice cropping pattern in that area. This gap could be used for producing green manure. Besides, other appropriate fertilizer management programs should be associated to reduce nutrient depletion from soils of that area. In the study area, very high status of available Cu, Mn and Fe prevailed as it was in the previous year of consideration. The soil Cu and Fe have not declined; moreover there was an indication of slight increased soil Fe over time. It might be due to decreasing trend of soil pH in the study area; as pH is one of the most influential factors of iron availability in soil. In lower soil pH, there is a tendency of higher availability of iron in soil. Generally, micronutrient status in surface soils (0-15 cm) was found higher compared to subsoil (15-30 cm) status. The reason behind such condition could be attributed due to addition of fertilizers and manure to top soil. In addition to that, the probable reason for decreasing micronutrient content with soil depth could be due to the accumulation of biomass in the surface layer of soils leading to higher organic matter and clay content in the top soil than sub-soils. Similar investigation was reported by Vijayakumar et al. (2011). Typical micronutrient soil-profile distribution was likely a result of greater decomposition of soil organic matter and crop residues that contribute to micronutrient accumulation to the surface layers. Secondly, root distributions and rooting depth play an important role in shaping micronutrient profiles because nutrients taken up by deep roots are transported into the above-ground parts and re-deposited on the soil surface through stem flow and through fall (Garcia et al., 2014).

**Relationship of micronutrients status with other soil properties and their interrelations**

Among the micronutrients Zn and Cu had significant positive relation with clay content in top soil, while in sub soil only Cu had significant positive relation. This might be due to the availability of binding sites for different cations on the clay particles. Sharma et al. (2004) found that the total content of Zn, Cu, Fe and Mn increased with an increase in soil clay content. Mustapha and Fagam (2007) also found significant positive relation between soil Zn and clay content. Soil available B was negatively correlated with clay content while Fe and Mn associated positively but the relations were non-significant. Worku et al. (2016) also found negative significant correlation between soil B and clay content ($r=-0.46^{**}$). Soil pH was negatively correlated with Zn, Cu and Fe in top soil, and Zn, Cu, Fe and Mn in sub surface soil. Worku et al. (2016) also found weak as well as negative association ($r =$
between Cu and pH values. Njukeng et al. (2013) also reported non-significant negative correlation between soil pH and Fe content (r = –0.04). Available Cu and Fe had strong association with soil organic matter content. This result is in agreement with Worku et al. (2016).

In case of interrelationship among the micronutrients, available Zn showed positive relation with Cu and Mn in top soil. In addition to that Fe was strongly correlated with available Zn. This finding has an agreement with Srivastava et al. (2017), who reported positive relation of Zn with Mn (r=0.031) and Cu (r= 0.098). The available B content had negative relation with most of the micronutrients studied, since positive charge of micronutrients like Fe, Mn and Cu influence antagonism for the availability of non metal B ion to the plants. These results were also supported by Srivastava et al. (2017) and Vijayakumar et al. (2011).

CONCLUSION
Like macronutrients soil micronutrient deficiency has also arisen in floodplain soils of Bangladesh with advancement of time. The relationships between soil micronutrients and other soil parameters as well as soil macronutrients which were explored through this study may help in soil management practices in the study areas.

ACKNOWLEDGEMENT
We gratefully acknowledge the financial support provided by Coordinated Project on Soil Fertility and Fertilizer Management for Crops and Cropping Patterns (BAU component), NATP, BARC during the research period.

REFERENCES


EFFECT OF AGE OF SEEDLINGS AT STAGGERED TRANSPLANTING AND NUTRIENT MANAGEMENT ON YIELD PERFORMANCE OF AROMATIC FINE RICE (cv. BRRI dhan38)

A. Roy, M.A.R. Sarkar and S.K. Paul

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ABSTRACT

An experiment was conducted at the Agronomy Field Laboratory, Bangladesh Agricultural University, Mymensingh during July to December 2014 with a view to finding out the effect of age of seedlings at staggered transplanting and nutrient management on growth and yield of aromatic fine grained rice (cv. BRRI dhan38). The experiment consisted of three ages of seedlings (30, 45 and 60 day-old) at staggered transplanting and six nutrient managements viz. control (no nutrients), recommended dose of inorganic fertilizers, 50% of recommended dose of inorganic fertilizers + cowdung @ 5 t ha\(^{-1}\), 75% of recommended dose of inorganic fertilizers + cowdung @ 5 t ha\(^{-1}\), 50% of recommended dose of inorganic fertilizers + poultry manure @ 2.5 t ha\(^{-1}\) and 75% of recommended dose of inorganic fertilizers + poultry manure @ 2.5 t ha\(^{-1}\). The experiment was laid out in a randomized complete block design with three replications. The effect of age of seedlings at staggered transplanting, nutrient management and their interactions were significant on crop characters, yield components and yield of aromatic fine rice. The tallest plant was recorded due to transplanting 30-day old seedlings fertilized with 75% of recommended dose of inorganic fertilizers + poultry manure @ 2.5 t ha\(^{-1}\). The highest leaf area index (6.55), number of total tillers hill\(^{-1}\) (12.56), number of effective tillers hill\(^{-1}\) (8.54), panicle length (24.07cm) and number of grains panicle\(^{-1}\) (141.3) were recorded in 30-day old seedlings fertilized with 75% of recommended dose of inorganic fertilizers + poultry manure @ 2.5 t ha\(^{-1}\) while the lowest values were recorded in 60-day old seedling with control. In case of sterile spikelets panicle\(^{-1}\), 60-day old seedlings with control treatment showed the highest value (30.94). The highest grain (3.85 t ha\(^{-1}\)) and straw (5.29 t ha\(^{-1}\)) yields were obtained in 30-day old seedlings fertilized with 75% of recommended dose of inorganic fertilizers + poultry manure @ 2.5 t ha\(^{-1}\). Therefore, 30-day old seedlings
fertilized with 75% of recommended dose of inorganic fertilizers + poultry manure @ 2.5 t ha\(^{-1}\) appeared as the promising technique for appreciable growth and grain yield of aromatic fine grained rice (cv. BRRI dhan38).

**Keywords:** Aromatic fine grained rice, seedlings, staggered transplanting, nutrient, yield.

**INTRODUCTION**

Rice contributes 95% of total food production in Bangladesh. About 77.07% of cropped area of Bangladesh is used for rice production, with annual production of 33.83 million ton from 11.41 million ha of land which contributes about 19.70% of the country’s GDP (BBS, 2013). Aromatic rice contributes a small but special group of rice which covers 2% of the national rice acreage of Bangladesh and 12.5% of the total transplant Aman rice cultivation (Ashrafuzzaman et al., 2009). Aromatic rice is rated best in quality and fetches much higher price than high quality non-aromatic rice in the domestic and international market. The demand of aromatic rice for internal consumption and also for export is increasing day by day. Most of the aromatic rice varieties in Bangladesh are traditional photo-period sensitive type and grown during Aman season (Kabir et al., 2004). In Bangladesh, Chinisagar, Badshabhog, Kataribhog, Kalizira, Tulsimla, Dulabhog, Basmati, Banglamati (BRRI dhan50), BRRI dhan34, BRRI dhan37, BRRI dhan38, Binadhan-9 and Binadhan-13 are grown as aromatic rice. Some of them have special appeal for their aroma. These varieties are cultivated by the farmers to meet domestic consumption mainly and very little amount goes to export purpose.

Staggered transplanting means, planting different fields in a community or in a farm over a period of several weeks, in contrast with simultaneous planting where all fields are planted over a period of a week or less. In Bangladesh, sometimes transplanting of Aman rice is delayed due to late recession of flood water, and unavailability of seedlings. In such cases more seedlings can be raised in the nursery bed to transplant in the main field at a later date than the optimum one so that, the damage caused by the flood is minimized. Here staggering of planting date with the seedlings of same source to be used because at this stage farmers are in shortage of time for raising new seedlings. The use of seedlings from the same source are planted at optimum date and thereafter at different dates are termed as staggered planting of rice seedlings having different ages. Transplanting of healthy seedlings of optimum age ensures better rice yield. When seedlings are transplanted at the right time, tillering and growth proceed normally. Age of seedling at the time of transplanting is an important factor for uniform stand establishment of rice (Ginigaddara, and Ranamukhaarachchi, 2011). Age of seedling influences yield components and yield of rice (Mishra and Salokhe, 2008; Amin and Haque, 2009 and Faghani et al., 2011).

The yield of aromatic fine grained rice can be increased with the improved cultivation practices like proper age of seedlings and proper nutrient management.
Optimum age of seedlings supports the plants to uptake more nutrients from the soil. Almost all soils of Bangladesh are deficient in nitrogen mainly due to low level of organic matter caused by continuous intensive cropping with high yielding varieties and adding of less amount of organic matter. Poultry manure and cowdung may play a vital role in soil fertility improvement as well as supplying primary, secondary and micronutrients in addition to N, P and K. It may supply sufficient amount of S, Zn and B for growth of rice plants. The application of cowdung and poultry manure to soil is considered a good management practice in any agricultural production system because of the stimulation of soil microbial growth and activity, subsequent mineralization of plant nutrients, and increased soil fertility and quality (Islam et al., 2007). Yield and grain protein content increased due to application of manure with inorganic fertilizers (Pal et al., 2015; Roy et al., 2015 and Biswas et al., 2016). The application of 75% of recommended dose of inorganic fertilizers + 50% cowdung showed superiority in terms of the growth, yield and quality of aromatic rice (Sarkar et al., 2014). So the efficient nutrient management increases crop yield and at the same time reduces fertilization cost. Therefore, the present study was carried out to delineate the effect of age of seedlings at staggered transplanting and nutrient management on the yield performance of aromatic fine rice (cv. BRRI dhan38).

**MATERIALS AND METHODS**

The experiment was conducted at the agronomy field laboratory of Bangladesh Agricultural University (BAU), Mymensingh during July to December 2014 to study the effect of age of seedlings at staggered transplanting and nutrient management on the growth and yield of aromatic fine grained rice (cv. BRRI dhan38). The experimental field is located at 24°75’ N latitude and 90°50’E longitude at an altitude of 18m. The experimental field belongs to the Old Brahmaputra Floodplain (AEZ-9). The soil of the experimental land belongs to the Sonatala series of non-calcareous dark grey floodplain. The land was medium high, silt loam in texture and more or less neutral in reaction (pH 6.5), and low in organic matter (1.29%). Aromatic rice variety BRRI dhan38 was used as plant material. The experiment consisted of three age of seedling at staggered planting viz. 30- (A₁), 45- (A₂) and 60- day old seedlings (A₃) and four nutrient managements viz. Control (no nutrients) (F₀), Recommended dose of inorganic fertilizers (i.e. 150, 97, 70, 60 and 12 kg Urea, TSP, MoP, Gypsum and ZnSO₄, respectively ha⁻¹) (F₁), 50% of recommended dose of inorganic fertilizers + cowdung @ 5 t ha⁻¹ (F₂), 75% of recommended dose of inorganic fertilizers + cowdung @ 5 t ha⁻¹ (F₃), 50% of recommended dose of inorganic fertilizers + poultry manure @ 2.5 t ha⁻¹ (F₄) and 75% of recommended dose of inorganic fertilizers + poultry manure @ 2.5 t ha⁻¹ (F₅). The experiment was laid out in a randomized complete block design with three replications. The size of unit plot was 4.0 m × 2.5 m. The distances between blocks and plots were 1 m and 75 cm, respectively. Sprouted seeds were sown in the prepared wet nursery bed on 23 June 2014. Proper care was taken to raise the seedlings in the nursery bed. Weeds were
removed and irrigation was given in the nursery bed as and when necessary. The experimental land was prepared by a power tiller 10 days before transplanting. It was then ploughed well with the help of country plough to make the soil nearly ready for transplanting. Weeds and stubble were removed and the field was then leveled by laddering. The experimental field was then divided into unit plots which were spaded one day before transplanting for incorporating the basal fertilizers. The specified amount of cowdung, poultry manure, triple superphosphate, muriate of potash, gypsum and zinc sulphate were applied at final land preparation. Urea was applied in three equal splits, at 15, 35 and 50 days after transplanting (DAT). Seedlings were transplanted on the well puddled experimental plots following staggering of transplanting on 23 July, 8 August and 23 August 2014, maintaining the spacing of 25 cm × 15 cm using three seedlings hill⁻¹. Intercultural operations were done for ensuring and maintaining the vigorous growth of the crop. Intensive care was taken throughout the growing season. The leaf area was measured by an automatic leaf area meter (Type AAN-7, Hayashi Dam KO Co., Japan). LAI was calculated as the ratio of total leaf area and total ground area of the sample as described by Hunt (1978) and Yoshida (1981). LAI = LA/P, Where, LA = Total leaf area of the leaves of all sampled plants (cm²), P = Area of the ground surface covered by the plant (cm²).

Maturity of the crop was determined when 90% of the grain became golden yellow in color. Five hills (excluding border hills and central 1 m² area) were selected randomly from each plot and uprooted prior to harvest to record data on crop characters and yield contributing characters. After sampling, central one square meter area was harvested to record the data on yield. The crop was harvested on 24 December 2014 with sickle at proper maturity. Then the crop was threshed, cleaned and sun dried to record the grain yield per square meter. The grain yield was adjusted to 14% moisture content. Straws were sun dried to record the straw yield per square meter. Grain and straw yields were then converted to t ha⁻¹. The recorded data were analyzed following analysis of variance technique and mean differences were adjudged by Duncan’s Multiple Range Test (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Effect of seedling age at staggered transplanting

Plant height was significantly influenced by seedling age at staggered transplanting (Table 1). The tallest plant (106.30 cm) was found when 30-day old seedlings were transplanted followed in order by 45-day old seedlings at staggered transplanting. The tallest plant was found with 30-day old seedlings due to early recovery of transplanting shock and better growth of the plants. The maximum number of non-effective tillers hill⁻¹ (2.39) was found when 60-day old seedlings were transplanted and the minimum number of non-effective tillers hill⁻¹ (1.62) was obtained when 30-day old seedlings were transplanted (Table 1). Number of non-effective tillers hill⁻¹ exhibited a trend of increase with the increase in seedling age. Similar results were
also observed by Haque (2002). Older seedlings produced more non-effective tillers than the younger ones due to the short duration of vegetative period as they were generated from the tertiary tillers and thus those plants rapidly switched over to reproductive phase leaving behind many tillers non-bearing.

**Effect of nutrient management**

Plant height was significantly affected different levels of manures with inorganic fertilizers (Table 1). The tallest plant (107.1 cm) was recorded in treatment $F_5$ (75% inorganic fertilizer + poultry manure @ 2.5 t ha$^{-1}$) which was at par with $F_3$ (75% of recommended dose of inorganic fertilizers + cowdung @ 5 t ha$^{-1}$) and $F_2$ (50% of recommended dose of inorganic fertilizers + cowdung @ 5 t ha$^{-1}$) whereas the shortest one (99.70 cm) was recorded in control treatment $F_0$ (no manures and no inorganic fertilizers). This might have occurred due to application of manure which regulated the exuberant vegetative growth. The number of non-effective tillers hill$^{-1}$ showed opposite trend (ranged from 1.85 to 2.39) and the highest value was obtained in $F_0$ (no manures and no fertilizers) while the lowest one was produced in $F_5$ (75% inorganic fertilizer + poultry manure @ 2.5 t ha$^{-1}$).

**Effects of interaction**

Leaf area index (LAI), number of total tillers hill$^{-1}$, number of effective tillers hill$^{-1}$, panicle length and grains panicle$^{-1}$ were significantly influenced by the interaction between age of seedlings at staggered transplanting and nutrient management (Table 2). The highest LAI (6.55), number of total tillers hill$^{-1}$ (12.56) and effective tillers hill$^{-1}$ (11.42) were recorded in 30-day old seedlings with $F_5$ treatment (75% inorganic fertilizer + poultry manure @ 2.5 t ha$^{-1}$) while the lowest values were recorded in 60-day old seedling with $F_0$ (no manure and no fertilizer). Younger seedlings produced more tillers than the older ones due to quick regeneration of seedlings and plant vigour. So, number of effective tillers hill$^{-1}$ gradually decreased as the age of seedling increased (Table 2). The longest panicle (24.07 cm) and grains panicle$^{-1}$ (141.3) were obtained in 30-day old seedlings with $F_5$ treatment (75% inorganic fertilizer + poultry manure @ 2.5 t ha$^{-1}$) while the corresponding lowest values were recorded in 60-day old seedlings with $F_0$ treatment (control). Number of grains panicle$^{-1}$ exhibited a trend of decrease with the increase in seedling age. These finding are in conformity with that of Luna et al. (2017). Younger seedlings produced more grains panicle$^{-1}$ than older ones due to longer vegetative period when spikelets are formed in the spike before emergence of the panicles. The maximum number of sterile spikelets panicle$^{-1}$ (30.94) was obtained in 60-day old seedlings with $F_0$ treatment (control) and the minimum number of sterile spikelets panicle$^{-1}$ (11.68) was found in 30-day old seedlings with treatment $F_5$ (75% inorganic fertilizer + poultry manure @ 2.5 t ha$^{-1}$). Number of sterile spikelets panicle$^{-1}$ exhibited a trend of increase with the increase in seedling age. Similar findings were also reported by Yoshii and Sandier (1998). Older seedlings produced more sterile spikelets panicle$^{-1}$ than the younger ones due to the short duration of vegetative period and the plants rapidly switched over to
reproductive and ripening phase leaving behind many spikelets sterile. Integration of manure and inorganic fertilizer enhanced production of number of effective tillers hill$^{-1}$ and grains panicle$^{-1}$ was also reported elsewhere (Shaha et al., 2014, Sarkar et al., 2014 and Marzia et al., 2016). Weight of 1000-grains was not significantly influenced by the interaction between age of seedlings and nutrient management. The weight of 1000-grains ranged from 13.42 g. to 16.24g.

Grain yield, straw yield and harvest index were significantly influenced by the interaction between age of seedlings at staggered transplanting and nutrient management. It was found that the highest grain yield (3.85 t ha$^{-1}$) was obtained from 30-day old seedlings with F$_5$ (75% inorganic fertilizer + poultry manure @ 2.5 t ha$^{-1}$) treatment, which was as good as 30-day old seedlings with F$_5$ (75% of recommended dose of inorganic fertilizers + cowdung @ 5 t ha$^{-1}$) and 45-day old seedlings with F$_5$ (75% inorganic fertilizer + poultry manure 2.5 t ha$^{-1}$) and the lowest grain yield (2.73 t ha$^{-1}$) was observed in 60-day old seedlings with control (no manure and fertilizer). The highest grain yield occurred due to the contribution of more numbers of effective tillers hill$^{-1}$ and grains panicle$^{-1}$ in F$_5$ treatment. Straw yield and harvest index showed similar trend as that of grain yield. Straw yield (5.29 t ha$^{-1}$) and harvest index (47.63 %) were the highest in 30-day old seedlings with F$_5$ (75% inorganic fertilizer + poultry manure @ 2.5 t ha$^{-1}$) and the lowest values were observed in 60-day old seedlings with F$_0$ nutrient management. All parameters exhibited a regular trend of decrease with the increase in seedling age. These finding are in agreement with that of Upadhyay et al. (2003) and Singh and Singh (1998). Older seedlings remained more days in the nursery bed and as a result basal node was formed in the seedlings. Again it took more time to get established in the main field. On the contrary, the younger seedlings stayed in the nursery bed from a short period and thus nodes were not formed and they quickly recovered the transplanting shock in the main field. Thus they started re-growth quickly which ultimately helped in favour of better growth of plant, yield components and yield. Again panicle initiation started earlier within the plant and more spikelets were formed which ultimately resulted in more number of spikelets panicle$^{-1}$. Thus the yield components were improved and sterility percentage was decreased, which were mainly responsible for the improvement of grain yield. The longest plant and the highest number of total tillers in 30-day old seedlings with F$_5$ (75% inorganic fertilizer + poultry manure @ 2.5 t ha$^{-1}$) were responsible for the highest straw yield while the highest harvest index in this treatment occurred due to higher grain yield indicating efficient translocation of assimilates for grain production. Application of poultry manure combined with inorganic fertilizer encouraged the vegetative growth of rice in terms of plant height and number of effective tillers hill$^{-1}$, which ultimately resulted in the increase of grain yield. These findings are in accordance with that of Sarkar et al. (2014) and Pal et al. (2016). The highest harvest index (45.04%) was obtained from nutrient management treatment F$_5$ (75% inorganic fertilizer + poultry manure @ 2.5 t ha$^{-1}$), which was statistically identical with F$_4$ (50% inorganic fertilizer + poultry manure @ 2.5 t ha$^{-1}$).
and F$_3$ (75% inorganic fertilizer + cowdung @ 5 t ha$^{-1}$) and the lowest one (33.92%) was obtained in F$_1$ (recommended dose of fertilizer), which was statistically identical with F$_0$ (control treatment).

Table 1. Effect of age of seedlings at staggered transplanting and nutrient management on plant height and number of non-bearing tillers hill$^{-1}$

<table>
<thead>
<tr>
<th>Age of seedling (days)</th>
<th>Plant height (cm)</th>
<th>No. of non-effective tillers hill$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>106.3a</td>
<td>1.62b</td>
</tr>
<tr>
<td>45</td>
<td>103.7b</td>
<td>2.39a</td>
</tr>
<tr>
<td>60</td>
<td>100.2c</td>
<td>2.39a</td>
</tr>
<tr>
<td>CV (%)</td>
<td>3.51</td>
<td>12.17</td>
</tr>
</tbody>
</table>

Nutrient management

<table>
<thead>
<tr>
<th>Nutrient management</th>
<th>Plant height (cm)</th>
<th>No. of non-effective tillers hill$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>F$_0$</td>
<td>99.70d</td>
<td>2.40a</td>
</tr>
<tr>
<td>F$_1$</td>
<td>102.4bcd</td>
<td>2.18abc</td>
</tr>
<tr>
<td>F$_2$</td>
<td>104.2abc</td>
<td>2.13bc</td>
</tr>
<tr>
<td>F$_3$</td>
<td>105.6ab</td>
<td>1.97cd</td>
</tr>
<tr>
<td>F$_4$</td>
<td>101.4cd</td>
<td>2.29ab</td>
</tr>
<tr>
<td>F$_5$</td>
<td>107.1a</td>
<td>1.86d</td>
</tr>
<tr>
<td>CV (%)</td>
<td>3.51</td>
<td>12.17</td>
</tr>
</tbody>
</table>

In a column, values having the same letters under each treatment do not differ significantly whereas values with dissimilar letter differ significantly as per DMRT.

** = Significant at 1% level of probability, NS = Not significant.

F$_0$ = Control (no manures and fertilizers), F$_1$ = Recommended dose of inorganic fertilizers (i.e 150, 97, 70, 60 and 12 kg Urea, TSP, MoP, Gypsum and ZnSO$_4$, respectively ha$^{-1}$), F$_2$ = 50% of recommended dose of inorganic fertilizers + cowdung @ 5 t ha$^{-1}$, F$_3$ = 75% of recommended dose of inorganic fertilizers + cowdung @ 5 t ha$^{-1}$, F$_4$ = 50% of recommended dose of inorganic fertilizers + poultry manure @ 2.5 t ha$^{-1}$, F$_5$ = 75% of recommended dose of inorganic fertilizers + poultry manure @ 2.5 t ha$^{-1}$. 
Table 2. Effect of interaction between age of seedlings at staggered transplanting and nutrient management on LAI, crop characters, yield components and yield of aromatic fine rice cv. BRRI dhan38

<table>
<thead>
<tr>
<th>Nutrient Management</th>
<th>Age of seedling (days)</th>
<th>Leaf area index</th>
<th>Number of total tillers hill⁻¹</th>
<th>Number of effective tillers hill⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>F₀</td>
<td>30</td>
<td>4.38de 5.67c</td>
<td>6.03b 6.22b 5.45c 6.55a 7.13efg 9.91c 10.87b 11.55b 8.95d 12.56a</td>
<td>0.5 g 25 d 32 c 0.18b 0.05 e 11.42a</td>
</tr>
<tr>
<td>F₁</td>
<td>45</td>
<td>3.98fg 4.10ef</td>
<td>4.20def 4.27def 4.04fg 4.47d 5.67ij 6.00hij 6.68gh 7.62e 5.86hij</td>
<td>8.46d 3.04kl 3.56 j 4.25hi 3.34kl 6.31f</td>
</tr>
<tr>
<td>F₂</td>
<td>60</td>
<td>3.75g 3.99fg 4.03fg 4.09efg 4.95fg 4.13ef 5.44j 5.88hij 6.46ghi 6.90efg</td>
<td>5.57j 7.36ef 2.96l 3.46jk 4.07i 4.56h 3.12kl 5.08g</td>
<td></td>
</tr>
<tr>
<td>F₃</td>
<td></td>
<td>CV (%) 3.79</td>
<td>6.09 4.20</td>
<td></td>
</tr>
<tr>
<td>F₄</td>
<td></td>
<td>Panicle length (cm)</td>
<td>Number of grains panicle⁻¹</td>
<td>Number of sterile spikelets panicle⁻¹</td>
</tr>
<tr>
<td>F₅</td>
<td></td>
<td>CV (%) 3.36</td>
<td>2.55 3.63</td>
<td></td>
</tr>
<tr>
<td>F₆</td>
<td></td>
<td>Grain yield (t ha⁻¹)</td>
<td>Straw yield (t ha⁻¹)</td>
<td>Harvest index (%)</td>
</tr>
<tr>
<td>F₇</td>
<td></td>
<td>CV (%) 2.14</td>
<td>3.01 1.75</td>
<td></td>
</tr>
</tbody>
</table>

Mean values under each parameter having the same letter do not differ significantly whereas mean values with dissimilar letters differ significantly as per DMRT.

F₀ = Control (no manures and fertilizers), F₁ = Recommended dose of inorganic fertilizers (i.e. 150, 97, 70, 60 and 12 kg Urea, TSP, MoP, Gypsum and ZnSO₄, respectively ha⁻¹), F₂ = 50% of recommended dose of inorganic fertilizers + cowdung @ 5 t ha⁻¹, F₃ = 75% of recommended dose of inorganic fertilizers + cowdung @ 5 t ha⁻¹, F₄ = 50% of recommended dose of inorganic fertilizers + poultry manure @ 2.5 t ha⁻¹, F₅ = 75% of recommended dose of inorganic fertilizers + poultry manure @ 2.5 t ha⁻¹.
Functional relationship between leaf area index (LAI) at 60 days after transplanting (DAT) and grain yield of aromatic fine rice cv. BRRI dhan38

Leaf area index (LAI) is also an important growth parameter for the determination of yield of aromatic rice. It is closely related to the amount of photosynthesis and to the surface available for plant for photosynthesis, the basic process of higher yield. The relationship of LAI and grain yield of aromatic fine rice was determined by using the respective data of nutrient management. Experimental results revealed that grain yield showed significantly positive correlation with LAI. In figure 1, the regression equation indicates that an increase in LAI would lead to an increase in the grain yield of BRRI dhan38. The functional relationship was significant at $p \leq 0.01$. The functional relationship can be determined by the regression equation $Y = 1.0141x - 1.2327$ ($R^2 = 0.9854$). The functional relationship revealed that 99% of the variation in yield could be explained from the variation in Leaf area index at 60 DAT. Similar relationship was reported by Ray et al. (2015). This indicates that Leaf area index (LAI) might be critical growth characteristics in yield performance of aromatic rice.

![Figure 1. Functional relationship between leaf area index (LAI) at 60 DAT and grain yield aromatic fine rice cv. BRRI dhan38](image)
CONCLUSION

It can be concluded that 30-day seedlings fertilized with 75% inorganic fertilizer + poultry manure @ 2.5 t ha\(^{-1}\) may be used for better performance compared to other combinations in respect of grain yield of aromatic fine rice (cv. BRRI dhan38). In case of staggered transplanting, 60-day old seedlings fertilized with 75% inorganic fertilizer + poultry manure @ 2.5 t ha\(^{-1}\) appeared as the promising technique to obtain an appreciable grain yield of 3.39 t ha\(^{-1}\) under late transplanted condition.

REFERENCE


EFFECT OF ORGANIC AND INORGANIC NUTRIENT SOURCES ON GROWTH, YIELD AND QUALITY OF RADISH (*Raphanus sativus* L.) VARIETIES IN CHITWAN, NEPAL

S. Subedi*, A. Srivastava, M.D. Sharma & S.C. Shah
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**ABSTRACT**

A study was conducted in Horticulture farm of Agriculture and Forestry University, Rampur, Chitwan, Nepal, during November 2015 to February 2016 by using three commercial radish varieties (All Season White, Mino Early Long White and Pyuthane Red) and five nutrient sources combination of organic manure (Poultry and Farm yard manure) and recommended dose of inorganic fertilizer (100:80:40 NPK kg ha⁻¹) replicated three times in a factorial RCBD design. Most of the plant growth and yield parameters were found superior in treatments consisting poultry manure (PM) alone and in combinations with inorganic fertilizer. Treatment consisting farm yard manure (FYM) was found inferior in most of the cases. At the time of harvest, significantly higher plant height (37.5 cm), number of leaves per plant (24.77), root diameter (39.01 mm), average leaf length (35.03 cm), average leaf width (12.86 cm) was observed in treatment consisting PM (50%) and RDF (50%). Similarly, root yield (73.98 t ha⁻¹) and shoot yield (62.52 t ha⁻¹) was also found higher in the same treatment. Among the three commercial radish varieties, Mino Early Long White was found superior in most of the growth and yield parameters viz., number of leaves per plant, biological yield, root yield, root length, root diameter, marketable root, smooth root and excellent fleshyed root percentage.

**Key words:** Farm yard manure, poultry manure, quality root, radish varieties

**INTRODUCTION**

Radish (*Raphanus sativus* L.) is one of the most popular root vegetable of the world which can be grown from tropical to temperate region. It is grown for the consumption of its young fresh tender tuberous root which can be used as cooked or raw food as a salad. It contains good amount of vitamin- C and minerals like P, Ca

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and K. It also has refreshing and diuretic properties and it can be used in homeopathy for neurological, headache, sleeplessness and chronic diarrhea (Ayub, et al., 2013 and Kumar et al., 2014).

Radish is one of the most popular root vegetable of Nepal which can be grown in winter season in Terai and throughout the year in hills of Nepal. The popularity of radish cultivation could be due its wider adaptability, low cost of production, short crop duration and can be grown in almost all type of soil without much care. Diseases and pest problems are also less in radish as compare to other vegetable crops (Shrestha and Shakya, 2004). Radish is a short duration and quick growing crop, so, the root growth and development should be uninterrupted. For this, optimum nutrition should be provided through organic, inorganic and bio-fertilizer sources. Chemical fertilizers are expensive and resulted in poor health condition of soil and water if used repeatedly. So, alternative cheap organic sources of nutrients should be used (Kumar et al., 2014).

For the use of organic manure, there are no clear recommendations available. Organic manures differ in their nutrient sources, composition as well as the mineralization rate. So, precise calculation of rate of use is important. Farmers tend to apply either too much or too low organic fertilizers. Due to which soil condition is influenced and amount of nutrients provided by organic manure is either insufficient or over dose for plants (Mbatha, 2008). Appropriate variety selection and use of optimum amount of nutrient is a crucial factor for obtaining higher yield from radish. For the optimum growth of radish roots inside the soil, optimum fertilization with both inorganic and organic sources of nutrient is necessary (Chapagain, et al., 2010). This research work was mainly focus on nutrient management (both organic and inorganic) along with appropriate varietal selection of radish for the late season sowing in Terai condition.

**MATERIALS AND METHODS**

A study was conducted in Horticulture farm of Agriculture and Forestry University, Rampur, Chitwan, Nepal, during November 2015 to February 2016. The location is inner Terai (plain) area with humid sub-tropical climate. The average temperature during the field research was 20.4°C. Rainfall occurred only once during research (7.70mm). The experimental site has acidic soil with low organic matter, low nitrogen and high phosphorus content.

The experiment was carried out in a RCBD with two factors viz. three radish varieties viz., All Season White, Mino Early Long White and Pyuthane Red and five nutrient sources combinations include 100% N through recommended dose of fertilizer (RDF) for radish (100:80:40 NPK ha\(^{-1}\)) (Anonymous, 2012), 100% N through PM, 50% N through RDF + 50% N through PM, 100% N through FYM and 50% N through RDF + 50% N through FYM.
Table 1. Physico-chemical properties of soil sample of the experimental field

<table>
<thead>
<tr>
<th>Properties</th>
<th>Contents</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic matter (%)</td>
<td>1.54</td>
<td>Low</td>
</tr>
<tr>
<td>Available nitrogen (%)</td>
<td>0.08</td>
<td>Low</td>
</tr>
<tr>
<td>Available phosphorus (kg ha(^{-1}))</td>
<td>360</td>
<td>High</td>
</tr>
<tr>
<td>Available potassium (kg ha(^{-1}))</td>
<td>146</td>
<td>Medium</td>
</tr>
<tr>
<td>pH</td>
<td>5.5</td>
<td>Low (Acidic)</td>
</tr>
</tbody>
</table>

(Rating was done according to Khatri Chetri, 1991).

The FYM and PM used in this experiment were analyzed at Regional Soil Testing Laboratory, Pokhara and the results were found as following.

Table 2. Nutrient content of different organic manures used in the experiment

<table>
<thead>
<tr>
<th>Organic manures</th>
<th>Nitrogen</th>
<th>Phosphorus</th>
<th>Potash</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poultry manure</td>
<td>1.06</td>
<td>0.79</td>
<td>0.52</td>
</tr>
<tr>
<td>FYM</td>
<td>0.76</td>
<td>0.57</td>
<td>0.67</td>
</tr>
</tbody>
</table>

After final field preparation, all the organic manure and chemical fertilizer were applied before sowing and mixed well in soil. Sowing was done at 28\(^{th}\) November, 2015 at the spacing of 40 x 20 cm. Thinning, weeding, hoeing, irrigation and other intercultural operations were done as per recommended. Final harvesting was done 60 days after sowing. Ten sample plants were taken from each plot for data recording during field growth and after harvesting. The observations were recorded during filed growth, canopy height, leaf number, rosette diameter. After harvesting of the crop, root length, diameter, leaf length, width, leaf yield, root and leaf dry weight, harvest index, marketable root, smooth surfaced roots and forked roots percentage were recorded.

\[
\text{ Marketable root } \% = \frac{\text{Marketable roots}}{\text{Sampled roots}} \times 100
\]

\[
\text{ Forked root } \% = \frac{\text{Total forked roots}}{\text{Total Sampled roots}} \times 100
\]

\[
\text{ Smooth root } \% = \frac{\text{Total smooth roots}}{\text{Total Sampled roots}} \times 100
\]

All the data were analyzed by using GENSTAT 16\(^{th}\) edition and MS-EXCEL 2010.
RESULTS

Plant height and number of leaves per plant

The individual effects showed significant but interaction between varieties and nutrient sources non-significant on plant height and number of leaves per plant. (Table 1). During early growth, varieties did not show any response but at the time of harvest, highest plant height was recorded in Pyuthane Red (36.40 cm). In case of different nutrient sources, the maximum plant height was obtained from N₄ closely followed by N₂ at 25 DAS but at harvest (60DAS) treatment N₃ and N₂ were found similar. No. of leaves showed maximum in N₂ followed by N₄ but no significant difference at harvest except N₄ treatment which produced lower number of leaves/plant.

Table 1. Effect of varieties and nutrient sources on plant height and number of leaves per plant at 25 DAS and 60 DAS (harvesting) at AFU, Rampur, Chitwan, 2015-2016

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Plant height (cm)</th>
<th>No. of leaves (plant⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25 DAS</td>
<td>60 DAS</td>
</tr>
<tr>
<td>Varieties</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V₁: All Season White</td>
<td>14.46</td>
<td>33.28ᵇ</td>
</tr>
<tr>
<td>V₂: Mino Early Long White</td>
<td>14.30</td>
<td>29.31ᶜ</td>
</tr>
<tr>
<td>V₃: Pyuthane Red</td>
<td>13.89</td>
<td>36.40ᵃ</td>
</tr>
<tr>
<td>SEM ±</td>
<td>0.39</td>
<td>1.03</td>
</tr>
<tr>
<td>Nutrient sources</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N₁ (RDF₁₀₀% chemical)</td>
<td>11.17ᶜ</td>
<td>32.51ᵇᶜ</td>
</tr>
<tr>
<td>N₂ (PM₁₀₀%)</td>
<td>15.56ᵃᵇ</td>
<td>36.23ᵃᵇ</td>
</tr>
<tr>
<td>N₃ (PM₅₀% + RDF₅₀%)</td>
<td>14.30ᵇ</td>
<td>37.50ᵃ</td>
</tr>
<tr>
<td>N₄ (FYM₁₀₀%)</td>
<td>15.94ᵃ</td>
<td>27.68ᵈ</td>
</tr>
<tr>
<td>N₅ (FYM₅₀% + RDF₅₀%)</td>
<td>14.11ᵇ</td>
<td>31.06ᶜᵈ</td>
</tr>
<tr>
<td>SEM ±</td>
<td>0.50</td>
<td>1.33</td>
</tr>
<tr>
<td>CV (%)</td>
<td>10.60</td>
<td>12.10</td>
</tr>
</tbody>
</table>

Means with same letter within column do not differ significantly at p = 0.05 by DMRT.

PM = Poultry manure, FYM = Farm yard manure, SEM = Standard error of means, CV = Coefficient of variance.

Root length, diameter and root yield

The variety Mino Early Long White produced significantly highest root length (23.56 cm) and root yield (74.49 t ha⁻¹) but root diameter insignificant. Among the nutrient
sources, root length was found insignificant but root diameter showed higher in N$_3$ followed by N$_2$ and N$_5$. The maximum root yield was obtained from N$_3$ but at par to N$_2$ and N$_5$, respectively. Root yield was influenced by root length and diameter as well as poultry manure. Farm yard manure and RDF failed to show higher root yield. (Table 2).

**Leaf length, width and leaf yield**

The Pyuthane Red variety (V$_1$) was produced maximum length of leaf longer (33.9 cm) but at par to V$_3$ where wider leaves (12.23 cm) from V$_3$ followed by V$_2$ (Table 2). Among different nutrient sources, N$_3$= PM$_{50\%}$ + RDF$_{50\%}$ produced maximum length (35.03 cm) followed by N$_2$ and wider leaves (12.86 cm) from N$_3$ which ultimately resulted maximum leaf yield (62.52 t ha$^{-1}$). The interaction effect of varieties and nutrient sources was found non-significant for leaf length, width and leaf yield.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Root length (cm)</th>
<th>Root diameter (mm)</th>
<th>Root yield (t ha$^{-1}$)</th>
<th>Leaf length (cm)</th>
<th>Leaf width (cm)</th>
<th>Leaf yield (t ha$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Varieties</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V$_1$: All Season White</td>
<td>21.42$^b$</td>
<td>33.99</td>
<td>47.77$^b$</td>
<td>31.48$^a$</td>
<td>9.75$^b$</td>
<td>35.79$^b$</td>
</tr>
<tr>
<td>V$_2$: Mino Early Long White</td>
<td>23.56$^a$</td>
<td>37.78</td>
<td>74.49$^a$</td>
<td>27.77$^b$</td>
<td>11.51$^a$</td>
<td>49.58$^a$</td>
</tr>
<tr>
<td>V$_3$: Pyuthane Red</td>
<td>20.00$^b$</td>
<td>36.27</td>
<td>53.35$^b$</td>
<td>33.90$^a$</td>
<td>12.23$^a$</td>
<td>50.20$^a$</td>
</tr>
<tr>
<td><strong>SEM ±</strong></td>
<td>0.65</td>
<td>1.10</td>
<td>4.38</td>
<td>0.94</td>
<td>0.31</td>
<td>3.32</td>
</tr>
<tr>
<td><strong>Nutrient sources</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N$<em>1$ (RDF$</em>{100%}$ chemical)</td>
<td>19.99</td>
<td>33.83$^{bc}$</td>
<td>49.98$^b$</td>
<td>30.51$^b$</td>
<td>11.46$^b$</td>
<td>43.00$^{bc}$</td>
</tr>
<tr>
<td>N$<em>2$ (PM$</em>{100%}$)</td>
<td>22.44</td>
<td>37.64$^{ab}$</td>
<td>68.53$^a$</td>
<td>34.28$^a$</td>
<td>11.46$^b$</td>
<td>54.49$^{ab}$</td>
</tr>
<tr>
<td>N$<em>3$ (PM$</em>{50%}$ + RDF$_{50%}$)</td>
<td>22.53</td>
<td>39.01$^a$</td>
<td>73.98$^a$</td>
<td>35.03$^a$</td>
<td>12.86$^a$</td>
<td>62.52$^a$</td>
</tr>
<tr>
<td>N$<em>4$ (FYM$</em>{100%}$)</td>
<td>21.04</td>
<td>33.13$^c$</td>
<td>42.95$^b$</td>
<td>25.95$^c$</td>
<td>9.35$^c$</td>
<td>25.90$^d$</td>
</tr>
<tr>
<td>N$<em>5$ (FYM$</em>{50%}$ + RDF$_{50%}$)</td>
<td>22.30</td>
<td>35.46$^{abc}$</td>
<td>57.25$^{ab}$</td>
<td>29.49$^b$</td>
<td>10.67$^b$</td>
<td>40.03$^c$</td>
</tr>
<tr>
<td><strong>SEM ±</strong></td>
<td>NS</td>
<td>1.43</td>
<td>5.65</td>
<td>1.21</td>
<td>0.39</td>
<td>4.29</td>
</tr>
<tr>
<td><strong>CV (%)</strong></td>
<td>11.60</td>
<td>11.90</td>
<td>29.00</td>
<td>11.70</td>
<td>10.70</td>
<td>28.50</td>
</tr>
</tbody>
</table>

Means with same letter within column do not differ significantly at p=0.05 by DMRT.

PM = Poultry manure, FYM = Farm yard manure, SEM = Standard error of means, CV = Coefficient of variance
Dry weight of root and leaf per plant

The variety Mino Early Long White (V₂) produced significantly highest root dry weight (15.13 g plant⁻¹) and leaf dry weight (12.23 g plant⁻¹) as compared to other varieties. Nutrient sources effect was non-significant for root dry weight but in case of leaf dry weight PM₁₀₀% treatment produced maximum value (11.56 g plant⁻¹) but at par to other treatments except N₄ followed by N₅ and N₃, respectively (Table 3).

Percentage of marketable roots, smooth roots and forked roots

In case of individual factors, higher marketable roots (84.67%) and smooth surfaced roots (44.89%) were produced by Mino Early Long White and lowest percentage was produced by Pyuthane Red (68.60% and 55.36%, respectively) (Table 3). The effect of nutrient sources on marketable root, smooth root and forked root was found insignificant. The interaction effect of varieties and nutrient sources was also found non-significant.

Harvest index

The FYM containing treatments, FYM₁₀₀% (60.88%) and FYM₅₀%+RDF₅₀% (57.69%) showed significantly higher harvest index than the other treatments (Table 3). Varietal effects was also found significant in case of harvest index. Mino Early Long White (V₂) produced significantly highest harvest index (60.72%) followed by All Season White (57.27%) and Pyuthane Red (51.54%).

Table 3. Effect of varieties and nutrient sources on root and leaf dry weight, marketable, smooth, forked root percentage and harvest index at AFU, Rampur, Chitwan, 2015/2016

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Root dry weight (g plant⁻¹)</th>
<th>Leaf dry weight (g plant⁻¹)</th>
<th>Marketable root (%)</th>
<th>Smooth root (%)</th>
<th>Forked root (%)</th>
<th>Harvest index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Varieties</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V₁: All Season White</td>
<td>11.00b</td>
<td>8.43b</td>
<td>84.67a</td>
<td>67.94b</td>
<td>31.28</td>
<td>57.27b</td>
</tr>
<tr>
<td>V₂: Mino Early Long White</td>
<td>15.13a</td>
<td>12.23a</td>
<td>94.33a</td>
<td>84.89a</td>
<td>37.72</td>
<td>60.72a</td>
</tr>
<tr>
<td>V₃: Pyuthane Red</td>
<td>10.67b</td>
<td>8.47b</td>
<td>68.60b</td>
<td>55.36b</td>
<td>33.69</td>
<td>51.54c</td>
</tr>
<tr>
<td>SEM ±</td>
<td>1.00</td>
<td>0.82</td>
<td>4.47</td>
<td>5.81</td>
<td>6.61</td>
<td>1.08</td>
</tr>
<tr>
<td>Nutrient sources</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N₁ (RDF₁₀₀%-chemical)</td>
<td>11.33</td>
<td>10.56a</td>
<td>81.94</td>
<td>66.39</td>
<td>35.56</td>
<td>53.93b</td>
</tr>
<tr>
<td>N₂ (PM₁₀₀%)</td>
<td>13.00</td>
<td>11.56a</td>
<td>89.60</td>
<td>70.87</td>
<td>34.21</td>
<td>56.02b</td>
</tr>
<tr>
<td>Treatments</td>
<td>Root dry weight (g plant(^{-1}))</td>
<td>Leaf dry weight (g plant(^{-1}))</td>
<td>Marketable root (%)</td>
<td>Smooth root (%)</td>
<td>Forked root (%)</td>
<td>Harvest index</td>
</tr>
<tr>
<td>---------------------------</td>
<td>-------------------------------------</td>
<td>------------------------------------</td>
<td>---------------------</td>
<td>-----------------</td>
<td>-----------------</td>
<td>---------------</td>
</tr>
<tr>
<td>N(<em>3) (PM(</em>{50%}) + RDF(_{50%}))</td>
<td>13.78</td>
<td>9.94(^{ab})</td>
<td>77.78</td>
<td>69.44</td>
<td>49.17</td>
<td>54.03(^{b})</td>
</tr>
<tr>
<td>N(<em>4) (FYM(</em>{100%}))</td>
<td>11.89</td>
<td>6.89(^{b})</td>
<td>85.56</td>
<td>74.17</td>
<td>23.33</td>
<td>60.88(^{a})</td>
</tr>
<tr>
<td>N(<em>5) (FYM(</em>{50%}) + RDF(_{50%}))</td>
<td>11.33</td>
<td>9.61(^{ab})</td>
<td>77.78</td>
<td>66.11</td>
<td>28.89</td>
<td>57.69(^{ab})</td>
</tr>
<tr>
<td>SEM(^{±})</td>
<td>1.30</td>
<td>1.05</td>
<td>5.78</td>
<td>7.50</td>
<td>8.53</td>
<td>1.39</td>
</tr>
<tr>
<td>CV (%)</td>
<td>31.90</td>
<td>32.60</td>
<td>21.00</td>
<td>32.40</td>
<td>74.80</td>
<td>7.40</td>
</tr>
</tbody>
</table>

Means with same letter within column do not differ significantly at p=0.05 by DMRT.
PM = Poultry manure, FYM = Farm yard manure, SEM = Standard error of means, CV = Coefficient of variance

**Variatral comparison**

The varietal comparison of radish was done on the basis of various morphological and sensory characters. The detail comparison is given below.

Table 4. Characterization of three commercial radish varieties at AFU, Rampur, Chitwan, 2015-2016

<table>
<thead>
<tr>
<th>Characterization</th>
<th>All season white</th>
<th>Mino early long white</th>
<th>Pyuthane red</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf attitude</td>
<td>Semi erect</td>
<td>Spreading</td>
<td>Erect</td>
</tr>
<tr>
<td>Leaf colour</td>
<td>Dark green</td>
<td>Faint green</td>
<td>Green</td>
</tr>
<tr>
<td>Hairiness of leaf</td>
<td>Rare</td>
<td>Dense</td>
<td>Rare</td>
</tr>
<tr>
<td>Nature of spine</td>
<td>Soft</td>
<td>Soft</td>
<td>Soft</td>
</tr>
<tr>
<td>Shape of leaf segment</td>
<td>Triangular</td>
<td>Triangular</td>
<td>Triangular</td>
</tr>
<tr>
<td>Leaf shape</td>
<td>Flat round</td>
<td>Flat round</td>
<td>Oval</td>
</tr>
<tr>
<td>Leaf margin</td>
<td>Serrated</td>
<td>Serrated</td>
<td>Entire</td>
</tr>
<tr>
<td>Spinyness of the stalk</td>
<td>Rare</td>
<td>Dense</td>
<td>Absent</td>
</tr>
<tr>
<td>Colour of stalk</td>
<td>Green</td>
<td>Green</td>
<td>Red</td>
</tr>
<tr>
<td>Root shape</td>
<td>Long</td>
<td>Long</td>
<td>Medium</td>
</tr>
<tr>
<td>Skin colour</td>
<td>White</td>
<td>White</td>
<td>Red</td>
</tr>
<tr>
<td>Flesh colour</td>
<td>White</td>
<td>White</td>
<td>Reddish white</td>
</tr>
<tr>
<td>Shape of root shoulder</td>
<td>Convex</td>
<td>Convex</td>
<td>Convex</td>
</tr>
<tr>
<td>Pungency of root</td>
<td>Moderate pungent</td>
<td>Moderate pungent</td>
<td>Less pungent</td>
</tr>
<tr>
<td>TSS ((^{0}) Brix)</td>
<td>5.12</td>
<td>5.22</td>
<td>6.14</td>
</tr>
</tbody>
</table>
DISCUSSION

The Pyuthane red variety has longer and entire leaves with erect growth habit, which produced highest plant height, leaf length, width and leaf yield, while All Season White and Mino Early Long White has semi-erect growth habit and serrated leaves. Highest number of leaves was produced by Mino Early Long White which ultimately produced highest leaf dry weight per plant. Similarly, longer and wider roots with maximum root yield was produced by same variety which performed better in Chitwan condition than the other two varieties. Besides, the variety Mino Early Long White performed better than other two varieties due to higher percentage of marketable roots and smooth surfaced roots. Pyuthane Red variety performed poor in Chitwan condition due to lowest percentage of marketable and smooth surfaced roots. The variety All Season White was produced lowest percentage of forked roots.

Among the different nutrient sources used, PM containing treatments performed best than other treatments either alone or in combination with chemical fertilizer. Treatment comprises of 50% PM and 50% RDF produced longer and wider leaves as well as roots which gives ultimately highest yield of root and leaf. Higher plant height was observed in PM treated plots alone and with combination with inorganic fertilizer than the other treatments. This might be due to the higher nutrient content (NPK and other micro nutrients) and higher organic matter content of PM which are essential for plant growth. Similar results also reported by Zeid et al., 2015, who also found highest plant height of radish when poultry manure is used either alone or in combination with inorganic fertilizer.

The results showed that, longer and wider roots were produced by treatments consisting poultry manure alone or in combination with chemical fertilizer produced longest and widest roots. Production of longest and widest roots by PM consisting treatments leads to highest leaf and root yield. The applied organic manure improves soil physical condition by increasing soil porosity and decreasing bulk density which make suitable for smooth root penetration and growth inside the soil. The chemical fertilizer used along with organic manure supports the plant growth and contributes to higher yield. Among the different organic manure, PM helps to improve soil condition, increase water holding capacity of soil and provide more macro as well as micro nutrients than FYM. PM produced better results than pure inorganic fertilizer treatment, because it can provide all 13 types of soil micronutrients in considerable amount which inorganic fertilizer can’t provide (Chastain, et al., 1999). Sylvestre et al. (2015) also reported that highest root yield of carrot was found when treated with PM and inorganic fertilizer as well as poultry manure leads to ease of root penetration and good soil moisture.

CONCLUSION

From this experiment the hybrid variety mino early long white of radish performed best in Chitwan late sowing condition due to higher yield and quality production.
Besides, poultry manure should be used along with inorganic fertilizer with precise calculation of recommended dose of nutrition. Poultry manure alone can produce better results than chemical fertilizer alone.

ACKNOWLEDGEMENTS
This research was supported under grant from Department of Research and Extension (DOREX). I would like to express my sincere gratitude to Department of Horticulture for providing an opportunity to conduct this research and express my sincere appreciation to Campus Administration. I would like to thank my advisory team especially Prof. Dr. Arvind Srivastava for his inspirations and assistances during research and manuscript preparation.

REFERENCES
ON-FARM YIELD PERFORMANCE OF IMPROVED VARIETIES OF VEGETABLES IN SYLHET REGION

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ABSTRACT

A total of five separate field trials were conducted at farm farmers’ field in Sylhet area during three consecutive crops seasons of 2014-15, 2015-16 and 2016-17, respectively to evaluate the yield performance of improved varieties with the existing cultivars of five vegetables at farmers' field. Each experiment was laid out in randomized complete block design with six dispersed replications. The unit plot size was 5m x 8m. The results showed that improved variety of tomato (var. BARI Tomato-14) produced higher average fruit (55.60 t ha$^{-1}$) yield with the yield increase of 16.93% over control. In case of country bean, the local variety Goalgadda performed better and produced higher green pod yield (14.31 t ha$^{-1}$) compared to that of BARI Sheem-6. The brinjal variety BARI Bt Begun-2 was the best yielder with an average fruit yield of 25.62 t ha$^{-1}$ i.e. 107.62% increase over non-Bt as check. In case of yield trial with Capsicum, locally grown cultivar California Wonder performed better and produced comparatively higher yield (14.02 t ha$^{-1}$) than var. BARI Mistimorich-1. In case of summer hyacinth bean viz., BARI Sheem-7 gave higher pod yield of 14.96 t ha$^{-1}$ compared to that of the check variety (11.41 t ha$^{-1}$) in researcher-managed trial.

Keywords: Country bean, summer hyacinth bean, capsicum, tomato, Bt begun, acidic soil

INTRODUCTION

Bangladesh has considerable potential for growing horticultural crops (Shahabuddin and Dorosh, 2002, Alam, 2005). The Sylhet region is mostly under the agro-ecological zone 20 (Eastern Surma Kushiyara Floodplain) and the soils of this region are strongly acidic (pH 4.5-5.5). The climate of this region is suitable for potato, tomato, cabbage, aroids and other vegetable production (Nazrul and Shaheb, 2014, Nazrul et al., 2013, Nazrul et al., 2013, Shaheb et al., 2012, Sarker, et al., 2012). In addition, agriculture is the only economic activity of most small farmers in this region. The region produces vegetables not sufficient enough to fulfill the local demand over the

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year. As a result, the price of vegetable is always higher in the market and the demand is fulfilled through the supply from other parts of the country. Vegetable growers often earn higher incomes than those of cereal crops alone. But improved production technologies such as high yielding varieties and improved cultivation practices are not available to the farmers in right time. Many new vegetable varieties are now available to the growers from different sources. But performance of those varieties with improved management practices is not available to the farmers for cultivation under different soil and climatic conditions of the country. Therefore, for creation of awareness among the vegetable farmers about the high yielding varieties along with their production technologies on-farm trial is necessary. Hence, filed trials were conducted with objective to evaluate the yield performance of improved varieties of five vegetables with their existing cultivars at farmers' field in Sylhet region.

**MATERIALS AND METHODS**

A total of five separate field trials were conducted at farmers’ field in Sylhet area for the three consecutive crops seasons of 201-15, 2015-16 and 2016-17 to evaluate the yield performance of improved varieties with the existing cultivars of five vegetables. The study area lies at 24°70' N latitude and 91°67' E longitude under the Surma-Kushiyara Floodplain of Bangladesh. The soil of experimental plots was non-calcareous gray with low organic matter content (1.23%), low soil pH (4.5-5.3), very low total N (0.06%), low content of P (9.46), K (0.13) and S (10.07) where as Zn (1.13) and Boron (0.51) medium and optimum; respectively (Table 1). Each experiment was laid out in randomized complete block design with six dispersed replications. The unit plot size was 5m x 8m.

The monthly average, maximum and minimum temperature of the experimental site are indicated in figure 1. The climatic data of Sylhet shows that the mean annual minimum temperature is11.55°C and the mean annual maximum temperature is 34.23°C and the annual mean temperature nearly is 17.54 °C.

As indicated in figure 2, rainfall of the area is uni-modal, usually occurring during April to October, and total annual rainfall reached to 4217 mm; whereas in December no rain at all and the lowest amount of rainfall occurred in January followed by February. However, during rest of the months, total rainfall was ranged from 100 to just below 800 mm. Rainfall increased gradually from the month of May and continued up to September.
Table 1. Chemical properties of experimental soil

<table>
<thead>
<tr>
<th>Replications</th>
<th>pH</th>
<th>OM (%)</th>
<th>Total N (%)</th>
<th>K (meq/100g soil)</th>
<th>P (µg/g soil)</th>
<th>S</th>
<th>Zn</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.40</td>
<td>1.56</td>
<td>0.07</td>
<td>0.11</td>
<td>2.46</td>
<td>10.80</td>
<td>1.24</td>
<td>0.33</td>
</tr>
<tr>
<td>2</td>
<td>4.50</td>
<td>1.07</td>
<td>0.05</td>
<td>0.12</td>
<td>12.53</td>
<td>12.31</td>
<td>1.11</td>
<td>0.51</td>
</tr>
<tr>
<td>3</td>
<td>5.53</td>
<td>0.98</td>
<td>0.06</td>
<td>0.16</td>
<td>11.32</td>
<td>8.31</td>
<td>1.00</td>
<td>0.56</td>
</tr>
<tr>
<td>4</td>
<td>5.51</td>
<td>1.26</td>
<td>0.06</td>
<td>0.11</td>
<td>11.99</td>
<td>8.31</td>
<td>1.15</td>
<td>0.54</td>
</tr>
<tr>
<td>5</td>
<td>4.70</td>
<td>1.21</td>
<td>0.07</td>
<td>0.14</td>
<td>9.87</td>
<td>11.52</td>
<td>1.08</td>
<td>0.62</td>
</tr>
<tr>
<td>6</td>
<td>5.48</td>
<td>1.27</td>
<td>0.06</td>
<td>0.13</td>
<td>8.59</td>
<td>9.14</td>
<td>1.22</td>
<td>0.48</td>
</tr>
<tr>
<td>Average</td>
<td>-</td>
<td>1.23</td>
<td>0.06</td>
<td>0.13</td>
<td>9.46</td>
<td>10.07</td>
<td>1.13</td>
<td>0.51</td>
</tr>
</tbody>
</table>

SA = Low, VL = Very Low, L = Low, M = Medium, OP = Over Potent

Source: Bangladesh Metrological Department, Sylhet.

Figure 1. Minimum, maximum and mean temperatures (°C) pattern in Sylhet of Bangladesh
Tomato

Two improved tomato varieties viz. BARI Tomato-14 and BARI Tomato-15 were tested with locally popular hybrid variety Raja, seeds was purchased from local market. The crop was fertilized with 151-58-52-23-0.5 kg ha\(^{-1}\) of NPKSzn (FRG, 2012) and cow dung at 5 t ha\(^{-1}\). Half of cow dung and P & entire amount of S and Zn were applied during final land preparation. The remaining half of cow dung and P was applied to the pits before a week of planting. N and K were applied in 3 equal installments at 21, 35 and 50 days after seedling transplanting. Thirty days old seedlings were planted during 25-30 November with a spacing of 60 cm x 40 cm. Fruits were harvested during 20 January to 20 March in each year. Three irrigations at 25 days interval from the transplanting of seedlings; one weeding at 20 DAP; two sprays of Score fungicide @ 1.5 m l\(^{-1}\) for controlling late blight disease and all other intercultural activities were carried out as and when needed.

Country bean

Two varieties of country bean viz. BARI Sheem-6 and local check (Goalgadda) were evaluated. The trial was laid out in RCB design with four dispersed replications. The unit plot size was 5 m x 8 m. The pit was prepared at a spacing of 1.5 m x 1.5 m. The fertilizers were used @ 28-40-75-12-2-1kg ha\(^{-1}\) of NPKSznB and cow dung @ 5 t ha\(^{-1}\) was used. Half of the quantity of cow dung was applied during final land preparation. The remaining half of the cow dung, the entire amount of P, Zn and B and half of N and K were applied during pit preparation. The rest of N and K were applied as top dressing at 30 days after planting. Intercultural operations like watering, stalking, preparation of trellis were done as and when necessary for each
dispersed replications. Seeds were sown during 25-29 August and harvesting started from third week of November and continued up to first week of March in each year.

**Brinjal**

Two Bt brinjal varieties *viz.* BARI Bt Begun-1 and BARI Bt Begun-2 against non-Bt counterparts *viz.* BARI Begun-1 and BARI Begun-4 was evaluated. One row of non-Bt counterpart of each variety was planted as border crops. The unit plot size was 20 m × 20 m for each Bt Begun variety with spacing of 100 cm × 80 cm. Stable bleaching powder @ 25 kg ha⁻¹ was applied 20 days before transplanting as a preventive measure against bacterial wilt. Thirty days old seedlings were planted during 17-22 November and fruits were harvested from 19 February to 25 April in each year. NPKSBZn @ 138-40-100-18-1.7-3.6 kg ha⁻¹ of and cow dung 10 t ha⁻¹ were used. One third MoP and rest of the fertilizers except urea were applied during final land preparation. Remaining two-third of MoP was divided into three splits and applied at 20 DAP, at flowering and fruiting stage. Urea was applied in four equal installments at 20 DAP, at flowering and two times at fruiting stage. Plant protection measures and other intercultural operations were taken as a when necessary as per recommendation.

**Capsicum**

The capsicum var. BARI Mistimorich-1 was evaluated with locally popular cultivated hybrid variety California Wonder; seeds were purchased from local market. Thirty 30 days old seedlings of capsicum were planted during 22-26 November and raised under nylon net at seed bed. The unit plot size was 5m×8 m with maintaining the spacing of 50 cm × 40 cm. The crop was fertilized with 100-66-100-20-2-5000 kg ha⁻¹ of NPKSZn (FRG, 2012). Half of N, full dose of other fertilizers and cow dung were applied as basal in the form of urea, triple super phosphate, muriate of potash, gypsum and zinc sulphate, respectively. The remaining N was top dressed at 25 and 50 days after transplant of seedlings followed by irrigation. Plant protection and management practices were followed as per recommendation (Azad et al., 2017). Harvesting of fruits was started from 24 January and continued up to 29 March each year.

**Summer country bean**

Performance of BARI Sheem-7 was compared with locally popular Patasheem. The unit plot size was 8 m x 10 m with 6 dispersed replications. The pit was prepared by maintaining spacing of 1.5mx 1.5 m. The seeds of both varieties were sown in the pits during 10-15 April. NPKS fertilizers @ 28-40-75-5 kg ha⁻¹ of along with cow dung @10 t ha⁻¹ were applied. The entire amount of cow dung and gypsum was applied during final land preparation. The total quantity of P and half of N and K were applied at 4-5 days before sowing seeds to the pits and mixed thoroughly with soils. The rest of N and K were applied as top dressing at 30 days after seed sowing. Intercultural operations like watering, staking, preparation of trellis were done as and
when necessary for each dispersed replications. The insecticide Sumithion @1.5 ml l^{-1} of water was applied for controlling Jassid and white fly. The flowering started during first week of July and harvesting started during second week of July in each year.

The yield data were rerecorded on whole plot basis and mean values were adjudged using least significant difference (LSD) test or using t-test in case of two treatments at 5% level of significance.

**RESULTS AND DISCUSSIONS**

**Tomato**

The marketable fruit yields of tested tomato varieties grown under trial are presented in table 2. The results revealed that var. BARI Tomato-14 and BARI Tomato-15 fetched an average fruit yield of 55.60 and 48.93 t ha^{-1}, respectively against 47.55 t ha^{-1} in local check. Similar findings were reported by Anonymous, (2016). The results revealed that BARI Tomato-14 exhibited the best performance at farmer’s field. The data showed that significant increase of tomato yield i.e. up to 16.93% over control. The fruit yield increase of 16.93% in case of var. BARI Tomato-14 followed by 2.90% in BARI Tomato-15.

Table 2. Fruit yield of winter tomato varieties at farmer’s field under acidic soil of Sylhet during 2014-15, 2015-16 and 2016-17

<table>
<thead>
<tr>
<th>Variety</th>
<th>Fruit yield (t ha^{-1})</th>
<th>Average fruit yield (t ha^{-1})</th>
<th>Fruit yield increased over local control variety (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2014-15</td>
<td>2015-16</td>
<td>2016-17</td>
</tr>
<tr>
<td>BARI Tomato-14</td>
<td>63.11</td>
<td>51.50</td>
<td>52.20</td>
</tr>
<tr>
<td>BARI Tomato-15</td>
<td>55.48</td>
<td>45.30</td>
<td>46.00</td>
</tr>
<tr>
<td>Raja (hybrid)</td>
<td>53.80</td>
<td>43.07</td>
<td>45.77</td>
</tr>
<tr>
<td>Local</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CV (%)</td>
<td>4.25</td>
<td>3.93</td>
<td>7.53</td>
</tr>
<tr>
<td>LSD (0.05%)</td>
<td>5.54</td>
<td>1.83</td>
<td>2.53</td>
</tr>
</tbody>
</table>

**Country bean**

Locally adopted variety performed better and produced higher yield of green pod (14.31 t ha^{-1}) compared to that of BARI Sheem-6 (11.64 t ha^{-1}) in during the trial years. An average fruit yield of 14.31 t ha^{-1} was recorded with 18.24% low yield over local check variety. The yield decrease indicating high feasibility of its adoption among farmers. This variety has become most popular in the area which is exporting aboard as commercial variety.
Table 3. Green pod yield of country bean varieties at farmer’s field under acidic soil of Sylhet during 2014-15, 2015-16 and 2016-17

<table>
<thead>
<tr>
<th>Variety</th>
<th>Green pod yield (t ha$^{-1}$)</th>
<th>Average pod yield (t ha$^{-1}$)</th>
<th>Yield decreased over local (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2014-15</td>
<td>2015-16</td>
<td>2016-17</td>
</tr>
<tr>
<td>BARI Sheem-6</td>
<td>11.73</td>
<td>11.85</td>
<td>11.35</td>
</tr>
<tr>
<td>Local check (Goalgadda)</td>
<td>14.55</td>
<td>14.86</td>
<td>13.52</td>
</tr>
<tr>
<td>t-value</td>
<td>4.42</td>
<td>4.52</td>
<td>3.15</td>
</tr>
</tbody>
</table>

**Brinjal**

Over the three years trial period, Bt varieties performed better and produced comparable higher fruit yields than non- Bt varieties. The highest average fruit yields 25.62 and 22.84 t ha$^{-1}$ were produced by BARI Bt Begun-2 and BARI Bt Begun-1, respectively; while non-Bt was the lowest yielder. The results are fully in agreement with the finding of trials conducted in different areas of Bangladesh (Anonymous, 2015). The results revealed that brinjal var. BARI Bt Begun-2 produced on an average yield of 25.62 t ha$^{-1}$ with 107.62% yield increase over non-Bt as check.

Table 4. Fruit yield of Bt Begun varieties at farmer’s field under acidic soil of Sylhet during 2014-15, 2015-16 and 2016-17.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Fruit yield (t ha$^{-1}$)</th>
<th>Average fruit yield (t ha$^{-1}$)</th>
<th>Fruit yield increase over non-Bt(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2014-15</td>
<td>2015-16</td>
<td>2016-17</td>
</tr>
<tr>
<td>BARI Bt Begun-1</td>
<td>23.80</td>
<td>21.40</td>
<td>23.11</td>
</tr>
<tr>
<td>BARI Begun-1</td>
<td>11.20</td>
<td>13.38</td>
<td>12.20</td>
</tr>
<tr>
<td>BARI Bt Begun-2</td>
<td>26.30</td>
<td>25.09</td>
<td>25.47</td>
</tr>
<tr>
<td>BARI Begun-4</td>
<td>12.80</td>
<td>12.42</td>
<td>11.79</td>
</tr>
<tr>
<td>CV (%)</td>
<td>5.98</td>
<td>14.57</td>
<td>4.74</td>
</tr>
<tr>
<td>LSD$_{(0.05)}$</td>
<td>1.53</td>
<td>4.11</td>
<td>2.18</td>
</tr>
</tbody>
</table>
Capsicum
The results revealed that California Wonder (Local check) performed better and produced higher fruit yields than BARI Mistimorich-1. The former variety provided maximum average fruit yield (14.02 t ha\(^{-1}\)) with 22.77 % increase yield over the var. BARI Mistimorich-1.

Table 5. Fruit yield performance of capsicum varieties at farmer’s field under acidic soil of Sylhet during 2014-15, 2015-16, 2016-17

<table>
<thead>
<tr>
<th>Variety</th>
<th>Fruit yield (t ha(^{-1}))</th>
<th>Average fruit yield (t ha(^{-1}))</th>
<th>Yield decreased over local (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2014-15</td>
<td>2015-16</td>
<td>2016-17</td>
</tr>
<tr>
<td>BARI Mistimorich-1</td>
<td>11.48</td>
<td>11.76</td>
<td>11.03</td>
</tr>
<tr>
<td>Local check (California Wonder)</td>
<td>13.92</td>
<td>14.45</td>
<td>13.68</td>
</tr>
<tr>
<td>t-value</td>
<td>2.43</td>
<td>2.00</td>
<td>4.03</td>
</tr>
</tbody>
</table>

Summer country bean
Performance of summer country bean var. BARI Sheem-7 was compared with locally popular cultivar Patasheem as check. It was observed that BARI Sheem-7 performed better with 31.11 % increase yield over existing local cultivar. Similar findings were also reported by Islam et al. (2015) in field crops under charland situations.

Table 6. Fruit yield performance of summer country bean varieties at farmer’s field under acidic soil of Sylhet during 2014-15, 2015-16 and 2016-17

<table>
<thead>
<tr>
<th>Variety</th>
<th>Fruit yield (t ha(^{-1}))</th>
<th>Average fruit yield (t ha(^{-1}))</th>
<th>Yield increase over local cultivar(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2014-15</td>
<td>2015-16</td>
<td>2016-17</td>
</tr>
<tr>
<td>BARI Sheem-7</td>
<td>15.36</td>
<td>15.46</td>
<td>14.07</td>
</tr>
<tr>
<td>Local check (Patasheem)</td>
<td>11.69</td>
<td>11.87</td>
<td>10.68</td>
</tr>
<tr>
<td>t-value</td>
<td>5.04</td>
<td>3.83</td>
<td>5.63</td>
</tr>
</tbody>
</table>
CONCLUSION

The results of the trials conducted for 3-years revealed that tomato var. BARI Tomato-14; brinjal var. BARI Bt Begun-2; locally grown Capsicum var. California Wonder and summer country bean var. BARI Sheem-7 performed better under the soil and climatic conditions of Sylhet region.

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DETERMINATION OF MULTIPLE ORGANOCHLORINE PESTICIDE RESIDUES IN SHRIMP USING MODIFIED QuEChERS EXTRACTION AND GAS CHROMATOGRAPHY

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ABSTRACT

Determination of organochlorine pesticide residues in shrimp is very important to ensure the consumer’s safety and to fulfill the importer’s demand. Therefore, a simple and efficient multiple organochlorine pesticide residues analytical method using quick, easy, cheap, effective, rugged and safe (QuEChERS) extraction technique and Gas Chromatography coupled with Electron Capture Detector (ECD) has been developed and validated for the determination of 19 organochlorine pesticides (α- BHC, δ- BHC, β- BHC, γ- BHC, Heptachlor, Aldrin, Heptachlor Epoxide, γ- Chlordane, α- Chlordane, α- Endosulfan, 4,4 DDE, Dieldrin, Endrin, 4,4 DDD, β- Endosulfan, 4,4 DDT, Endosulfan sulphate, Methoxychlor, and Endrin Ketone) in shrimp. The method was validated by evaluating the accuracy, precision, linearity, limit of detection (LOD) and limit of quantification (LOQ). The average recoveries of the selected pesticides ranged from 84% to 106% with RSDr ≤ 14% in four fortification levels of 0.05, 0.1, 0.2 and 0.3 mg kg⁻¹. The linearity was ≥ 0.996 for all of the selected pesticides with matrix matched calibration standards. The LOD ranged from 0.003 to 0.009 mg kg⁻¹ and the LOQ was 0.05 mg kg⁻¹. This method was applied successfully for the residue analysis of 40 shrimp samples collected from different regions in Bangladesh.

Keywords: Shrimp, organochlorine pesticide residues, modified QuEChERS extraction, GC-ECD, method validation.

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INTRODUCTION

Fish play a crucial role in the Bangladeshi diet, providing more than 60% of animal source food, representing a crucial source of micro-nutrients, and possessing an extremely strong cultural attachment. Fish (including shrimp and prawn) is the second most valuable agricultural crop in Bangladesh. The culture and consumption of fish therefore has important implications for national food and nutrition security, poverty and growth (Belton et al., 2011). The prime source of high-quality protein is fish, which provides 14–16% of the animal protein consumed worldwide. Over one billion people across the world consume fish as their primary source of animal protein (Helfman et al., 1997). Thus fish either harvested from natural source(s) or cultured artificially and the fish products have great importance as human food worldwide. The fisheries sector especially shrimp production in Bangladesh plays a significant role in providing employment to rural poor, reducing poverty and enhancing export earning. The sector annually contributes 544 million US Dollar to the national economy. The shrimp industry also provides direct employment to over 1 million people. Increasingly stringent standards for food safety rules are being adopted by EU, USA and Japan, the three main importers of shrimp form Bangladesh. Kaphalia et al. (1990) reported that the majority of people were indirect consumers of pesticides through food intake.

In Bangladesh, shrimp production is linked with rice cultivation. For the cultivation of rice, the farmers of our country are using pesticides mostly belonging to organocarbamate, organophosphate and synthetic pyrethroid pesticides. In the long past organochlorine pesticides (OCPs) like endrin were used in rice while other OCPs were used legally in other crops until 1977 when the last OC insecticide heptachlor was banned. The OCPs are lipophilic in nature, their hydrophobicity, low chemical and biological degradation rates have led to their widespread accumulation in food chain (John et al., 2001; Bedi et al., 2005; Aulakh et al., 2006). The exposure of OCPs in humans creates severe health hazards particularly breast cancer, testicular cancer, endocrine dysfunction, births defects, lower sperm count (Brody and Rudel, 2003; Ahmed et al., 1996; Garry et al., 2004; Soto et al., 1998).

Most of the organochlorine pesticides (OCPs) were banned in 1970s for their long persistence in the environment (Annonymous, 1979 and Annonymous, 1989). But because of their long persistence, OCPs are still detectable in fish from various waterways (Zhang et al., 2014; Prodhan et al., 2010; Prodhan et al., 2009; Kaur et al., 2008; Antunes and Gil 2004; Osuna-Flores and Riva, 2001; Chan et al., 1999; Berg et al., 1999; Sapozhnikova et al., 2004). Although not as persistent in the environment as OC pesticides, many pesticides of the other three groups are also suspected to be present in fish samples. As required by importing countries as well as for our own need it is important to know the pesticide residue status in shrimp.
In order to detect and quantify pesticide residues quickly and easily, multi-residue methods are required. The multi-residue methods used for the analysis of pesticide residues should be validated prior to analyze the samples. In the analysis of pesticide residues, effective extraction and clean-up techniques are essential. Nowadays, the quick, easy, cheap, effective, rugged and safe (QuEChERS) technique, which was first introduced by Anastassiades et al., 2003, is widely used for the extraction and clean-up of food matrices (Anastassiades et al., 2003). Therefore, the QuEChERS extraction techniques followed by GC-ECD were chosen for the determination of OCPs in shrimp. Up until now, few multi-residue methods were developed for the determination of OCPs in shrimp (Osuna-Flores and Riva, 2001; Zhang et al., 2014). However, in this developed method we have incorporated a large number of OCPs that were not incorporated with the previously developed methods. With this view, the present study was initiated to develop and validate an analytical method for the determination of 19 organochlorine pesticide residues in shrimp and to monitor OCPs residues status in shrimp in Bangladesh.

MATERIALS AND METHODS

Chemicals and reagents

Reference standards of Organochlorine Pesticide Mix (α- BHC, δ- BHC, β- BHC, γ-BHC, Heptachlor, Aldrin, Heptachlor Epoxide, γ- Chlordane, α- Chlordane, α-Endosulfan, 4,4 DDE, Dieldrin, Endrin, 4,4 DDD, β- Endosulfan, 4,4 DDT, Endosulfan sulphate, Methoxychlor, and Endrin Ketone) were obtained from SIGMA-Aldrich, Germany through SF Scientific, Dhaka, Bangladesh. Analytical grade Acetonitrile (MeCN), methanol, Sodium chloride (NaCl), anhydrous magnesium sulphate (MgSO₄) and Primary Secondary Amine (PSA) were also obtained from SIGMA-Aldrich, Germany through SF Scientific, Dhaka, Bangladesh.

Sample preparation procedures

The quick, easy, cheap, effective, rugged and safe (QuEChERS) extraction technique, which was first introduced by Anastassiades et al. (2003), is widely used for the extraction and cleanup of food matrices. The QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method modified by Prodhan MDH et al. (2015) was used for the extraction and clean-up of organochlorine pesticide residues from shrimp matrix. The method is described below:

Ten gm of properly homogenized eggplant sample was taken in a 50ml screw-capped polypropylene centrifuge tube and 10 ml acetonitrile (MeCN) was added into the centrifuge tube. The centrifuge tube was closed properly and shaken vigorously for 30 sec. by vortex mixer. Then 4g anhydrous MgSO₄, 1g NaCl were added into the centrifuge tube and it was shaken by vortex mixer for 1 minute. Afterwards, the
extract was centrifuged for 5 min at 5000 rpm. An aliquot of 3 ml of the MeCN layer was transferred into a 15 ml micro centrifuge tube containing 600 mg anhydrous MgSO₄ and 120 mg Primary Secondary Amine (PSA). The content of the centrifuge tube was thoroughly mixed by vortex for 30 sec. and centrifuged for 5 minutes at 4000 rpm. After centrifuge, a 1 ml supernatant was filtered by a 0.2 µm PTFE filter, and then it was taken in a clean HPLC vial for injection.

Preparation of pesticide standard solution

Mixed pesticide standard stock solutions of α- BHC, δ- BHC, β- BHC, γ- BHC, Heptachlor, Aldrin, Heptachlor Epoxide, γ- Chlordane, α- Chlordane, α- Endosulfan, 4,4 DDE, Dieldrin, Endrin, 4,4 DDD, β- Endosulfan, 4,4 DDT, Endosulfan sulphate, Methoxychlor, and Endrin Ketone were prepared in hexane: toluene (50:50) at a concentration of 200 mg l⁻¹ and stored at -20°C until use. An intermediate mixed standard solution of 10 mg l⁻¹ in acetone was prepared from the mixed standard solution of 200 mg l⁻¹. Then working standard solutions of 0.5, 1.0, 2.0, 3.0, and 5.0 mg l⁻¹ in acetonitrile were prepared by transferring the appropriate amount from 10 mg l⁻¹ intermediate mixed standard solution into five separate 5-ml volumetric flasks.

Preparation of matrix matched calibration standard solution

Matrix matched calibration standards were prepared by adding 100 µl of the mixed pesticide standards working solutions of 0.5, 1.0, 2.0, 3.0, and 5.0 mg l⁻¹ and 900 µl of the blank extract to reach the final concentrations of 0.05, 0.10, 0.2, 0.3 and 0.5 mg l⁻¹, respectively. Calibration standards in acetonitrile having the same concentrations as in the matrix matched calibration standards were also prepared. All the standard solutions were kept in a freezer at -20°C until use. A typical chromatogram containing 19 organochlorine pesticides prepared with matrix-matched calibration standard is presented in figure 1.

Operating condition of GC

A Gas Chromatograph (GC-2010 Shimadzu) coupled with Electron Capture detector (GC-ECD) was used for the identification and quantification of selected organochlorine pesticides. Separations were done by RTX-CL capillary column (30 m long, 0.25 mm i.d. and 0.25 µm film thicknesses), nitrogen was used as carrier (column flow 1.5 ml/min.) and make up gas as well. The injector and detector temperatures were set to 250 °C and 330 °C, respectively and the column oven temperature was programmed, which was started from 180 °C and went up to 220°C with incremental rate of 5 °C (12 min hold), then it raised to 260°C with incremental rate of 5 °C. All the injections (1 µl) were done in spit mode. The total run time was 28 min. Identification of the analyte in the samples was done by comparing the retention time of the corresponding matrix matched calibration standard and quantification was done by external calibration curves maid with 5 point matrix matched calibration standard.
Figure 1. GC-ECD chromatogram of matrix matched standard of organochlorine pesticides in shrimp matrix: 1) α- BHC, 2) δ- BHC, 3) β- BHC, 4) γ- BHC, 5) Heptachlor, 6) Aldrin, 7) Heptachlor Epoxide, 8) γ- Chlordane, 9) α- Chlordane, 10) α- Endosulfan, 11) 4,4 DDE, 12) Dieldrin, 13) Endrin, 14) 4,4 DDD, 15) β- Endosulfan, 16) 4,4 DDT, 17) Endosulfan sulphate, 18) Methoxychlor, and 19) Endrin Ketone.

Method validation

The method was validated by evaluating the accuracy, precision, linearity, limit of detection and the limit of quantification.

Accuracy and precision

The accuracy of the method was calculated as percent recovery of pesticides from spiked samples. A 10-g homogenized sample was spiked prior to the extraction procedure by the addition of a mixed pesticide standard working solution to reach the final fortification levels of 0.05, 0.10, 0.20 and 0.30 mg kg⁻¹. For each level of fortification, five replicates were analyzed. After fortification, the sample was equilibrated by shaking and then allowed to settle for 30 min prior to the extraction procedures in order to ensure the sufficient contact of the analytes with the whole matrix. Then, the samples were prepared according to the method described earlier. Precision in case of repeatability (RSDᵣ) was determined at four fortification levels of 0.05, 0.10, 0.20 and 0.30 mg kg⁻¹ with 5 replicates on the same day. Precision in case of Reproducibility (RSDₓ) was determined at two fortification levels of 0.05 and 0.20 mg kg⁻¹ with 5 replicates during a period of 2 months interval.
Limit of detection (LOD) and limit of quantification (LOQ)

The Limit of Detection (LOD) was calculated according to EURACHEM guidelines (EURACHEM 1998). In order to determine the LOD of each analyte 10 independent blank samples fortified at the lowest acceptable concentration of 0.05 mg kg\(^{-1}\) were processed and the LOD was expressed as the analyte concentration corresponding to 3 times the standard deviation. LOQ was determined according to the European Commission (EC) document number SANTE/11945/2015 (European commission 2015). LOQ was set as the lowest fortification level for each pesticide giving an acceptable accuracy (mean recoveries for individual pesticides being in the range of 70-120%) and precision (RSD \(\leq 20\%\)).

RESULTS

Method validation

Accuracy and precision

A very good accuracy and precision was found for all of the analytes at four fortification levels of 0.05, 0.10, 0.2, and 0.30 mg kg\(^{-1}\). The average recoveries ranged from 84 to 106\% with relative standard deviations (RSD\(_r\)) \(\leq 14\%\) for all of the analytes (Table 1). Reproducibility (Interday accuracy and precision) was determined at two fortification levels of 0.05 and 0.20 mg kg\(^{-1}\) with 5 replicates. A very good accuracy and precision was also found. The average recoveries ranged from 85.92 to 104.63\% and RSD\(_r\) were \(\leq 5\%\) for all of the analytes (Table 2).

 Calibration curve and linearity

Five point calibration curves were prepared by matrix matched standards and analyzed in triplicate. Calibration curves were made by plotting the mean peak area of the selected pesticides versus concentration. Linearity was evaluated by calculating the correlation coefficient, intercept and slope of the regression line. Linearity was very good and coefficients of determination were \(\geq 0.996\) for all of the selected pesticides with matrix matched calibration standards. The correlation coefficients for all of the selected pesticides are summarized in table 3.
Table 1. Mean recovery (%) and RSD (%) of the selected pesticides in shrimp matrix at different fortification levels

<table>
<thead>
<tr>
<th>Name of Pesticide</th>
<th>Fortification level</th>
<th>Mean (%)</th>
<th>RSD (%)</th>
<th>Mean (%)</th>
<th>RSD (%)</th>
<th>Mean (%)</th>
<th>RSD (%)</th>
<th>Mean (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.05 mg kg(^{-1})</td>
<td></td>
<td></td>
<td>0.1 mg kg(^{-1})</td>
<td></td>
<td>0.2 mg kg(^{-1})</td>
<td></td>
<td>0.3 mg kg(^{-1})</td>
<td></td>
</tr>
<tr>
<td>α- BHC</td>
<td>98.86</td>
<td>3.52</td>
<td>96.44</td>
<td>4.90</td>
<td>89.64</td>
<td>6.01</td>
<td>90.25</td>
<td>2.59</td>
<td></td>
</tr>
<tr>
<td>δ- BHC</td>
<td>88.31</td>
<td>3.40</td>
<td>94.12</td>
<td>3.86</td>
<td>89.63</td>
<td>6.75</td>
<td>90.08</td>
<td>2.74</td>
<td></td>
</tr>
<tr>
<td>β- BHC</td>
<td>103.85</td>
<td>4.62</td>
<td>95.22</td>
<td>3.88</td>
<td>89.34</td>
<td>5.50</td>
<td>90.97</td>
<td>3.58</td>
<td></td>
</tr>
<tr>
<td>γ- BHC</td>
<td>102.68</td>
<td>2.98</td>
<td>84.07</td>
<td>5.71</td>
<td>87.42</td>
<td>11.33</td>
<td>88.52</td>
<td>4.78</td>
<td></td>
</tr>
<tr>
<td>Heptachlor</td>
<td>90.90</td>
<td>4.44</td>
<td>96.85</td>
<td>4.13</td>
<td>90.33</td>
<td>4.31</td>
<td>89.79</td>
<td>2.61</td>
<td></td>
</tr>
<tr>
<td>Aldrin</td>
<td>95.60</td>
<td>4.36</td>
<td>94.12</td>
<td>3.86</td>
<td>86.18</td>
<td>4.53</td>
<td>85.78</td>
<td>4.02</td>
<td></td>
</tr>
<tr>
<td>Heptachlor Epoxide</td>
<td>100.36</td>
<td>2.19</td>
<td>96.61</td>
<td>2.46</td>
<td>93.67</td>
<td>6.48</td>
<td>90.61</td>
<td>3.21</td>
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<tr>
<td>γ- Chlor dane</td>
<td>90.66</td>
<td>6.60</td>
<td>99.07</td>
<td>2.07</td>
<td>93.01</td>
<td>3.87</td>
<td>90.13</td>
<td>3.35</td>
<td></td>
</tr>
<tr>
<td>α- Chlor dane</td>
<td>98.32</td>
<td>3.32</td>
<td>98.32</td>
<td>3.32</td>
<td>88.75</td>
<td>4.83</td>
<td>90.35</td>
<td>3.62</td>
<td></td>
</tr>
<tr>
<td>α- Endosulfan</td>
<td>97.04</td>
<td>11.93</td>
<td>94.59</td>
<td>3.79</td>
<td>90.65</td>
<td>4.78</td>
<td>90.05</td>
<td>3.55</td>
<td></td>
</tr>
<tr>
<td>4,4 DDE</td>
<td>86.24</td>
<td>4.51</td>
<td>98.73</td>
<td>3.84</td>
<td>90.47</td>
<td>3.84</td>
<td>90.20</td>
<td>3.90</td>
<td></td>
</tr>
<tr>
<td>Dieldrin</td>
<td>103.12</td>
<td>9.52</td>
<td>87.20</td>
<td>4.12</td>
<td>88.32</td>
<td>5.85</td>
<td>89.34</td>
<td>3.52</td>
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<tr>
<td>Endrin</td>
<td>84.01</td>
<td>5.79</td>
<td>96.57</td>
<td>6.02</td>
<td>89.81</td>
<td>2.94</td>
<td>90.39</td>
<td>3.70</td>
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</tr>
<tr>
<td>4,4 DDD</td>
<td>102.76</td>
<td>8.05</td>
<td>100.95</td>
<td>5.69</td>
<td>88.68</td>
<td>6.39</td>
<td>91.80</td>
<td>2.74</td>
<td></td>
</tr>
<tr>
<td>β- Endosulfan</td>
<td>106.16</td>
<td>10.29</td>
<td>99.79</td>
<td>5.60</td>
<td>89.75</td>
<td>3.62</td>
<td>90.93</td>
<td>3.40</td>
<td></td>
</tr>
<tr>
<td>4,4 DDT</td>
<td>97.40</td>
<td>13.68</td>
<td>99.22</td>
<td>8.30</td>
<td>89.23</td>
<td>5.16</td>
<td>88.12</td>
<td>3.44</td>
<td></td>
</tr>
<tr>
<td>Endosulfan sulphate</td>
<td>90.51</td>
<td>10.35</td>
<td>96.90</td>
<td>3.37</td>
<td>86.91</td>
<td>14.27</td>
<td>86.83</td>
<td>7.38</td>
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<tr>
<td>Methoxychlor</td>
<td>98.78</td>
<td>13.16</td>
<td>100.55</td>
<td>10.45</td>
<td>94.55</td>
<td>5.16</td>
<td>89.54</td>
<td>3.47</td>
<td></td>
</tr>
<tr>
<td>Endrin ketone</td>
<td>94.45</td>
<td>6.77</td>
<td>95.54</td>
<td>4.25</td>
<td>89.08</td>
<td>4.93</td>
<td>90.23</td>
<td>4.27</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Interday accuracy [Mean Recovery (%)] and precision [RSD\(_R\) (%)] of the selected pesticides in shrimp matrix at different fortification levels at different days

<table>
<thead>
<tr>
<th>Pesticides</th>
<th>Fortification level</th>
<th>Mean (%)</th>
<th>RSD (%)</th>
<th>Mean (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.05 mg kg(^{-1})</td>
<td></td>
<td></td>
<td>0.2 mg kg(^{-1})</td>
<td></td>
</tr>
<tr>
<td>α- BHC</td>
<td>99.25</td>
<td>0.78</td>
<td>91.06</td>
<td>1.65</td>
<td></td>
</tr>
<tr>
<td>δ- BHC</td>
<td>93.80</td>
<td>2.28</td>
<td>91.56</td>
<td>1.07</td>
<td></td>
</tr>
<tr>
<td>β- BHC</td>
<td>102.78</td>
<td>1.14</td>
<td>94.15</td>
<td>2.44</td>
<td></td>
</tr>
<tr>
<td>γ- BHC</td>
<td>100.59</td>
<td>1.24</td>
<td>93.10</td>
<td>1.38</td>
<td></td>
</tr>
<tr>
<td>Heptachlor</td>
<td>96.31</td>
<td>2.10</td>
<td>95.82</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Aldrin</td>
<td>99.01</td>
<td>1.20</td>
<td>90.00</td>
<td>1.09</td>
<td></td>
</tr>
<tr>
<td>Heptachlor Epoxide</td>
<td>99.25</td>
<td>1.55</td>
<td>101.12</td>
<td>0.77</td>
<td></td>
</tr>
</tbody>
</table>
The limit of detection (LOD) of each analyte is presented in Table 3. The LOD ranged from 0.003 to 0.009 mg kg\(^{-1}\). The Limit of Quantification (LOQ) for all of the selected pesticides was set to 0.05 mg kg\(^{-1}\) which was achieved the acceptable accuracy (mean recoveries for individual pesticides in the range of 84\% to 106\%) and precision (RSD \(\leq 14\%\)).

Application of the method for real sample analysis

The proposed method was used for the analysis of shrimp samples collected from different market places in Bangladesh. A total of 40 samples were analyzed. Among the analyzed samples 38 (95\% of the total no. of samples) contained no detectable residues of the pesticides sought and 2 (5\% of the total no. of samples) had pesticides residues. None of the sample was found contaminated at a level above the EU-MRLs (European commission 2005). The detected pesticide was 4, 4 DDT. The ranges of the detected residues were 0.057-0.95 mg kg\(^{-1}\).

DISCUSSION

The described method in this study is an efficient and effective multi-residue analytical method using Gas Chromatography coupled with Electron Capture Detector (GC-ECD) for the determination of 19 organochlorine pesticide residues in shrimp. A very good accuracy and precision was found for all of the analytes using this proposed method.
Table 3. Retention Time (RT), Limit of Detection (LOD), Limit of Quantification (LOQ) and Coefficient of determination ($R^2$) of the selected pesticides for Shrimp matrix

<table>
<thead>
<tr>
<th>Pesticides</th>
<th>RT</th>
<th>LOD (mg kg$^{-1}$)</th>
<th>LOQ (mg kg$^{-1}$)</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha$- BHC</td>
<td>5.48</td>
<td>0.003</td>
<td></td>
<td>0.996</td>
</tr>
<tr>
<td>$\delta$- BHC</td>
<td>6.33</td>
<td>0.007</td>
<td></td>
<td>0.997</td>
</tr>
<tr>
<td>$\beta$- BHC</td>
<td>6.55</td>
<td>0.005</td>
<td></td>
<td>0.996</td>
</tr>
<tr>
<td>$\gamma$- BHC</td>
<td>7.33</td>
<td>0.009</td>
<td></td>
<td>0.997</td>
</tr>
<tr>
<td>Heptachlor</td>
<td>7.46</td>
<td>0.005</td>
<td></td>
<td>0.998</td>
</tr>
<tr>
<td>Aldrin</td>
<td>8.36</td>
<td>0.004</td>
<td></td>
<td>0.999</td>
</tr>
<tr>
<td>Heptachlor Epoxide</td>
<td>10.31</td>
<td>0.006</td>
<td></td>
<td>0.999</td>
</tr>
<tr>
<td>$\gamma$- Chlordane</td>
<td>11.07</td>
<td>0.007</td>
<td></td>
<td>0.9998</td>
</tr>
<tr>
<td>$\alpha$- Chlordane</td>
<td>11.73</td>
<td>0.005</td>
<td></td>
<td>0.997</td>
</tr>
<tr>
<td>$\alpha$- Endosulfan</td>
<td>11.97</td>
<td>0.007</td>
<td></td>
<td>0.999</td>
</tr>
<tr>
<td>4,4 DDE</td>
<td>12.67</td>
<td>0.008</td>
<td></td>
<td>0.999</td>
</tr>
<tr>
<td>Dieldrin</td>
<td>13.37</td>
<td>0.006</td>
<td></td>
<td>0.998</td>
</tr>
<tr>
<td>Endrin</td>
<td>15.15</td>
<td>0.007</td>
<td></td>
<td>0.998</td>
</tr>
<tr>
<td>4,4 DDD</td>
<td>16.33</td>
<td>0.006</td>
<td></td>
<td>0.998</td>
</tr>
<tr>
<td>$\beta$- Endosulfan</td>
<td>16.69</td>
<td>0.004</td>
<td></td>
<td>0.996</td>
</tr>
<tr>
<td>4,4 DDT</td>
<td>18.83</td>
<td>0.009</td>
<td></td>
<td>0.999</td>
</tr>
<tr>
<td>Endosulfan sulphate</td>
<td>22.05</td>
<td>0.008</td>
<td></td>
<td>0.999</td>
</tr>
<tr>
<td>Methoxychlor</td>
<td>24.96</td>
<td>0.007</td>
<td></td>
<td>0.999</td>
</tr>
<tr>
<td>Endrin ketone</td>
<td>25.67</td>
<td>0.004</td>
<td>0.05</td>
<td>0.999</td>
</tr>
</tbody>
</table>

The average recoveries ranged from 84 to 106% with RSD$_t$ ≤ 14% and RSD$_R$ ≤ 5%, thus fulfilling the requirement set by SANTE document no. SANTE/11945/2015 for accuracy and precision (European commission 2015). Nineteen organochlorine pesticides ($\alpha$- BHC, $\delta$- BHC, $\beta$- BHC, $\gamma$- BHC, Heptachlor, Aldrin, Heptachlor Epoxide, $\gamma$- Chlordane, $\alpha$- Chlordane, $\alpha$- Endosulfan, 4,4 DDE, Dieldrin, Endrin, 4,4 DDD, $\beta$- Endosulfan, 4,4 DDT, Endosulfan sulphate, Methoxychlor, and Endrin Ketone) were incorporated in this method that helps the scientist/analysts for quick determination of multiple pesticide residues in shrimp. In addition, this analytical method was applied successfully to monitor the selected organochlorine pesticide residues in 40 shrimp samples collected from different regions of Bangladesh. Among the analyzed samples, 2 (5% of the total no. of samples) had pesticides residues. None of the sample was found contaminated at a level above the EU-MRLs (European commission 2005). The detected pesticide was 4, 4 DDT. The ranges of
the detected residues were 0.057-0.95 mg kg\(^{-1}\). Thus the proposed method can be used successfully to monitor multiple organochlorine pesticide residues in shrimp.

The findings of the present study are in a good agreement with the observation of Sankar et al. (2006). They have collected fish from five different locations from the Caligut region, India and analyzed for the quantification of organochlorine (OC) insecticides and heavy metal (HM) residues. The highest concentrations of OC insecticides detected in the edible portion of fish were 10.47, 70.57 and 28.35 ng g\(^{-1}\) in marine, brackish water and freshwater, respectively. BHC and heptachlor epoxide formed the major share of OC insecticides in the marine fish while BHCs contributed to the major share in the freshwater and brackish water fish. The DDT ranged from 0.05 to 80 ng g\(^{-1}\) in the samples irrespective of the habitat. The concentrations of OC insecticides and HMs in the samples, in general, were below the EU-MRLs (European Commission, 2005).

Battu et al. (1984) have detected OC insecticides in fresh water fish in Ludhiana, India and residues of both DDT and HCH in all the samples with the maximum levels of DDT at 3.02 mg kg\(^{-1}\), while Kannan et al. (1992) reported mean levels of HCH and DDT at 0.002 and 0.015 mg kg\(^{-1}\), respectively in fish. In Pakistan, Saqqib et al. (2005) have detected DDE, aldrin and dieldrin residues in fish tissues while Jabber et al. (2001) reported DDT, aldrin, dieldrin, lindane and heptachlor in different organs of fish (muscle, liver, gut and egg samples) in Bangladesh. Among the analyzed four organs, they have found residues in the following order: egg > gut > muscle > liver. Higher levels of residues have found during the dry season due to high lipid content in fishes. They have also observed a positive correlation between pesticide residues and lipid contents of fish. The concentrations of pesticide residues in muscle, liver and gut were below the FAO/WHO (1993) recommended permissible limit except in eggs.

Pesticides residues remain in fish including other food item have become a consumers’ safety issue. The exposure of OCPs in food products to the consumers creates severe health hazards particularly breast cancer, testicular cancer, endocrine dysfunction, births defects, lower sperm count (Garry et al., 2004; Brody and Rudel, 2003; Ahmed et al., 1996; Soto et al., 1998). Thus, the food safety issues concerning pesticide residues needs to be considered along with food production and an effort including integrated pest management and stringent quality control system comprising rational use of pesticides and their regular monitoring in the environmental samples including fish should be ensured.

**CONCLUSION**

The described method in this study is an efficient and effective multi-residue analytical method using GC-ECD for the determination of 19 organochlorine
pesticide residues in shrimp. A very good accuracy and precision was found for all analytes using this proposed method. The average recoveries ranged from 84 to 106% with RSD ≤ 14% and RSDp ≤ 5%, thus fulfilling the requirement set by SANTE document number SANTE/11945/2015 for accuracy and precision (European commission 2015). Nineteen organochlorine pesticides were incorporated in this method that helps the scientist/analysts for quick determination of multiple pesticide residues in shrimp. In addition, this analytical method was applied successfully to monitor the selected organochlorine pesticide residues in shrimp in Bangladesh. Thus the proposed method can be used successfully to monitor multiple organochlorine pesticide residues in shrimp.

REFERENCES


RICE GROWTH STAGES AND TEMPERATURE AFFECT THE ABUNDANCE OF LEAFHOPPERS AND PLANTHOPPERS

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ABSTRACT
Relative abundance of leaf and plant hoppers species regarding plant growth stages and temperature were studied in the rice variety BINA10 field at the research farm of Patuakhali Science and Technology University (PSTU) during October 2013 to April 2014. The percent relative abundance of leafhoppers was in the rank order of M. dorsalis> C. brevis> N. virescens> N. malayanus> N. cincticeps> N. nigropictus at seedling, early and maximum tillering stages while N. virescens> C. brevis> M. dorsalis> N. nigropictus> N. malayanus> N. cincticeps at reproductive stage. The percent relative abundance of planthoppers was in the rank order of N. nervosa> N. lugens> S. furcifera at seedling and reproductive stages while N. lugens> N. nervosa> S. furcifera at early tillering and N. nervosa> S. furcifera and N. lugens at maximum tillering stages. The abundance of all leafhopper species showed highly negative relationship with temperature. This relationship can be expressed by 81% (R^2=0.806) for N. virescens, 80% (R^2= 0.801) for N. nigropictus, 65% (R^2=0.653) for N. cincticeps, 60% (R^2=0.600) for C. brevis and 70% (R^2=0.698) for M. dorsalis. Among three planthopper species, population of S. furcifera showed highly negative relationship with temperature and this relationship can be expressed by 71% (R^2=0.707). The abundance of N. nervosa showed poor positive relationship with temperature. This relationship can be expressed by 3 % for N. nervosa (R^2=0.030). N. lugens showed poor negative relationship with temperature and this relationship can be expressed by 31% (R^2=0.310) for N. lugens.

Keywords: BINA dhan10, growth stage, leafhopper, planthopper, temperature

INTRODUCTION
Rice is the major cereal crop of the world and considered as staple food especially in Asian countries (Smil, 2005). It is mainly used for human consumption. Rice grain is

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a source of protein, oil, sugar (Ahmad, 1989) and its outer layer is rich in thiamin (vitamin B₁), deficiency of which results in a disease called “Beriberi” in human beings (Montgomery et al., 1980). The average yield of rice in Bangladesh is quite low which is 3.02 mt ha⁻¹ (BBS, 2014) compared to other rice growing countries of the world. Two hundred sixty six insect species have been identified so far as pest of rice in Bangladesh (Islam et al., 2003). Of these, 42 species are considered as crucial insect pests. Among the key pests, both leaf and plant hoppers cause direct damage by feeding as well as by transmitting viruses. Green leafhopper is one of the most serious pests of rice both in the tropics and temperate zone known to be a vector of rice viruses and MLO- mycoplasma like organisms (Kawabe, 1986). Planthoppers especially brown planthopper (BPH) has become a serious pest of high yielding variety of rice (Alam et al., 1983). BPH remains at the base of the plant and suck sap from the mesophyll of all stages of the growing plant. It also blocks the xylem and phloem by laying egg masses in the midrib of the leaf blade and by this reduces the yield potentiality of rice plant (Wu et al., 2001). When the pest density becomes high, the plants turn yellow and dry up rapidly. At early infestation, round yellow patches appear which soon turn brownish due to the drying up of the plants. This condition is called Hopperburn. The patches of infestation then may spread out and cover the entire field.

The leaf hoppers feed on the leaves and upper parts of the rice plant, whereas the plant hoppers confine themselves to the basal parts. In the warm and humid tropics, different species of leafhoppers and planthoppers remain active year round, and their population fluctuates according to the availability of food plants, natural enemies and environmental conditions. Considering the above facts, the present study was undertaken to know the abundance of leaf and plant hoppers on different growth stages of rice variety BINA dhan10 at different temperature.

MATERIALS AND METHODS

The field study was undertaken in some selected rice fields in the research farm of Patuakhali Science and Technology University (PSTU) during October 2013 to April 2014. The laboratory works were carried out in the Department of Entomology, Patuakhali Science and Technology University, Dumki, Patuakhali on the taxonomic classification, identification of different species of rice leafhoppers and planthoppers. The rice variety BINA dhan10 was used as study material. The experiment was designed in a randomized complete block design with 3 replications. Each rice field was treated as treatment replication. In such a way, three rice fields were used as three replications in the study.

The different species of leafhopper and planthopper were collected by a fine mesh nylon sweep net. Sweeping was done from the plant canopy level including the interspaces between plants as well as close to basal region of the plants as far as possible. In each field, 10 complete sweeps were made to collect hopper populations.
Sampling was done at four stages of rice viz. seedling, early tillering (initial tillering), maximum tillering (prior to booting stage) and panicle initiation stage. Sampling was done during morning hours at all study fields on all sampling dates. The samples of 10 complete sweeps from each field were collected and preserved separately in labeled container. The samples were sorted, counted and identified in the laboratory of the Department of Entomology, PSTU under microscope. The relative abundance of leafhoppers and planthoppers were calculated.

**Relative abundance**

Relative abundance of insect pests was calculated using the following formula:

\[
\text{Relative abundance (\%) = \frac{\text{Total number individuals of each species}}{\text{Total number individuals of all species}} \times 100}
\]

The collected data were analyzed statistically by using the MSTAT-C computer package. The treatment means were compared by LSD test.

**RESULTS AND DISCUSSION**

**Relative abundance of leafhoppers**

The percent relative abundance of leafhoppers on BINA dhan10 was in the rank order of *M. dorsalis* (30.84\%) > *C. brevis* (20.75\%) > *N. virescens* (16.14\%) > *N. malayanus* (11.52\%) > *N. cincticeps* (9.22\%) > *N. nigropictus* (8.65\%) (Table 1). In early tillering stage, the percent relative abundance of leafhoppers on BINA dhan10 was in the rank order of *M. dorsalis* (35.48\%) > *N. virescens* (29.03\%) > *N. nigropictus* (12.90\%) > *N. malayanus* (6.45\%) > *N. cincticeps* (3.23\%) > *C. brevis* (0\%) (Table 1).

In maximum tillering stage, the percent relative abundance of leafhoppers on BINA dhan10 was in the rank order of *M. dorsalis* (48.48\%) > *N. virescens* (21.21\%) > *N. cincticeps* (12.12\%) > *N. nigropictus* (3.03\%) and *N. malayanus* (3.03\%) > *C. brevis* (0\%) (Table 1). In reproductive stage, the relative abundance of leafhoppers on BINA dhan10 was in the rank order of *N. virescens* (28.00\%) > *C. brevis*(24.00\%) > *M. dorsalis* (16.00\%) > *N. nigropictus* (12.00\%) and *N. malayanus* (12.00\%) > *Nephotettix cincticeps* (8.00\%) (Table 1).
Table 1. Relative abundance of leafhoppers per 10 complete sweeps at different growth stages of BINA dhan10

<table>
<thead>
<tr>
<th>Leafhoppers</th>
<th>Seedling stage</th>
<th>Early tillering stage</th>
<th>Maximum tillering stage</th>
<th>Reproductive stage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Relative abundance (%)</td>
<td>No.</td>
<td>Relative abundance (%)</td>
</tr>
<tr>
<td>Nephotettix virescens</td>
<td>56</td>
<td>16.14</td>
<td>9</td>
<td>29.03</td>
</tr>
<tr>
<td>Nephotettix nigropictus</td>
<td>30</td>
<td>8.65</td>
<td>4</td>
<td>12.90</td>
</tr>
<tr>
<td>Nephotettix cincticeps</td>
<td>32</td>
<td>9.22</td>
<td>1</td>
<td>3.23</td>
</tr>
<tr>
<td>Nephotettix malayanus</td>
<td>40</td>
<td>11.52</td>
<td>2</td>
<td>6.45</td>
</tr>
<tr>
<td>Coelidia brevis</td>
<td>72</td>
<td>20.75</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Maiestas dorsalis</td>
<td>107</td>
<td>30.84</td>
<td>11</td>
<td>35.48</td>
</tr>
<tr>
<td>Total</td>
<td>347</td>
<td>-</td>
<td>31</td>
<td>-</td>
</tr>
</tbody>
</table>

Relative abundance of planthoppers

In seedling stage, relative abundance of planthoppers in rice ecosystem is presented in Table 2. The percent relative abundance of planthoppers on BINA dhan10 was in the rank order of *N. nervosa* (40%) > *N. lugens* (35%) > *S. furcifera* (25%). In early tillering stage, the percent relative abundance of planthoppers in BINA dhan10 was in the rank order of *N. lugens* (60%) > *N. nervosa* (26.67%) > *S. furcifera* (13.33%) (Table 2).

In maximum tillering stage, the percent relative abundance of planthoppers on BINA dhan10 was in the rank order of *N. nervosa* (50%) > *S. furcifera* (25%) and *N. lugens* (25%) (Table 2). In reproductive stage, the relative abundance of planthoppers on BINA dhan10 was in the rank order of *N. nervosa* (44.44%) > *N. lugens* (33.33%) > *S. furcifera* (22.22%) (Table 2).
ABUNDANCE OF LEAFHOPPERS AND PLANTHOPPERS

Table 2. Relative abundance of planthoppers per 10 complete sweeps at different growth stages of BINA dhan10

<table>
<thead>
<tr>
<th>Planthoppers</th>
<th>Seedling stage</th>
<th>Early tillering stage</th>
<th>Maximum tillering stage</th>
<th>Reproductive stage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Relative abundance (%)</td>
<td>No.</td>
<td>Relative abundance (%)</td>
</tr>
<tr>
<td><em>Nisia nervosa</em></td>
<td>8</td>
<td>40</td>
<td>4</td>
<td>26.67</td>
</tr>
<tr>
<td><em>Sogatella furcifera</em></td>
<td>5</td>
<td>25</td>
<td>2</td>
<td>13.33</td>
</tr>
<tr>
<td><em>Nilaparvata lugens</em></td>
<td>7</td>
<td>35</td>
<td>9</td>
<td>60</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>20</td>
<td>-</td>
<td>15</td>
<td>-</td>
</tr>
</tbody>
</table>

Mean number of Leafhoppers in different growth stages of BINA dhan10

There was significant difference among the population of *Nephotettix virescens* in different growth stages of BINA dhan10 with mean ranged from 6.00 to 47.50 per 10 complete sweeps. Significantly the highest number of *N. virescens* was recorded in seedling stage (47.50) whereas reproductive stage showed the lowest population (6.00). No significant difference was at early tillering and maximum tillering stages 11.00 and 10.50, respectively (Table 3).

The mean number of *Nephotettix nigropictus* population in different crop growth stages of BINA dhan10 ranged from 5.50 to 59.50 per 10 complete sweeps. Significantly the highest number of *N. nigropictus* population was found in seedling stage (59.50) while the lower number of *N. nigropictus* population was recorded in reproductive stage (5.50). No significant difference was at early tillering and maximum tillering stages (Table 3).

The mean number of *Nephotettix cincticeps* population in different crop growth stages of BINA dhan10 ranged from 6.50 to 68.00 per 10 complete sweeps. Significantly the highest number of *N. cincticeps* population was found in seedling stage (68.00) while the lowest number of *N. cincticeps* population was recorded in early tillering stage (6.50). No significant difference was at maximum tillering and reproductive stages (Table 3).

The mean number of *Maiestas dorsalis* population in different crop growth stages of BINA dhan10 ranged from 7.50 to 93.50 per 10 complete sweeps. Significantly the highest number of *M. dorsalis* population was found in seedling stage (93.50) while the lowest number of *M. dorsalis* population was recorded in the maximum tillering stage (7.50). No significant difference was at early tillering and reproductive stages (Table 3).
The mean number of *Coelidia brevis* population in different crop growth stages of BINA dhan10 ranged from 4.50 to 55.00 per 10 complete sweeps. Significantly the highest number of *C. brevis* was found in seedling stage (55.00) while the lowest population was recorded in maximum tillering stage (4.50). No significant difference was at early tillering and reproductive stages (Table 3).

**Table 3. Mean number of Leafhoppers in different growth stages of BINA dhan10**

<table>
<thead>
<tr>
<th>Growth stage</th>
<th>Mean number / 10 complete sweeps</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Nephotettix virescens</em></td>
</tr>
<tr>
<td>Seedling stage</td>
<td>47.50a</td>
</tr>
<tr>
<td>Early tillering stage</td>
<td>11.00b</td>
</tr>
<tr>
<td>Maximum tillering stage</td>
<td>10.50b</td>
</tr>
<tr>
<td>Reproductive stage</td>
<td>6.00c</td>
</tr>
<tr>
<td>LSD value</td>
<td>4.41</td>
</tr>
<tr>
<td>Level of significance</td>
<td>**</td>
</tr>
<tr>
<td>CV (%)</td>
<td>9.79</td>
</tr>
</tbody>
</table>

**Mean number of planthoppers as influenced by different growth stages of BINA dhan10**

*Nisia nervosa* differed significantly among the four crop growth stages of BINA dhan10 with the mean ranged from 3.50 to 9.50 per 10 complete sweeps. The highest number of *Nisia nervosa* population was found in maximum tillering stage (9.50) which was statistically similar with reproductive stage (9.00). The lowest number of *Nisia nervosa* population was recorded in early tillering stage (3.50) which was statistically identical to reproductive stage (9.00). The lowest number of *Nisia nervosa* population was recorded in early tillering stage (3.50) followed by seedling stage (7.50) (Table 4).

*Sogatella furcifera* differed significantly among the four crop growth stages of BINA dhan10 with the mean ranged from 3.50 to 5.00 per 10 complete sweeps. Significantly the highest number of *S. furcifera* population was found in maximum tillering stage (5.00) while the lowest number of *S. furcifera* population was recorded in early tillering stage (3.50) which was statistically identical to reproductive stage (3.50) followed by the seedling stage (4.50) (Table 4).
Table 4. Mean number of planthoppers in different growth stages of BINA dhan10

<table>
<thead>
<tr>
<th>Growth stage</th>
<th>Mean number / 10 complete sweeps</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Nisia nervosa</em></td>
</tr>
<tr>
<td>Seedling stage</td>
<td>7.50b</td>
</tr>
<tr>
<td>Early tillering stage</td>
<td>3.50c</td>
</tr>
<tr>
<td>Maximum tillering stage</td>
<td>9.50a</td>
</tr>
<tr>
<td>Reproductive stage</td>
<td>9.00a</td>
</tr>
<tr>
<td>LSD value</td>
<td>1.48</td>
</tr>
<tr>
<td>Level of significance</td>
<td>**</td>
</tr>
<tr>
<td>CV (%)</td>
<td>7.81</td>
</tr>
</tbody>
</table>

** = Significant at 5% level by LSD

*Nilaparvata lugens* differed significantly among the four crop growth stages of BINA dhan10 with the mean ranged from 6.50 to 9.50 per 10 complete sweeps. Significantly the highest number of *N. lugens* population was found in seedling stage (9.50) while the lowest number of *N. lugens* population was recorded in early tillering stage (6.50) which was statistically identical to reproductive stage (6.50) followed by the maximum tillering stage (8.00) (Table 4).

**Relationship between temperature and abundance of leaf and plant hoppers species**

Correlation between temperature and abundance of leaf and plant hoppers species is presented in Table 5. The abundance of all leafhopper species showed highly negative relationship with temperature. This relationship can be expressed by 81% ($R^2=0.806$) for *N. virescens*, 80% ($R^2=0.801$) for *N. nigropictus*, 65% ($R^2=0.653$) for *N. cincticeps*, 60% ($R^2=0.600$) for *C. brevis* and 70% ($R^2=0.698$) for *M. dorsalis*. It indicated that population of different leafhopper species decreased with the increase of temperature. Similarly, the abundance of all planthopper species showed negative relationship with temperature. Among three planthopper species, population of *S. furcifera* showed highly negative relationship with temperature and this relationship can be expressed by 71% ($R^2=0.707$). The abundance of *N. nervosa* showed poor positive relationship with temperature. This relationship can be expressed by 3 % for *N. nervosa* ($R^2=0.030$). *N. lugens* showed poor negative relationship with temperature and this relationship can be expressed by 31% ($R^2=0.310$) for *N. lugens* (Table 5).
Table 5. Correlation between temperature and population abundance of different leafhopper and planthopper species

<table>
<thead>
<tr>
<th>Leafhopper species</th>
<th>Regression equation</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. virescens</td>
<td>$Y = -3.752x + 114.4$</td>
<td>0.806</td>
</tr>
<tr>
<td>N. nigropictus</td>
<td>$Y = -4.878x + 144.7$</td>
<td>0.801</td>
</tr>
<tr>
<td>N. cincticeps</td>
<td>$Y = -5.294x + 158.0$</td>
<td>0.653</td>
</tr>
<tr>
<td>C. brevis</td>
<td>$Y = -4.204x + 126.8$</td>
<td>0.600</td>
</tr>
<tr>
<td>M. dorsalis</td>
<td>$Y = -6.713x + 208.2$</td>
<td>0.698</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Planthopper species</th>
<th>Regression equation</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. nervosa</td>
<td>$Y = 0.139x + 3.208$</td>
<td>0.030</td>
</tr>
<tr>
<td>S. furcifera</td>
<td>$Y = -0.246x + 9.461$</td>
<td>0.707</td>
</tr>
<tr>
<td>N. lugens</td>
<td>$Y = -0.431 + 17.53$</td>
<td>0.310</td>
</tr>
</tbody>
</table>

The findings of the present study are supported by Soekhardjan et al. (1974) who reported that, in general there is an increase in the level of green leafhopper infestation with the increase of the age of the rice plants. Sabir et al. (2006) found that the maximum population of whitebacked planthopper (Sogatella furcifera Horv.), green leafhopper (Nephotettix cincticeps Uhl.) and white leafhopper (Cofana spectra Dist.) per 10 net sweeps, respectively in October. Khan (2013) found that the highest percent relative abundance of green rice leafhopper (GLH) and spider. Among the insect pest species, the population of GLH and short horned grasshopper was most prevalent in the rice field. Among natural enemies, damsel fly, spider and Ichneumonid wasp were the most prevalent while mirid bug, lady bird beetle and ground beetle were low in rice habitat. The occurrence of insect pests and natural enemies was the highest in maximum tillering stage and the lowest in early tillering stage. Abundance of insect pests and their natural enemies were more in high yielding rice varieties namely accession no. 20 as compared to local rice cultivars viz. Lalmota, moulata (Khan, 2013). Srinavasa et al. (1991) reported 3 hopper pests of rice viz. Nephotettix spp., Nilaparvata lugens and Scirpophaga incertulas. They reported that Nephotettix spp. and N. lugens were present throughout the year but showed peaks of abundance in November and May; S. incertulas was also present throughout the year with low incidence in March, and had peaks in November and June. Sabir et al. (2006) stated that the maximum and minimum temperature and rainfall are vital for bringing a change in the population of green leafhopper, leaffolder, stem-borer, whitebacked planthopper and white leafhopper while relative humidity has shown a positive response on the population. Hafizal and Idris (2014)
reported that Delphacidae (planthopper) and Cicadellidae (leafhopper) population are main insect pests of rice plants. As phloem-feeder insects, their population abundance can be influenced by rice growth development and abiotic factors such as temperature and humidity. It was evident that the mean temperature and relative humidity were varied slightly during rice growth period but not significantly affecting the population abundance of both hoppers. Changes of temperature influenced the abundance of Delphacidae, but not cicadelids. The abundance of Delphacids and Cicadelids population among rice growth stages differed significantly and was positively correlated with rice growth stages. Delphacids and Cicadelids had the highest and lowest abundance during maturing and reproductive stages, respectively.

**CONCLUSION**

The highest populations of leaf hoppers were in seedling stage and the lowest in early tillering stage. The highest numbers of plant hoppers population were found in seedling stage and the lowest numbers were in reproductive stage. All leafhopper species and one plant hopper species *S. furcifera* showed highly negative relationships with temperature. Plant hopper species *N. nervosa* showed poor positive and *N. lugens* showed poor negative relationships with temperature.

**REFERENCES**


EFFECTS OF PROBIOTICS-ENCAPSULATED LIVE FEED ON GROWTH AND SURVIVAL OF JUVENILE *Clarias batrachus* (Linnaeus, 1758) AFTER DIFFERENTIAL EXPOSURE TO PATHOGENIC BACTERIA

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¹Entomology Research Unit, Department of Zoology, The University of Burdwan, Burdwan, India
²Aquaculture Laboratory, Department of Zoology, The University of Burdwan, Burdwan, India

ABSTRACT

Growth and survival of *Clarias batrachus* juveniles (10-day old) fed probiotic *Bacillus cereus* (KR809412) encapsulated live feed (chironomid larvae) have been evaluated after differential exposure to the pathogenic *Aeromonas hydrophila* (MTCC 1739). Catfish juveniles were stocked at a density of 30 fish per tank in five experimental groups (T1-T5) along with a control group in triplicate and fed twice @ 5% of body weight day⁻¹ for four weeks. Groups T1 and T2 were fed probiotic-encapsulated (PR) or pathogen-inoculated (PGN) live feed respectively, for initial three weeks. During this period groups T3 (PGN-PR-PR), T4 (PR-PGN-PR), and T5 (PR-PR-PGN) were differentially exposed to the pathogen. Live feed without probiotic and pathogen was offered to the control group throughout the experimental period and all other treatment groups (T1-T5) during the 4th week. Continuous exposure to probiotics in group T1 resulted in significantly higher (P<0.05) specific growth rate (SGR, % d⁻¹) and survivability than other groups, whereas, pathogen exposed and probiotic deprived group (T2) noticed with the lowest SGR and the highest mortality. Among other treatment groups (T3, T4 and T5), group T4 resulted in improved SGR and survivability. The coefficient (r value) of 0.867 along with regression slope suggested a positive correlation (0.01 levels) between RNA: DNA and SGR. The study might suggest protective effects of probiotic *B. cereus* in pathogen exposed *C. batrachus* juveniles.

Keywords: *Clarias batrachus*, *Bacillus cereus*, probiotic, *Aeromonas hydrophila*, live feed

INTRODUCTION

Walking catfish, *Clarias batrachus* (commonly known as magur) is an economically important group of fish with high nutritional value and recuperation ability (Sahoo et
al., 2010). High mortality of magur juveniles has been observed due to less availability of food and poor nutrient utilization (Sahoo et al., 2010) along with ecological imbalance in the breeding ground and habitat degradation (Ahmed et al., 2012). Moreover, magur juveniles are susceptible to the pathogen, majority of which are bacteria (Ikpi and Offem, 2011). Although antibiotics are traditionally used to solve this problem (Hu et al., 2007), the use of antibiotics has been criticized since they can alter the gut microbiota and might lead to develop resistant bacteria population (Verschuere et al., 2000). Alternatively, likely application of gut associated bacteria as probiotics in catfish has been apprehended in some of the recent investigations (Banerjee et al., 2015; Dey et al., 2016). Besides, magur juveniles require protein rich food to ensure proper growth and survival (Kiri Ratnikom and Kiri Ratnikom, 2012). Application of potent extracellular enzyme-producing and pathogen inhibitory autochthonous bacteria through protein rich live food might hold promise to supply nutritional support and limit pathogenic microbial load to reduce mortality in catfish juveniles as suggested elsewhere (Cruz et al., 2012).

Hence, this study made an effort to assess the role of probiotic-encapsulated live feed (chironomid larvae) in improving growth and survivability of juvenile C. batrachus after differential exposure to pathogenic A. hydrophila.

MATERIALS AND METHODS

Experimental fish
Ten day old juveniles of C. batrachus were procured from a reputed fish farm (Blutech Dynamics, Dakshin Bijoynagar, South 24 Parganas, West Bengal, India) and stocked in fiber reinforce plastic (FRP) tanks (45 L). Juveniles were acclimatized for 10 days with feeding of zooplanktons and/or chopped Tubifex. Water quality parameters, viz., temperature, pH, total dissolved solids (TDS) and dissolved oxygen content (mg l⁻¹) from each experimental set were monitored at regular intervals (APHA, 2005). Faecal matter and remains were siphoned out daily.

Experimental procedure
Autochthonous, extracellular enzyme-producing Bacillus aryabhattai KP784311, B. flexus KR809411 and B. cereus KR809412 were previously isolated from the gut of adult C. batrachus (Dey et al., 2016) and their probiotic features had been documented (Dey et al., 2017). Antibacterial activity of the putative probiotic strains was tested against pathogenic Aeromonas hydrophila (MTCC 1739), by cross-streaking and agar well-diffusion as described by Mukherjee et al. (2016). The pathogenic strain was obtained from the Microbial Type Culture Collection, Chandigarh, India. The efficient antagonistic strain was selected for the present study.
The experiment was conducted for 4 weeks (28 days) in rectangular fibre reinforced plastic (FRP) tanks (90 cm × 30 cm × 30 cm). Overall, experimental fish were distributed randomly at a stocking density of 30 fish per FRP tank with three replicates for each experimental set (altogether 6 sets; control and T1-T5), and exposed to probiotic and/or pathogenic bacteria through the live feed (12–15 day old chironomid larvae) following the experimental design depicted in table 1. Probiotic encapsulation (PR) and pathogen inoculation (PGN) of the live feed was done separately either with the selected probiotic strain (B. cereus KR809412) or the pathogenic A. hydrophila (MTCC 1739) as described in Dey et al. (2017).

Table 1. Experimental design showing combination of feed in control group and treatment groups for 4 weeks

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Duration</th>
<th>1st week</th>
<th>2nd week</th>
<th>3rd week</th>
<th>4th week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>LF</td>
<td>LF</td>
<td>LF</td>
<td>LF</td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>PR</td>
<td>PR</td>
<td>PR</td>
<td>LF</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>PGN</td>
<td>PGN</td>
<td>PGN</td>
<td>LF</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>PGN</td>
<td>PR</td>
<td>PR</td>
<td>LF</td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>PR</td>
<td>PGN</td>
<td>PR</td>
<td>LF</td>
<td></td>
</tr>
<tr>
<td>T5</td>
<td>PR</td>
<td>PR</td>
<td>PGN</td>
<td>LF</td>
<td></td>
</tr>
</tbody>
</table>

PR = Probiotic encapsulated, PGN= Pathogen inoculated, LF= Live feed without any bacterial inoculation

Catfish juveniles were fed live feed twice (9.00h and 16.00h) @ 5% of body weight day\(^{-1}\) (Chepkirui–Boit et al., 2011). Fish juveniles were sampled prior to and after completion of the experiment. Growth was calculated as specific growth rate (SGR, % day\(^{-1}\)) = 100 [(\(\ln W_f - \ln W_i\))/T], where \(W_i\) and \(W_f\) are the initial and final wet weights of fish respectively; T is the trial period in days. RNA-DNA ratio was considered as an index of growth. DNA and RNA aliquots were prepared from the larval tissue (Esteves et al., 2000). DNA and RNA contents were determined following Bruton (1956) and Marham (1955), respectively.

Survivability was calculated as: (Final number of juveniles/ Initial number of juveniles) × 100. Growth (Length, weight and SGR) and survival rate were observed in each week and data analysed using one-way analysis of variance (ANOVA) and a post hoc analysis (Tukey HSD) followed by Zar (1999). To correlate RNA: DNA and SGR, correlation coefficient (r value) and regression analysis were performed.
RESULTS

Among the three probiotic strains, B. cereus KR809412 was noticed to inhibit the growth of A. hydrophila. Thus, B. cereus KR809412 was used as the probiotic strain in this study. Water quality parameters; temperature (25-28°C), pH (6.3-7.7), dissolved oxygen (5.8-6.8 mg l⁻¹) and TDS (1.21-1.25 ppm) varied within narrow range during the experiment. Feeding of B. cereus encapsulated midge larvae in group T1 resulted in significantly higher (P<0.05) specific growth rate (SGR, % d⁻¹) and maximum survivability in C. batrachus juveniles. While in contrast to the group T3, groups T4 and T5 exhibited improved growth rate and survivability. Group T2 displayed the lowest growth rate and the highest mortality. Weight gain, increment in total length, SGR (% day⁻¹) and survivability of the experimental fish at the end of 4th weeks have been presented in Table 2. The RNA content in the carcass increased over the initial value in all treatment groups, whereas, the DNA content did not indicate any significant change. The coefficient (r value) of 0.867 along with regression slope suggested a positive correlation (0.01 levels) between RNA: DNA and SGR (Figure 1).

Table 2. Growth parameters and RNA: DNA of catfish juveniles

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Parameters</th>
<th>Weight (g)</th>
<th>Length (cm)</th>
<th>Survivability (%)</th>
<th>Specific Growth Rate (% day⁻¹/fish)</th>
<th>RNA (μg ml⁻¹)</th>
<th>DNA (μg ml⁻¹)</th>
<th>RNA: DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>0.64±</td>
<td>3.10±</td>
<td>82.30±</td>
<td>1.10±</td>
<td>82</td>
<td>72.7</td>
<td>1.12b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.01c</td>
<td>0.04c</td>
<td>2.36d</td>
<td>0.40b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td></td>
<td>0.72±</td>
<td>3.20±</td>
<td>91.70±</td>
<td>3.50±</td>
<td>88</td>
<td>71.4</td>
<td>1.23d</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.01d</td>
<td>0.07e</td>
<td>1.42e</td>
<td>0.05f</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td></td>
<td>0.52±</td>
<td>2.52±</td>
<td>30.00±</td>
<td>0.80±</td>
<td>77</td>
<td>70.1</td>
<td>1.09a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.01a</td>
<td>0.02a</td>
<td>2.86a</td>
<td>0.05a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td></td>
<td>0.57±</td>
<td>2.64±</td>
<td>69.3±</td>
<td>1.72±</td>
<td>84</td>
<td>71.2</td>
<td>1.14b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.02b</td>
<td>0.01b</td>
<td>1.40b</td>
<td>0.01c</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td></td>
<td>0.66±</td>
<td>2.77±</td>
<td>81.60±</td>
<td>2.58±</td>
<td>84.3</td>
<td>72.3</td>
<td>1.16c</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.02c</td>
<td>0.01c</td>
<td>2.30d</td>
<td>0.10d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T5</td>
<td></td>
<td>0.60±</td>
<td>2.80±</td>
<td>73.00±</td>
<td>1.75±</td>
<td>81</td>
<td>71.2</td>
<td>1.13b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.06b</td>
<td>0.01d</td>
<td>1.40c</td>
<td>0.06c</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results are mean ± standard deviation of 3 determinations. Values with the same superscripts in the same vertical column are not significantly different (P <0.05).
DISCUSSION

The non-specific immune system of aquatic animals is developed with the application of probiotics that perhaps help the animals to resist infection from potential pathogens (Andani et al., 2012) and might explain the improvement in growth and survivability associated with the fish that received probiotics prior to exposure to the pathogenic *A. hydrophila* (T4 and T5) in the present study. Diverse strains of *Bacillus* spp. have been used as probiotics against the bacterial pathogens in fish (Aly et al., 2008). Probiotic *B. Subtilis* BT23 was noticed to antagonize *Vibrio harveyi* that reduced mortality in shrimps (Vaseeharan and Ramasamy 2003). The probiotic *B. cereus* used in the present investigation was antagonistic to *A. hydrophila*, which was in accordance with the reports portraying pathogen inhibitory activity of *B. cereus* and *B. circulans* isolated from the gut of different fish species (Laloo et al., 2010; Geraylou et al., 2014). Administration of the probiotic *B. cereus* resulted in maximum growth and survival when catfish juveniles were not exposed to the pathogen. However, improvement in growth and survivability in catfish juveniles previously exposed to probiotic *B. cereus* prior to *A. hydrophila* exposure might indicate protective effects of the probiotics against the pathogen. Probiotic potential of *Bacillus* sp. in Indian major carp, *L. rohita* challenged with pathogenic *A. hydrophila* has been documented (Nandi et al., 2017). However, to the authors’ knowledge, protective effects of probiotic *B. cereus* in pathogen exposed catfish, *C.*
Batrachus juveniles has not been documented previously. Production of extracellular enzymes to assist digestion could be the reason behind improvement in growth performance of hosts with the administration of probiotics (Ray et al., 2012). Enzyme-producing ability of B. cereus used in the recent study has been documented earlier (Dey et al., 2016). Therefore, although not addressed in this pilot study, improved growth and survivability in the probiotic exposed groups might be attributed to the enhanced nutrient utilization and lower stressor levels as indicated elsewhere (Al-Dohail et al., 2009). RNA:DNA might be considered as a reliable indication of growth trend (Bandyopadhyay and Das-Mohapatra, 2009). The ratio was the greatest in the fish reared as T1 that continuously received probiotic encapsulated live feed and didn’t expose to the pathogen. In conformity with the present study, Bandyopadhyay and Das-Mohapatra (2009) reported better growth as well as better RNA:DNA in an Indian major carp, Catla catla fed probiotic supplemented diet (B. circulans PB7; 2 × 10^5 cells 100 g^-1).

Furthermore, fish juveniles require good amount of nutrients because of their rudimentary digestive system (Govoni et al., 1986) and they use to prefer live food organisms that act as ‘living capsules’ of nutrition. Thus, administration of probiotics through bioencapsulated live feed might be an effective approach for introduction of large numbers of probiotic bacteria during rearing of the early stages (Ibrahim, 2015). However, more research is inevitable for a prolonged duration and with large number of replicates to assess the effects of mixed culture probiotics, as dominance of single strain in a continuous changing environment might be uncertain (Verschuere et al., 2000). Although higher survival against the pathogenic A. hydrophila exposure might specify immune-stimulatory property of the probiotic strain (Bandyopadhyay and Das-Mohapatra 2009), we need to look into stress and immune parameters to ascertain probiotic B. cereus as a potent immune-stimulant for likely use in aquaculture.

**CONCLUSION**

Controlled fish culture demands quality seed supply in optimum amount, which has now become difficult due to huge mortality and disease susceptibility. Inhibitory effect of a probiotic strain against one aeromonad fish pathogen was examined in the present investigation and the study pointed out that bacterial symbionts in fish may endow the host with some ecological benefits by enabling them to overcome the harmful effects of Aeromonas spp. C. batrachus juveniles were fed probiotic-encapsulated midge larvae and differentially exposed to pathogen inoculated midge larvae. Unfavourable effect of the pathogen was checked in those juveniles who were previously exposed to the probiotic bacteria.
ACKNOWLEDGEMENTS

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diet containing freshwater atyid shrimp (*Caridina nilotica*) during weaning. *Aquaculture nutrition*, 17, 82–89.


TRIBAL WOMENS INVOLVEMENT WITH PIG FARMING IN BANGLADESH: AN EVIDENCE OF MOULVIBAZAR DISTRICT

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ABSTRACT

The study examined the profitability and factors affecting farm income from pig farming in Moulvibazar district of Bangladesh. A multistage sampling technique was followed to select 70 tribal women entrepreneurs involved with pig farming. Primary data were collected through structured questionnaire and analyzed using descriptive statistics, independent sample t-test, benefit-cost analysis, and linear regression model. Average age of pig entrepreneurs was 39.47 years which indicates that they are young and agile. About 71% of Garo entrepreneurs had experience for pig farming. The cost and return analysis showed that in one year, the gross margin was Tk. 56743.70, while the benefit-cost ratio (BCR) was 1.19 indicating that the enterprise is profitable. Regression analysis revealed that three variables namely farm size, age of the respondent and experience of pig farming are significant factors affecting farm income. Thus, it was recommended that tribal women should be encouraged to practice pig farming to overcome their low income and unemployment situation.

Keywords: Pig farming, entrepreneur, tribal women, profitability.

INTRODUCTION

Small scale home based pig farming is an important livelihood source for pig farmers. In 2017, there were approximately 769.05 million pigs worldwide, where China has been produced about 51.85 million metric tons (USDA, 2018). Pig production has also been seen as a source of protein. Their fast growth rate which is only slightly exceeded by the best, carefully managed broilers, their proliferation which is unsurpassed by that of any other animal species except the birds, their very good efficiency of feed utilization which brings better returns per units of inputs than most animals and quality of their meat which is both tender and more nutritive in
terms of the contents of protein and the B-vitamins than those of other animals (Ogunniyi and Omoteso, 2011). Despite these attributes, pig production in Bangladesh has remained low due to Muslim population who constitute the majority of most areas of the country. But it is one of the most important livestock for the cobbler, sweeper, Christian, nomadic people and tribal community of the country who keep them for their livelihood and animal protein and also to maintain their social value. The killing of pigs is important to celebrate their main occasion of communally such as cremation, marriage and initiation rites. In addition, a pig farm contributes in many ways by providing high value of animal protein and additional income. Dietze (2011) stated that pigs provide income for women, strengthening their role in families as well as in local communities. It often requires high investment and can easily be raised within home yard areas. Pig farming increases easily household income and nutritional status of women and children. It is not an easy task of farming but also takes a lot of time for rearing. It needs to have a large area with a lot of grass and soil. It is therefore, pig production is very significant and may favour profitable for their religious belief.

Although it is difficult to get the exact number of pigs population in Bangladesh, but they become an enjoyable business and increasing day by day in tribal areas (Hossain et al., 2012). Regarding this, Patr et al. (2014) reported that the tribal population of North-Eastern region rear pigs as integral part of their livelihood, the majority of pig enterprises belong to lower income groups, and have small and medium land holding capacity because of zero to minimum inputs involvement and low remuneration. Due to unemployment, inadequate nutrition and poverty, scarcity of cultivable land in the tribal society (Hossain, 2002), pig farming is getting importance in tribal regions for improving their economic status. It is relatively easy and profitable farm to reduce poverty also. In study areas, some tribal communities are rearing pigs by receiving financial and technical support from local non-government organization (NGOs). Thus the pig farming continues to be primitive scavenging in nature because they are raised by tribal women who are educationally, economically and socially most backward.

Most of the tribal women are involved in pig farming under the poverty alleviation program of direct local. For this purpose, local NGOs provide financial and training facilities on pig rearing to tribal women for meeting their basic needs. An important way of helping is to reduce their production cost, so that the prices of locally reared pig become more competitive and profitable. A few literatures (Sarma, 2014; Kabir et al., 2006; Roy and Manna, 2014) are available in home and aboard. Sarma (2014) focused on entrepreneurial activity of tribal women who improved their socioeconomic condition by using local raw materials like jute, straw, wood, and paper etc. Kabir et al. (2006) analyzes the performance and role of small entrepreneurship development in socioeconomic development of rural poor women. They found that participation of rural women to different small enterprise activities contributed significantly to increase participation in economic activities and
household decision making. Ray & Manna (2014) conducted on the issues of women entrepreneurship and empowerment from the perspective of thriving, evolving and prospering small urban India. It is therefore, clear that most of the studies were conducted on women empowerment through enterprise development and uplift their socioeconomic status, which was not presented in the present study. On the other hand, Sylhet, one part of Bangladesh is totally neglected or untouched in terms of academic research where tribal women recently have developed pig farm and benefitted. So it is needed in-depth research whether pig farming is profitable for them or not and made a difference of socioeconomic conditions from tribal women who have not involved in pig farming. If pig farming is profitable and better livelihood option for tribal women, it will be easy to take a decision for further improvement. It was also a systematic and comprehensive study which has not been conducted in Sylhet region of Bangladesh yet. This study is therefore an attempt to examine the profitability associated with pig farming in Moulvibazar district. The specific objectives of this study are: 1) to examine the socioeconomic differences between two groups of tribal women; 2) to determine profitability of pig farming; and 3) to analyze the factors affecting income from pig farming.

**MATERIALS AND MATHODS**

**Study area, sampling techniques and data collection**

Moulvibazar district was purposively selected for the study considering the emerging importance of pig farming. A multistage sampling technique was used for the selection of pig producers. In the first stage, two upazila namely, Sreemangal and Kulaura were selected purposively. In the second stage, two unions from each upazila (Rajghat and Kalighat unions from Sreemangal upazila, and Kulaura and Kadirpur unions from Kulaura upazila) were selected purposively because of prominence of pig entrepreneur in the areas. After that the targeted tribal women entrepreneurs from two communities namely Garo and Khasia were randomly selected from 13 punji (the living place of Garo and Khasia communities) using the list of communities available in the local NGOs’ sampling frame. Out of 13 punji, 7 punji were considered from Kulaura and 6 from Sreemangal upazila. The fourth stage involved the random selection of 35 pig farms from the Garo and another 35 pig farms from the Khasia communities making a total of 70tribal women entrepreneurs using the list of pig farmers available with the local NGOs’ sampling frame. Primary data were collected through structured questionnaire from the selected pig entrepreneurs. Both descriptive and analytical methods were employed in order to analyze the data.

**Data analysis techniques**

**Descriptive statistics**

Descriptive techniques have been used to illustrate current situations, describe different variables separately. These included: frequency distribution, percentage, mean, and standard deviation. Analytical techniques have been utilized to investigate
relationship between two communities of tribal women and statistical difference/association among them.

**Independent t-test**

It is also a parametric test where data are collected in probability sampling technique. In this study, this technique was used to determine the differences between two tribal communities ‘Garo and Khasia’ on the selected characteristics of tribal women entrepreneur.

**Cost-benefit analysis**

This was used to estimate farm net revenue for pig production. Theoretically, net revenue (NR) is total revenue (TR) less the total cost (TC) (Sarma et al., 2014):

\[ NR = TR - TC \]

Total cost is the addition of the entire variable cost (VC) and fixed cost (FC) items;

\[ TC = TVC + TFC \]

Total revenue is the total amount of money that an entrepreneur received from the sale of stock;

\[ TR = \sum P Q \]

Where,

\[ P = \text{Price per pig, } Q = \text{Quantity of pig sold} \]

Gross margin (GM) = TR – TVC

Net farm income (NFI) = GM – TFC

The rate of return is a performance measure used to measure the amount of return on an investment relative to the investment cost. It is given by:

Rate of Returns (ROR) = NR/TC

Gross Ratio (GR) = TC/TR

Benefit cost ratio (BCR) = TR/TC

Pig farming is profitable if it’s BCR ≥ 1 (Boardman et al., 2006). The higher the BCR the more profitable the pig production enterprise is. Depreciation was calculated using the straight line method.

**Linear regression model**

Following linear regression model was used to analyze the factors affecting income from pig production in the tribal areas (Miles and Shevlin, 2001).

\[ Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5 + \beta_6 X_6 + \epsilon_i \]

Where,

Dependent variable

\[ Y = \text{Annual income from pig farm (Tk.)} \]
Independent variables:
\[ X_1 = \text{Age of the respondent (years);} \]
\[ X_2 = \text{Family size (number);} \]
\[ X_3 = \text{Educational level of the respondent (year of school);} \]
\[ X_4 = \text{Farm size (number of pig);} \]
\[ X_5 = \text{Experience of pig farming (number of years);} \]
\[ X_6 = \text{Pig feed (quantity);} \]
\[ \beta_0 = \text{Intercept;} \]
\[ \beta_1 \text{ to } \beta_6 = \text{Regression coefficients of the independent variable;} \]
\[ \varepsilon = \text{Disturbance term or error term.} \]

RESULTS AND DISCUSSION

Socioeconomic characteristics differences between Two Tribal Women Entrepreneur

Socioeconomic differences of the sample pig entrepreneurs were presented in Table 1. It is evident from the table that most (60 and 54 percent) of Garo and Khasia entrepreneurs in the study area were within the age group of 35-44 years, 6 and 37% of years were above 44 years of age respectively. The mean age was 39.47 years. This implies that most of the tribal women entrepreneur were young and agile and therefore, able to cope up with the stressful nature of pig production. The result of t-test (4.41) also shows that Garo entrepreneurs were significantly ahead compared to Khasia. Durno and Stuart (2005) stated that the risk bearing abilities and innovativeness of a farmer, the mental capacity to cope with the daily challenges and demands of farming business decreases with advancing age.

Table 1. Socioeconomic characteristics of tribal women entrepreneurs (n=70)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Garo</th>
<th>Khasia</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency</td>
<td>Frequency</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;35 years</td>
<td>12 (34)</td>
<td>3 (9)</td>
<td>39.47</td>
<td>5.65</td>
<td>4.41***</td>
</tr>
<tr>
<td>35 to 44 years</td>
<td>21 (60)</td>
<td>19 (54)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>45 and above years</td>
<td>2 (6)</td>
<td>13 (37)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Educational level</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illiterate</td>
<td>3 (9)</td>
<td>5 (14)</td>
<td>5.13</td>
<td>2.60</td>
<td>0.32</td>
</tr>
<tr>
<td>Primary (1-5)</td>
<td>17 (48)</td>
<td>17 (49)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secondary (6-10)</td>
<td>15 (43)</td>
<td>13 (37)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family size</td>
<td>4.96</td>
<td>1.42</td>
<td></td>
<td></td>
<td>0.42</td>
</tr>
<tr>
<td>Variables</td>
<td>Garo Frequency</td>
<td>Garo Frequency</td>
<td>Khasia Frequency</td>
<td>Mean</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>---------------------------------</td>
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<td>------------------</td>
<td>------</td>
<td>--------------------</td>
</tr>
<tr>
<td>Small family (up to 3)</td>
<td>3 (9)</td>
<td>8 (23)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium family (4 to 6)</td>
<td>27 (77)</td>
<td>20 (57)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large family (7 and above)</td>
<td>5 (14)</td>
<td>7 (20)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farm size</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11 to 20 pigs</td>
<td>28 (80)</td>
<td>30 (86)</td>
<td>18.81</td>
<td>2.09</td>
<td>0.06</td>
</tr>
<tr>
<td>21 to 30 pigs</td>
<td>7 (20)</td>
<td>5 (14)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experience of pig farming</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 to 5 years</td>
<td>10 (29)</td>
<td>17 (49)</td>
<td>6.13</td>
<td>1.69</td>
<td>1.64**</td>
</tr>
<tr>
<td>6 to 10 years</td>
<td>25 (71)</td>
<td>18 (51)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Training on pig rearing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>12 (34)</td>
<td>14 (40)</td>
<td></td>
<td></td>
<td>0.49</td>
</tr>
<tr>
<td>Yes</td>
<td>23 (66)</td>
<td>21 (60)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Sources of fund</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Personal savings</td>
<td>9 (26)</td>
<td>10 (29)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Friend/relatives</td>
<td>3 (9)</td>
<td>3 (11)</td>
<td></td>
<td></td>
<td>0.36</td>
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<tr>
<td>Bank loan</td>
<td>4 (11)</td>
<td>4 (8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Local NGOs</td>
<td>19 (54)</td>
<td>18 (51)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farm income (annual)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 25 thousand</td>
<td>2 (6)</td>
<td>0</td>
<td>30479.57</td>
<td>1984.45</td>
<td>0.78</td>
</tr>
<tr>
<td>25 to 30 thousand</td>
<td>12 (34)</td>
<td>12 (34)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 30 thousand</td>
<td>21 (60)</td>
<td>23 (66)</td>
<td></td>
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</tr>
</tbody>
</table>

Source: Field survey, 2015
Note: Figures within the parentheses indicate percentage of total

**Garo** and **Khasia** entrepreneurs’ literacy level reveals that only 9 and 14 percent, respectively had no formal education whereas the remaining 91 and 86 percent had formal education ranging from primary to secondary education. The result of mean and standard deviation of educational level were 5.13 and 2.60 for pig entrepreneurs respectively. Ajieh and Okwuolu (2015) also reported that majority of the pig farmers in Delta state are literates. In the study, the result of t-test (0.32) implies that the educational level of pig entrepreneurs had insignificant impact on their capacity to exploit latent opportunities and adaptation of improved technologies.

The distribution of pig entrepreneurs by size of their household shows that a larger percentage (77 and 57 percent) had between 4 to 6 members, 9 and 23 percent, respectively had a small family and the remaining 14 and 20 percent had a large
family respectively. The number of family members ranged from 3 to 8 within an average of 4.96 and 1.42. The national average size in Bangladesh is 4.5 (BBS, 2014) which was relatively lower compared to the average size of the study areas. The average farm size of 18.81 pigs implies that pig production in the study areas is on small scale level and had insignificant impact on pig production. Table 1 shows that 71 and 51 percent of Garo and Khasia entrepreneurs had between 6 to 10 years pig rearing experience, while 29 and 49 percent, respectively had only 1 to 5 years of experience. The mean pig rearing experience in the study areas was 6.13 and significant (1.64) which suggests that Garo entrepreneurs had considerable years of pig production experience than Khasia entrepreneurs. Table 1 also shows that 66 and 60 percent of Garo and Khasia entrepreneurs have received training on pig production by government agencies and local NGOs about pig production whereas 34 and 40 percent respectively have not. The implication of this is that the trained entrepreneurs will be better equipped and perform better than those without training.

Furthermore, source of funding of pig production in the study areas revealed that most (54 and 51 percent) of Garo and Khasia entrepreneurs finance their business from local NGOs, 26 and 29 percent source of their capital from personal savings, while 9 and 11 percent from friends and relatives respectively. This study disagrees with Ogunniyi and Omoteso (2011) who found that the source of capital of livestock farmers was either from friends and relatives or from their personal savings. Table 1 further show that the farm income ranged from Tk. 24000 to Tk. 33380 with the mean and coefficient of variance was Tk. 30479.57 and 6.51 percent, respectively. Based on annual income of farm, entrepreneurs are classified into three categories, namely low income (Tk.<25 thousand), medium income (25 – 30 thousand), and high income (Tk.>30 thousand) respectively. The majority of tribal women entrepreneur belonged to the medium to high classes (34 to 66 percent) respectively.

**Average annual costs and returns of pig farming**

Table 2 presented detail information on the cost, returns and profitability of pig farming in the study areas. The annual average total cost of production as shown in table 2 was Tk. 277875.48 per pig. On an average, the annual total cost was estimated at Tk. 273857.30 and Tk. 281893.66 per pig for Garo and Khasia operated farms respectively.
Table 2. Annual average cost and returns of pig farming (Tk./pig)

<table>
<thead>
<tr>
<th>Cost/Return</th>
<th>Garo entrepreneurs</th>
<th>Khasia entrepreneurs</th>
<th>All entrepreneurs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amount (Tk.)</td>
<td>Amount (Tk.)</td>
<td>Amount (Tk.)</td>
</tr>
<tr>
<td>Total Revenue (TR)</td>
<td>330687</td>
<td>328260</td>
<td>329473.5</td>
</tr>
<tr>
<td>Variable cost</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cost of stocking</td>
<td>29078.42 (10.62)</td>
<td>25965.23 (9.21)</td>
<td>27521.83 (9.90)</td>
</tr>
<tr>
<td>Feed</td>
<td>200869.6 (73.35)</td>
<td>213828.4 (75.85)</td>
<td>207349 (74.62)</td>
</tr>
<tr>
<td>Labour</td>
<td>27387.83 (10.00)</td>
<td>26785.21 (9.50)</td>
<td>27086.52 (9.75)</td>
</tr>
<tr>
<td>Veterinary expense</td>
<td>2434.78 (0.89)</td>
<td>2300.67 (0.82)</td>
<td>2367.72 (0.85)</td>
</tr>
<tr>
<td>Transportation</td>
<td>6572.65 (2.40)</td>
<td>5847.73 (2.07)</td>
<td>6210.19 (2.23)</td>
</tr>
<tr>
<td>Other cost</td>
<td>2252.17 (0.82)</td>
<td>2136.89 (0.76)</td>
<td>2194.53 (0.79)</td>
</tr>
<tr>
<td>Total Variable Cost (TVC)</td>
<td>268595.45 (98.08)</td>
<td>276864.1 (98.22)</td>
<td>272729.8 (98.15)</td>
</tr>
<tr>
<td>Fixed cost</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depreciation</td>
<td>1057.45(0.39)</td>
<td>1132.17(0.40)</td>
<td>1094.81(0.39)</td>
</tr>
<tr>
<td>Interest on loan</td>
<td>4204.40(1.54)</td>
<td>3897.39(1.38)</td>
<td>4050.89(1.46)</td>
</tr>
<tr>
<td>Total Fixed Cost (TFC)</td>
<td>5261.85(1.92)</td>
<td>5029.56(1.78)</td>
<td>5145.71(1.85)</td>
</tr>
<tr>
<td>Total Cost (TFC+TVC)</td>
<td>273857.30</td>
<td>281893.66</td>
<td>277875.48</td>
</tr>
<tr>
<td>GM = TR – TVC</td>
<td>62091.55</td>
<td>51395.90</td>
<td>56743.70</td>
</tr>
<tr>
<td>NFI = GM – TFC</td>
<td>56829.70</td>
<td>46366.34</td>
<td>51597.99</td>
</tr>
<tr>
<td>NR = TR – TC</td>
<td>56829.70</td>
<td>46366.34</td>
<td>51597.99</td>
</tr>
<tr>
<td>ROR = NR/TC</td>
<td>0.21</td>
<td>0.16</td>
<td>0.19</td>
</tr>
<tr>
<td>BCR = TR/TC</td>
<td>1.21</td>
<td>1.16</td>
<td>1.19</td>
</tr>
<tr>
<td>Gross Ratio = TC/TR</td>
<td>0.83</td>
<td>0.86</td>
<td>0.84</td>
</tr>
</tbody>
</table>

Source: Field survey, 2015

Note: Figures within the parentheses indicate percentage of total

The respective annual average variable costs and fixed costs were calculated at Tk. 272729.8 and Tk. 5145.7 per pig respectively. The major cost items of pig farming included were feed cost, cost of stocking, labour cost, veterinary expenses, and transportation cost etc. Table 2 shows that the average feed cost is the highest about 74.62 percent of total, whereas Garo and Khasia managed farms contributed about 73.35 and 75.85 percent of total respectively.

Tribal women entrepreneur usually used the wastage of consumed food as feed for pig. The supplementary feed were also used and purchased at market price. In the study, the value of home supplied and purchased feeds were considered according to the market price. They used mixed vegetables, rice, and green grass etc. as feed for pig. The annual average feed cost was estimated at Tk. 207349 per pig. Labour cost shared about 10 percent of total costs respectively for both Garo and Khasia managed farms. Veterinary expense is an important cost item for pig rearing when
diseases occur frequently but in reality it happened scarcely. The annual average veterinary cost of pig farming was estimated at Tk. 2368 per pig which shared about 1 percent of total costs. On the other hand, transportation used for selling and buying pigs etc. Table 2 also shows that the annual average miscellaneous cost include electricity bill, contact with buyer etc. of pig farm was calculated at Tk. 2194 per pig in which Garo entrepreneur shared higher than Khasia. Fixed cost included depreciation cost of housing and interest on loan which shares only 1.92 and 1.78 percent for Garo and Khasia operated farms respectively. The annual average total fixed cost was Tk. 5146 per pig which was lower than variable cost.

The annual average total revenue was Tk. 329474 per pig while Garo entrepreneur received higher than Khasia. Table 2 shows that the annual average gross margin was Tk. 56744 per pig, where Garo and Khasia managed pig farms were at Tk. 62092 and Tk. 51396 respectively. The annual net farm income was Tk. 51598 per pig. The rate of return on investment in the study area was 0.19 which implies that every Tk. 1 invested in the pig business yielded Tk.19 per pig as profit. The benefit cost ratio (BCR) of 1.19 shows that pig production is a profitable business in the study area since it is greater than one. The gross ratio of 0.84 implies that Tk. 84 is spent for every one Taka gained in the business. Result indicated that Garo entrepreneur were comparatively more benefited than Khasia for pig farming. Thus pig farming is a profitable venture in the study areas as indicated by the various profitability ratio techniques employed in the analysis. Several fields based studies on pig farming have been reported in many South East Asian countries like India (Kumaresan et al., 2007, 2009, Nath et al., 2013), China (Riedal et al., 2012), and Lao People’s Democratic Republic (Phengsavanh et al., 2011) indicating general household information, farm characteristics, performances of the pigs, pig health status, marketing system, constraints and opportunities for development. Tylor and Roese (2006) also reported that piggery was a profitable business which provided employment and return on investment, as Hu et al. (2004) studied that higher earnings from pig farming keeps the market attractive even for small producers, but recently Anower et al. (2017) identified that pig production is an additional income source of household. In the present study, an attempt has been taken to analyze whether pig farming is profitable for tribal women or not. The profitable farm might be encouraging business activities for tribal women to develop an entrepreneur which was done in Sylhet region of Bangladesh for the first time.

Factors affecting farm income from pig production

The result of linear regression analysis examined the characteristics of pig entrepreneurs and its impact on farm income of pig production. Different variables were expected to influence pig rearing, these are age of entrepreneurs, educational level, family size, experience of pig farming, farm size based on number of pigs, and pig feed. Durbin-Watson (2.199) test was used to detect to autocorrelation. The result revealed that there is no autocorrelation problem. A variance inflation factor (VIF)
detects multicollinearity in regression analysis. It estimates how much the variance of a regression coefficient is inflated due to multicollinearity in the model and range from 1 upwards. The numerical value for VIF indicated (in decimal form) what percentage of the variance (i.e. the standard error squared) is inflated for each coefficient. In the study, a VIF of 1.2 shows that the variance of educational level of pig entrepreneurs is 90 percent.

Table 3. Regression results of factors affecting of farm income of pig farming

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Coefficient</th>
<th>t-test</th>
<th>P-value</th>
<th>VIF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>10.02</td>
<td>102.17</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>Age of the entrepreneurs</td>
<td>0.002</td>
<td>1.13</td>
<td>0.262</td>
<td>1.13</td>
</tr>
<tr>
<td>Family size</td>
<td>-0.003</td>
<td>-0.65</td>
<td>0.518</td>
<td>1.01</td>
</tr>
<tr>
<td>Experience of pig farming</td>
<td>0.01</td>
<td>2.24**</td>
<td>0.029</td>
<td>1.05</td>
</tr>
<tr>
<td>Farm size</td>
<td>0.014</td>
<td>3.73***</td>
<td>0.000</td>
<td>1.15</td>
</tr>
<tr>
<td>Educational level of pig entrepreneurs</td>
<td>0.006</td>
<td>1.90*</td>
<td>0.061</td>
<td>1.20</td>
</tr>
<tr>
<td>Pig feed used</td>
<td>-0.037</td>
<td>-3.39***</td>
<td>0.001</td>
<td>1.11</td>
</tr>
</tbody>
</table>

Source: Field survey, 2015

Note: Dependent variable: Annual income from pig farming; *, **, and *** indicates the significance level of 10, 5 and 1 percent respectively.

Among the six factors affecting farm income of pig farming, four variables (educational level, farm size, experience of pig farming and used pig feed) were found significant. The coefficient of farm size is positive and significant at 1 percent, means that 1 percent in the number of pigs will increase output level by 0.014 percent (Table 3). The experience of pig farming has a positive effect on farm income and is statistically significant at 5% probability level. The result suggests that as entrepreneurs have high pig farming experience the number of pig increased through its effect on farm income. The result also explained that every one year of experience in pig farming leads to increase farm income by Tk. 0.01 per day per pig. This result is plausible and suggests that farm income of the pig farm in the study areas is more responsive to a number of pig. Furthermore, this result illustrates that farm income per year increases in responses to the increases in a number of pig.

Table 3 also shows that farm income in the study areas increases with increases in the entrepreneurs’ level of education. This implies that pig entrepreneurs who are educated achieved higher level of income than the uneducated ones. Finding agrees with Umeh et al. (2015) who explained that education is important for achieving effective utilization of inputs in pig production in Nigeria. The coefficient of pig feed is negative but significant at 1percent probability level. The result of feed used (0.037) confirms the importance of concentrates in pig production. This implies that farm income declines with increases feed suggesting.
CONCLUSION

The study was conducted to measure the profitability and factors affecting on farm income of pig in Moulvibazar district of Bangladesh. The study revealed that majority of tribal women entrepreneurs were middle aged and earned significantly from pig farm. It gives them year round work with extra income. The calculated net profit and the benefit-cost ratio (BCR) indicated that it is a profitable business for tribal women. Econometric result also indicate that number of pigs, experience and educational level of entrepreneurs significantly associated with farm income. This also implies that it is profitable and worth venturing as a source of year round income. It plays a vital role in creation of employment opportunities in tribal areas, animal protein supply, increasing income and standard of living, although there are room for improvement.

Based on findings of the study, adequate training programme on pig production can be organized for pig entrepreneurs in the study areas. Pig entrepreneurs should also be organized into formidable groups such as cooperative society to enjoy the marketing of pigs and purchasing inputs such as feeds, drugs and vaccines. If need based remedial measures could be taken, then pig farming would be a viable in commercial enterprise which in turn would play a vital role to overcome the problems of low income and unemployment situation of tribal women. Therefore, the government may provide necessary assistance on how to get access in receiving credit in order to increase their capital base to expand their scale of production.

ACKNOWLEDGEMENT

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REFERENCES


PASSIVE SURVEILLANCE ON OCCURRENCE OF DEADLY INFECTIOUS, NON-INFECTIOUS AND ZOONOTIC DISEASES OF LIVESTOCK AND POULTRY IN BANGLADESH AND REMEDIES

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ABSTRACT

Passive surveillance system was designed with the data (102,613 case records) collected from the Government Veterinary Hospitals, Bangladesh and frequency distribution of diseases was calculated during July 2010 to June 2013. Frequently occurring diseases/disease conditions reported in livestock were fascioliasis (10.66%), diarrhoea (7.92%), mastitis (7.42%), foot and mouth disease (6.42%), parasitic gastroenteritis (6.31%), coccidiosis (5.5%), Peste des petits ruminants (PPR, 5.32%), anthrax (4.19%) and black quarter (3.74%). Diarrhoea and coccidiosis were reported to occur throughout the year. The frequency of fascioliasis appeared higher in buffaloes (34%) followed by sheep (22%), goats (13%) and cattle (11%). PPR is a deadly infectious disease of goats and sheep, accounted for 20% and 13% infectivity in respective species. In chicken the most frequently occurring diseases reported were Newcastle disease (28%), fowl cholera (19%) and coccidiosis (11%). In ducks, duck viral enteritis (28%), duck viral hepatitis (17%), diarrhoea (15%), coccidiosis (10%) and intestinal helminthiasis (10%) were the commonest diseases reported in Bangladesh. Few other endemic diseases of livestock and poultry like Tuberculosis, brucellosis, avian influenza, duck anatipestifer, Marek’s disease, Gumboro disease, avian tuberculosis, mycoplasmosis, dermatophilosis etc. were not included in the hospital data sheet. Financial hurdles persist in a country like Bangladesh, imposing difficulties onto the surveillance and early reporting of the disease outbreaks; these diseases are, therefore, stubbornly prevalent. Development of technological and knowledgeable

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man power, in time surveillance and early warning of disease outbreak are the key to protect animal and public health and produce safe food of animal origin.

**Keywords:** Surveillance, zoonotic, infectious, non-infectious, diseases

**INTRODUCTION**

Diseases of livestock and poultry are the main hurdles of profitable animal agriculture in Bangladesh. Infectious, non-infectious, emerging, parasitic and zoonotic diseases of livestock and poultry are the leading cause of morbidity and mortality, restricted international trade of meat, milk, eggs, bone, fishes and feed of animal origin, condemnation of carcasses during slaughter and costs of their management. In general, sustainable and profitable livestock and poultry management requires independent state veterinary services and, modern surveillance technologies in order for quick and confirmatory detection of the diseases at early onset and designing control strategy. There are Veterinary Services under the Department of Livestock Services (DLS), Ministry of Livestock and Fisheries, Bangladesh but the surveillance system was not optimized due to conflict between Vet and non-Vet people working under the same ministry. Moreover, the public health services in Bangladesh is yet to organize to its requirement. The State Veterinary Services, Veterinary Faculties and Public Health Department are the wings for designing routine surveillance. Collection of information onto the diseases that are considered reportable by state statute and regulation (OIE 2008) are also ignored. The filed veterinarians and public health officers in the third world country including Bangladesh lack innovative ideas/ techniques of diseases surveillance and are not regularly soliciting modern surveillance information.

Most of the local, city, and state veterinary departments require Vets, para vets and animal health care providers as well as town, school, hospital and laboratory officials to report diseases to the local veterinary office. This reporting system in passive surveillance system is yet to develop in Bangladesh. It is interesting to note that the field veterinarians seldom report all cases, sometimes are unaware about the reportable diseases or may consider the report a breach of confidentiality, similar condition also existed in abroad (Wright and Thrusfield, 2016). There are few reports of infectious diseases but the timeliness of reports is often affected by the delay in receiving laboratory test results (active surveillance). In passive surveillance system, there should have coordinator, should provide training to Vets, para-Vets and animal health provider about how to fill in the surveillance forms, and may even send someone to periodically collect forms from animal’s health facilities. But little training has given to individual veterinary health provider who reports the information. The Vets, para-Vets and animal health providers require to interview farmers/ owners to filteractive case finding in the herd or animal population in passive surveillance (Amaku et al., 2015). This surveillance is required in order to detect sub-clinically infected/ sick animals and birds that may not notify to veterinary health care facilities and this may, therefore, be enabling emergence or epidemicity of a disease.
This research project designed a passive surveillance protocol against a number of deadly infectious, non-infectious, zoonotic and emerging diseases of livestock and poultry in Bangladesh. The passive surveillance data analyzed towards better understanding of the existence of important diseases, formulating necessary control and preventive measures. In developed country passive surveillance is the key epidemiological investigation to predict diseases flow in human and animals. Initially most surveillance for important communicable, trans-boundary, emerging or vector borne diseases is passive (Khan, 2015; OIE, 2008; Ouagal et al., 2010).

Development and implementation of global animal disease surveillance has been limited by the lack of information systems of real-time data capturing, reporting, sharing, analysis, and related decision- and policy-making (Perez et al., 2011). It is global trend to support results of passive surveillance through active surveillance. For active surveillance, samples collected from the field or on farm used in the laboratory for confirmatory detection of diseases. Active surveillance system provides important stimulus to animal health workers, especially Vets and para-Vets in the form of individual feedback or outbreak studies. Active surveillance requires substantially more time and resources in the laboratory and is, therefore, less commonly used in emergencies. But it is often more complete than passive surveillance (OIE, 2008). Active surveillance is often used if an outbreak has begun or is suspected to keep close track of the number of cases. This paper described the results of a passive surveillance with the data collected from Upazilla Veterinary Hospitals, Bangladesh. The output of this finding is assumed to generate suggestions and probable resolutions against a number of deadly infectious, non-infectious, zoonotic and emerging diseases of livestock and poultry in Bangladesh.

**MATERIALS AND METHODS**

**Passive surveillance**

Passive surveillance is the most common type of surveillance in human and veterinary medical emergencies. Most surveillance for communicable, trans-boundary, emerging, vector borne diseases are passive. There is diseases investigation and monitoring software for reporting diseases of livestock and poultry attended in the Upazilla Veterinary Hospital, Bangladesh. The Government Vets working in the Upazilla Veterinary Hospitals collect data in the patient register, fill in the surveillance questioner and submit it to the epidemiology unit, DLS by the end of each month. In this study data obtained from the epidemiology wing, DLS, were analyze targeting format (epidemiology, ecology), extend of freedom of the Vets and animal health workers during collection and reporting data etc. We have designed a disease investigation format and analyzed data collected from selected Upazilla Veterinary Hospitals, Bangladesh. A total of 102,613 hospital cases were analyzed through the disease investigation forms during the year 2010-2011, 2011-2012 and 2012-2013. The divisions included were Dhaka, Chittagong, Rangpur, Rajshahi,
Sylhet, Khulna and Barisal. The data were analyzed by statistical analysis software (SAS) at Chittagong Veterinary and Animal Sciences University (CVASU), Chittagong and Bangladesh Agricultural University, Mymensingh. We report herewith the contribution of seasons, age, geography and species of animals and birds onto the distribution of diseases of livestock and poultry and proposing possible remedies, preventive and control strategies.

RESULTS

Out of 102,613 hospital case reports analyzed during 2010 to 2013, cattle (N=49523) appeared to be the most frequently involved animal species attended in the government veterinary hospital followed by goats (N=24526), chickens (N=15936) and ducks (N=4619). Prevalence of all the diseases appeared higher at Dhaka division followed by Chittagong, Rangpur, Rajshahi, Sylhet, Khulna and Barisal division (Figure 1). The top 10 frequently occurring diseases recorded (Figure 2) in the study areas were liver fluke infestation (fascioliasis, 10.66%), diarrhea (7.92%), mastitis (7.42%), FMD (6.42%), parasitic gastroenteritis (6.31%), coccidiosis (5.5%), PPR (5.32%), Newcastle disease (ND, 4.36%), anthrax (4.19%) and black quarter (BQ, 3.74%). While evaluating seasonal influence onto the occurrence of diseases in livestock throughout the country during 2010 to 2013 (Figure 3), liver fluke infestation appeared higher during July and August. During winter, diarrhea appeared as the most commonly occurring malady in livestock. In chicken, ND is the most frequently reported disease during spring, summer and rainy seasons. The burden of common parasitic gastroenteritis appeared higher during rainy (June and July) and winter (November and December) seasons while river bank and bank of other water reservoirs started rising. The farmers allowed their ruminants to graze at the sites of natural water bodies during that time where the encysted cercaria of liver fluke made available to infect ruminants. The warm and humid environment and green grasses on Pasteur land by the sites of river, ponds, lakes, and grazing field also favor dissemination of endo-parasites in ruminants.

Frequency distribution of commonly occurring and important diseases of cattle across the study period (Figure 4) were liver fluke infestation, FMD, mastitis, black quarter and anthrax. Commonly occurring diseases of buffaloes (Figure 5) were liver fluke infestation followed by parasitic gastroenteritis and nonspecific diarrhoea. Goats were commonly infected with PPR (Figure 6) followed by liver fluke infestation, mastitis and diarrhoea. Frequency distribution of different diseases of poultry across the country during the study period included ND, Fowl cholera, coccidiosis and fowl pox (Figure 7). Ducks were commonly infested with duck viral enteritis followed by duck viral hepatitis, diarrhoea, coccidiosis and intestinal helminthiasis (Figure 8). The zoonotic diseases reported was mostly anthrax, incidence of anthrax was higher in Dhaka and lower in Sylhet divisions.
Figure 1. Frequency of commonly occurring diseases of livestock and poultry (2010-2011, 2011-2012 and 2012-2013) is stratified by different geographical areas (Divisions) of Bangladesh. The top most and commonest diseases reported were anthrax, liver fluke, mastitis, FMD, PPR, ND and BQ. Prevalence of all the diseases appeared higher at Dhaka division followed by Chittagong, Rangpur, Rajshahi, Sylhet, Khulna and Barisal division.

Figure 2. Top 10 frequently occurring (percentages are shown on the top of the bar) diseases of animals and birds in reporting areas of Bangladesh. The geo-climatic condition of Bangladesh favors the growth and dissemination of liver-fluke infestation (10.66%) in cattle, buffaloes, sheep and goats. FMD outbreaks (6.42%) in young calves and PPR outbreaks (5.32%) in yearling goats are the leading causes of death in calves (40-70%) and goats (50-70%) respectively.
Figure 3. Seasonal occurrence of diseases in livestock throughout the country during 2010 to 2013. The frequency of liver fluke infestation in all ruminants appeared higher during July and August of the year. The burden of parasitic gastroenteritis appeared higher during rainy and winter seasons.

Figure 4. Frequency of commonly occurring and important diseases of cattle across the study period. The highest incidence of disease observed were liver fluke infestation (11%), FMD (11%), mastitis (10%), black quarter (08%) and anthrax (08%). The lowest incidence of diseases recorded were HS (04%), lesions in Skin (05%), diarrhoea (06%) and parasitic gastroenteritis.
Figure 5. Frequency of commonly occurring and important diseases of buffaloes across the study period. Highest occurrence of diseases reported were liver fluke (34%) infestation followed by parasitic gastroenteritis (14%) and nonspecific diarrhoea (12%). The lowest incidence of diseases in buffaloes seen were anthrax (02%), HS (04%), coccidiosis (04%), dermatitis (04%) and FMD (08%).

Figure 6. Frequency distribution of different diseases in goats across the study period. The highest incidence of diseases reported were PPR (20%) followed by liver fluke infestation (13%), mastitis (10%) and diarrhoea (10%). The least incidence of diseases reported were coccidiosis (4%), unclassified respiratory signs (6%), dermal lesions (6%) and parasitic gastroenteritis (9%).
Figure 7. Frequency distribution of different diseases in chicken across the country during July 2010 to June 2013. The highest incidence of NCD (28%) was recorded in the Upazilla Veterinary Hospitals followed by fowl cholera (19%), coccidiosis (11%) and fowl pox (9%). The least commonly occurring diseases were chronic respiratory diseases (3%), intestinal helminthiasis (5%), diarrhoea (5%) and Infectious bursal diseases (8%).

Figure 8. Frequency distribution of different diseases in ducks across the country during 2010 to 2013. In ducks highest incidence of diseases recorded were duck viral enteritis (28%) followed by duck viral hepatitis (17%), diarrhoea (15%), coccidiosis (10%) and intestinal helminthiasis (10%). The least commonly occurring diseases were respiratory distresses (3%), CRD (4%) and duck cholera (7%).
It is customarily accepted that the existing and suspicious new cases or clinical signs should be reported to local (Upazilla), regional (city or division) and state Veterinary department (Department of Livestock Services) by the animal health care providers, institutional, research laboratories or market sources (Figure 9). Surveillance case definitions enable animal health officials to classify and count cases consistently across reporting jurisdictions. Surveillance case definitions are not intended to be used by healthcare providers for making a clinical diagnosis or determining how to meet an individual patient’s health needs. Not all the diseases has to be reported to Center for Disease Control and Prevention (CDC). The list of reportable conditions varies by state. The Council of State and Territorial Epidemiologists (CSTE) has to define and recommend the state health departments report cases of selected diseases to CDC’s National Notifiable Diseases Surveillance System (NNDSS). Every year, case definitions are updated using CSTE’s Position Statements and they provide uniform criteria of national notifiable infectious and non-infectious conditions for reporting purposes.

Figure 9. A flow chart of national diseases surveillance system. Only the notifiable diseases or disease conditions (https://wwwn.cdc.gov/nndss/conditions/notifiable/2018/) have to be reported to CDC. The main goal of such reporting is to protect public and animal health and safety through the prevention and control of disease, injury, and disability in the regional and international level.
DISCUSSION

The top 10 frequently occurring diseases of livestock and poultry in Bangladesh were liver fluke infestation, diarrhoea, mastitis, FMD, parasitic gastroenteritis, coccidiosis, PPR, ND, anthrax and BQ. Less than 2% of total livestock and poultry population were attended in the Upazilla Veterinary Hospitals and the prevalence of diseases reported does not truly reflect the real pictures. There is little opportunity in the Government veterinary health care facilities at Upazilla level to measure the effect of these diseases on weight gain, meat and milk yield, morbidity and mortality of animals and birds. The important zoonotic diseases only noted in the hospital data sheet during passive surveillance was anthrax. Brucellosis (Dey et al., 2013) and Tuberculosis in dairy cattle (Hossain et al., 2015), Leishmaniasis in goats (Labony et al., 2014) and canids (Khan et al., 2012) and avian influenza in chickens (Bari et al., 2009) and ducks (Ruba et al., 2015) were endemic in Bangladesh and extremely zoonotic, these diseases were not included in the hospital data sheet. The passive surveillance protocols were, therefore, unable to analyze frequency distribution of the existing zoonotic diseases of livestock and poultry in Bangladesh.

The hospital record proposed diarrhoea as second most commonly occurring condition in livestock but the actual cause(s) left unreported. The gastrointestinal round worm infestation, coccidiosis, balantidiasis, immature amphistomiasis, schistosomiasis, nitrate poisoning etc. may contribute diarrhea in livestock but these are underreporting. Babesiosis, anaplasmosis and thileriosis are commonly occurring tick borne diseases of livestock in Bangladesh (Karim et al., 2012) but have got little information in the data sheet. The higher rate of morbidity and mortality of goats due to PPR as we observed during our active surveillance (Dhar et al., 2015) was literally absent in the hospital data recording.

Anthrax (Zohora et al., 2012), FMD, PPR and BQ are preventable disease of livestock and require regular immunization. The farmers have restricted opportunity to vaccinate their animals against these diseases. Cattle in Bangladesh were commonly infected with FMD viral serotype O, A and Asia 1 (Islam et al., 2016; Pervin et al., 2011). Routine biannual vaccination of ruminant by using polyvalent vaccine containing FMD viral serotype O, A and Asia 1 is recommended. Following primary immunization of livestock with trivalent FMD vaccine a boost 15-21 days lateris recommended. Young sheep and goats were commonly infected with PPR with higher rate of mortality. Infected goats died following PPR mostly due to secondary infection caused by Pasteurella multocida (data was not shown). Goats over one year of age were commonly infested with Fasciola gigantic and may contribute higher rate of morbidity and mortality. Moreover, 25-30% goats found to carry anti PPR antibodies in their sera, they may neutralize vaccine virus following immunization and may be the leading cause of vaccine failure and death following PPR. Regular deworming of small ruminants and analyzing anti PPR antibodies before immunization with PPR vaccine is recommended.
A recent threat of dairy industry in Bangladesh is the occurrence of Tuberculosis (TB, Hossain et al., 2016), which is not addressed in hospital data sheet. Recent evidence suggested that there is emergence of drug resistance TB (multi drug and extensive drug resistance, about 8-10%) in man and animals (Cohen et al., 2015; Corbett et al., 2003; Espinal et al., 2001); this information in Bangladesh is virtually absent. Vets are routinely involved with the management of diseases of livestock including TB but unknowingly many vets of Bangladesh may have had TB from farm, zoo and slaughtered animals. Routine intra dermal tuberculin test in the elderly dairy cattle is recommended to prevent future zoonosis and possible emergence of multi drug and extensively drug resistance TB. Avian TB is also endemic in few elderly commercial poultry farms in Bangladesh (Haque et al., 2016) but was not included in the hospital data sheet. The bovine TB (*M. bovis*), human TB (*M. tuberculosis*), Para TB (*M. avium sub spvarpara tuberculosis*) and avian TB (*M. a. avium*) are endemic in Bangladesh and extremely zoonotic. State Veterinary Services or Public Health Department require empowering to test all the dairy, zoo and slaughtered animals and dispose the test positive animals immediate after testing. Test and slaughter of infected/ test positive animals and birds are needed to make a safe environment for the farmers, owners, veterinarians and human being getting close proximity to farms.

The ecosystem and availability of vectors or intermediate hosts of parasites made Bangladesh a heaven for parasitism (Alam et al., 2014; Hossain et al., 2015; Hossain et al., 2011; Khan, 2015; Samad et al., 2004); all animals require regular examination for the existence of specific nematode, trematode, cestode and ecto-parasites. It is not difficult to test and diagnose parasitism of livestock but little technical facilities were provided from the diagnostic shelf of Government and private veterinary health care services. The government Vets, para-Vets and animal health providers require training on certain ecological and epidemiological approaches of parasitic life cycle and to learn basics of parasitic disease diagnostics and management. Accurate reporting and management of parasitic diseases of livestock and poultry were, therefore, obtained. Buffaloes and sheep appeared resistant animals species to a number of infectious and parasitic disease compared to cattle and goats; their feeding, breeding and management practices are mostly neglected. Very few buffaloes and sheep were attended in the Upazilla veterinary hospitals and the diseases they carry were not either reported.

Farm animals living in and around forest areas are at increased risk of vector borne disease like trypanosomiasis (Surra), babesiosis, anaplasmosis, thileriosis, stephanofilariosis etc. but the disease information is lacking in the hospital data sheet; these may be due to lack of detection protocol or reporting system. There are diseases of cattle, buffaloes, sheep and goats transmitted through domestic and wild mammals and vectors like heart worm infestation (Yousuf et al., 2014) rabies, toxoplasmosis, hydatidosis, coeneurosis etc., these information is also lacking in the hospital recording. A similar study was carried out in Van Gujjars, India (Wright and
Thrusfield, 2016) with the perceptions that these diseases should be present, but were under-reported. The animal diseases in Bangladesh and India have much greater concern to the community like surra (trypanosomiasis), kala-ajar, bovine viral diarrhoea, salmonellosis, brucellosis, leptospirosis, endocrine disorders like anestrous, hypoplastic ovary, cystic ovarian syndrome etc. but were neglected or the field veterinarians were not much aware of these diseases.

In chicken the most frequently occurring diseases reported were ND, FC and coccidiosis. In ducks the diseases reported were duck viral enteritis, duck viral hepatitis, and intestinal helminthiasis. The ND, FC in chickens and duck viral enteritis and duck cholera in ducks were preventable by using appropriate vaccines but the preventive measures rarely practiced in most of the farm operation. The free range chickens and ducks (more than 60% of total poultry and duck population) rarely brought to the Government veterinary health care facilities and rarely vaccinate against these diseases. The ecosystem of Bangladesh also provides ampoule opportunity for the development and dissemination of parasitic disease of poultry and ducks (Alam et al., 2014; Musa et al., 2012). Using passive surveillance system the loads of parasitic diseases cannot be identified but information like progressive weight loss, reduce eggs and meat production may be indicatory. The active surveillance system to identify specific adult parasites and their load is helpful. It is simple to test fecal samples of livestock and poultry by direct microscopy (available in veterinary health care facilities) but little is practiced in the Upazilla Veterinary Hospital. Intestinal nematodes, cestodes and flukes are common in ducks rearing in open water bodies. All of the free range ducks and chicken requiring anthelmintic application at regular basis.

**CONCLUSIONS**

Before considering how best animal disease surveillance can be implemented, the veterinarians or data collector should first have a clear understanding of why they need to do surveillance. The important reasons why veterinary authorities undertake surveillance activities can be summarized into four general purposes; 1. Finding cases of new disease, 2. Early detection of disease, 3. Measuring the level of disease, and 4. Demonstrating freedom from disease (diseases are not truly present). These purposes can further be divided into two groups: 1). surveillance for diseases that are currently or usually not present and 2). surveillance of diseases that are endemic. The data we analyzed showed that a number of important diseases of livestock and poultry were neglected or under reported. Diseases that were not reported can be categorized into six subtype for better reporting and management of diseases includes;

1. **Exotic diseases** (known diseases that are not present in a country, but exist in other countries) like Bovine spongiform encephalopathy (BSE), visna, scrapie, bluetongue disease, chikungunya etc.
2. **Endemic diseases** (diseases existed in a geographic location under certain levels) like dermatophilosis, amphistomiasis due to *Gigantocotyle explanatum*, contagious bovine pleuro-pneumonia (CBPP), schistosomiasis, Linguatulaserrata infestation (Islam et al., 2018) etc.

3. **Emerging diseases** (recently identified diseases that have got much attention due to increased host range, enhance pathogenicity or spread) like Avian influenza, ND, Marek’s disease etc.

4. **New diseases** (diseases which have not previously been recognized): need to find out

5. **Epidemic diseases** (diseases which is present in a country or location where sudden outbreak occurs, and then do not occur for a certain period): goat pox and often confused with PPR in goats

6. **Zoonotic diseases** (diseases transmissible between man and animals) like Tuberculosis, Avian influenza, Malaria, toxoplasmosis, Brucellosis, mites infestation, heartworm infestation in dogs, etc.

When the diseases is not present in a country (or a zone or compartment within a country) there may have a number of benefits, including the ability to export animals/animal product or to cease disease control measures (such as a vaccination program). However, in order to get these benefits, the veterinary authorities must first be confident that the disease is truly absent. Demonstrating freedom from disease is difficult. However, if there is a tendency of the Vets, para-Vets or animal health providers to hide information about the occurrence of a disease or diseases in passive surveillance, that may cause serious havoc on national economy in the long run by sudden outburst which in turn costs a lot for their prevention, control and eradication and finally restricting global trade of animals and animal product. Early recognition of a disease incursion may be important for early response and prevention of epidemicity.

Our study showed that all of the diseases now endemic in livestock and poultry in Bangladesh was not recorded by the Vets or animal health providers in the hospital data sheet. There is lacking of routine active surveillance of animal diseases as well. Rarely the results of passive surveillance were supported by the observation of active surveillance. There is huge shortage of effective vaccines (less than 10% of requirement) against all of the endemic infectious diseases of livestock and poultry in Bangladesh. The free animal movement across the country may disseminate avian influenza in poultry and FMD and PPR in livestock. Lab based survey of filed samples revealed that avian influenza in ducks and chickens is now endemic in Bangladesh. There is crying need to develop FMD, PPR and Avian influenza institutes in Bangladesh to combat devastation on livestock and poultry sector each year. The Government and non-government research organizations have to enrich their surveillance system and have to report all the diseases to prevent future havoc.
Timely collection and analysis of the data of passive and active surveillance systems may enable us to detect most of the infectious, emerging, zoonotic and endemic diseases at early onset and provide warning and precautions accordingly. This may boosts biosecurity and health care facilities of millions of buffaloes, cattle, goats, sheep, chickens and ducks across the country. A successful adaptation of passive surveillance system onto the occurrence of diseases of livestock and poultry may enable us to provide safe food of animal origin and may boostup economic power of the country.

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SALINITY IMPACTS ON EXPERIMENTAL FODDER SORGHUM PRODUCTION

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ABSTRACT
Field experiment was conducted at research station of Soil, Water & Environment Discipline, Khulna University, during the dry season to see the growth performance of sorghum (*Sorghum bicolor* L. cv. Morokoshi) irrigating with saline water. For irrigation, river water (RW) containing EC value of 14.04 dS m⁻¹ was collected from the Rupsha river, Khulna and mixed with tap water [TW] containing EC value of 0.78 dS m⁻¹ at three different ratios (3:1, 1:1 and 1:3 v/v). After mixing, water containing five different EC values (0.78, 4.19, 7.18, 10.79 and 14.04 dS m⁻¹) were obtained and considered as salinity treatment. Harvesting and sampling was done 83 days after transplanting (DAT) by cutting four sorghum plants randomly selected from each plot. Different morphological parameters such as plant height, leaf number, leaf length, leaf width, stem diameter and plant biomass were measured and recorded. Soil samples were also collected from each plot. Under water salinity stress, all the agronomic attributes and plant biomass showed a decreasing tendency with increasing salt concentration in irrigation water but the growth was not harmfully affected at lower levels of salinity. Plant height and biomass was significantly decreased irrigating with water containing salinity 10.79 dS m⁻¹. After harvest it was found that irrigation with saline water up to 10.79 dS m⁻¹ did not show any increase of soil salinity. It was probably due to rainfall during the monsoon which was occurred at the later stage of the growing period. So, the fodder sorghum plant might be cultivated in the coastal regions of Bangladesh where fresh water irrigation is limited due to salinity problem as well as might be grown irrigating with saline water up to 10.79 dS m⁻¹.

Keywords: Biomass, coastal region, fodder, sorghum, morphology, salinity.

INTRODUCTION
Salinity is one of the major abiotic stress factors that affect plant growth and productivity, especially in arid and semi-arid tracts as well as coastal areas in tropical
regions of the world (Hafsi et al., 2010). Salinity is the accumulation of salt in soil and water. Increasing both soil and water salinity decrease crop production through adversely affecting plant growth and development (Ahmed, 2009; Jahanzad et al., 2013, Qadir et al., 2014). Over 800 million hectares of land are salt affected throughout the world, either by salinity (397 million ha) or by the associated condition of sodicity (434 million ha) (FAO, 2000). More than 30% of the cultivable land in Bangladesh lies in the coastal area. Out of 2.86 million hectares of coastal and off-shore lands about 1.06 million ha of arable lands are affected by varying degrees of salinity (SRDI, 2010). Over the last 35 years, salinity has increased around 26 percent in the coastal region of Bangladesh (Mahmuduzzaman et al., 2014).

In fact, in arid and semi-arid regions saline soils are abundant due to intensity of evaporation and insufficient amount of rainfall for substantial leaching (Dai, 2011). Salinity problem in tropical region is quite different than in arid and semiarid region. Saline or salt affected soils are common problem in coastal area of tropical regions as well as in Bangladesh. The main source of salinity here is of marine origin. Sea water surrounds the south coast, intrudes northward and causes salinity (Brady & Weil, 2002).

Farmers in the coastal areas mostly cultivate low yielding, traditional rice varieties during wet season. Most of the land remains fallow in the dry season (January- May) because of soil salinity, lack of good quality irrigation water and late draining condition of the field (Karim et al., 1990). Livestock contributed around 14% to agriculture GDP (FPMU, 2014) in Bangladesh. The major constraints to dairy cattle production are the shortages of feeds and fodder. In case of coastal region in Bangladesh the problem is severe and diverse. Since plant growth and development are adversely affected by both soil and water salinity, these conditions are unsuitable for crop cultivation as like as livestock feeds and fodder which are dependent on supplemental irrigation during the season December–May. The scope of cow rearing is limited due to the shortage of grazing and fodder field. Besides, peoples have been using virgin land and water for shrimp production through allowing the intrusion of saline water, and increasing salinity in surrounding areas and damaging the grazing areas for livestock. The grazing land is decreased up to 64% over last twenty years (Ghafur et al., 1999; Anwar, 2005).

The shortage of feeds and fodder in the coastal areas of Bangladesh, often affect livestock production and productivity, needs immediate attention, especially, in searching of salt tolerant fodder crops. Farmers either can use salt tolerant fodder species or can grow plant irrigating with available fresh water mixing with saline water to increase livestock productivity. 

Sorghum bicolor, a highly productive crop, grown for fodder, fiber and/or biofuel, ranks fifth in global cereal production and it shows a strong environmental stress tolerance to drought, heat, salinity and flooding (Igartua et al., 1994; Marambe & Ando, 1995; Belton et al., 2004; Netonda et al., 2004). The tillering characteristics
enable sorghum to completely regenerate the above-ground portions of the plant. Thus, sorghum plants have been kept alive for as long as 6 to 7 years where the climate is mild enough to avoid winterkill and when disease and insect protection have been provided (Saberi & Aishah, 2014). In the present research, we cultivated fodder sorghum in the south-west coastal region of Bangladesh under irrigation with saline water.

The objective of the present study was to see the growth performance of fodder sorghum under irrigation water salt stress.

MATERIALS AND METHODS

Description of the site

The experiment was carried out at the experimental field of Soil, Water and Environment Discipline, Khulna University, Bangladesh. The field was medium high land. The location lies in the agro-ecological zones (AEZ) 13, i.e. Ganges Tidal Floodplain. The experimental site is characterized by hot humid subtropical climate with abundant rainfall during monsoon.

Growing season

The experiment was conducted during the dry season (February–May).

Test plant

Forage sorghum (Sorghum bicolor L. cv. Morokoshi) was used as the test plant. The sorghum seeds were collected from the Plant Nutrition and Physiology Laboratory, Iwate University, Morioka, Japan.

Seed bed preparation

A seed bed (1m×1m) was prepared for seed germination and growing seedlings. The seed bed was ploughed and leveled properly by using traditional country spade. Weeds and stubbles were removed from the bed manually. The bed was kept moist through irrigation as and when required.

Seed sowing in seed bed

Before seed sowing, sorghum seeds were soaked in water for 6h then the seeds were sown on the seed bed by broadcasting method. After seeds sowing due care was taken to ensure no damage by birds and to raise healthy and strong seedling. Proper irrigation was done as and when required.

Experimental plot preparation

In the present experiment, there were four treatment combinations along with control. So, five experimental plots of equal size (1m×1m) were prepared by ploughing and leveling properly by using traditional country spade. All weeds, stubble, and crop residues were removed manually. During plot preparation the soil was well fertilized by applying chemical fertilizer (NPK) following the fertilizer recommendation guide (FRG, 2012) in Bangladesh. Then the field was made ready for transplanting.
Seedling transplantation

Fifteen days aged young seedlings were uprooted from the seed bed for transplanting. Before that the seed bed was moistened by the application of water so that the root system was not damaged. There were twelve plants in each plot. Since the experimental plot was small in size, transplantation method was followed to maintain equal space between and within the rows and columns to reduce the spacing effect.

Weeding

The experimental plot was kept free from weeds. Manual weeding was done on regular interval.

Collection of saline water for irrigation

The saline water was collected from the Rupsha river situated beside the Khulna city and the bulk water was immediately transported to the field laboratory of Soil, Water and Environment Discipline, Khulna University and stored in plastic container. Natural tide occur in this river and the river water (RW) was collected during high tide.

Treatments combination

Tap water (TW) containing EC value of 0.78 dS m$^{-1}$ was mixed with collected saline RW containing EC value of 14.04 dS m$^{-1}$ at three different ratios (3:1, 1:1 and 1:3) to change the salinity levels as different treatments. Treatment combinations are presented in Table 1. The table describes that control plants received irrigation water containing 0.78 dS m$^{-1}$ of EC. Whereas different salinity treated plants received irrigation water containing 4.19, 7.18, 10.79 and 14.04 dS m$^{-1}$ of EC, respectively.

Irrigation

For seedlings establishment on experimental field normal tap water (0.78 dS m$^{-1}$) was used for irrigation when required. Fifteen days after transplanting (DAT), irrigation was done with treated water i.e. saline water and tap water combination according to treatment (Table 1). For each experimental plot same volume of water was irrigated irrespective to treatment.

Harvesting and sampling

Harvesting was done 83 days after transplanting. Four plants were selected as sample from each experimental plot through random selection. The selected plants were separated from the plot carefully by cutting the stems 3cm above the soil surface. Different morphological parameters viz. plant height, leaf number, leaf length, leaf width, and stem diameter, and plant biomass i.e. the fresh weight of the whole plant were measured and recorded. The soil sample was also collected from each plot.
Table 1. Treatment combination and EC values after mixing

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Water mixing combination</th>
<th>EC (dS m⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td>Control (100% TW)</td>
<td>0.78</td>
</tr>
<tr>
<td>T1</td>
<td>75% TW + 25% RW (TW:RW, 3:1)</td>
<td>4.19</td>
</tr>
<tr>
<td>T2</td>
<td>50% TW + 50% RW (TW:RW, 1:1)</td>
<td>7.18</td>
</tr>
<tr>
<td>T3</td>
<td>25% TW + 75% RW (TW:RW, 1:3)</td>
<td>10.79</td>
</tr>
<tr>
<td>T4</td>
<td>100% RW</td>
<td>14.04</td>
</tr>
</tbody>
</table>

TW=Tap water; RW=River water

Data collection
For convenience of agronomic observation on the plant characters data were collected from four randomly selected plants from each of the plots. Data on plant characters were then collected as follows:

• Plant height (m): Plant height of the harvested plant samples was measured with the help of a meter scale from the ground level to the tip of the uppermost leaf. So, the height of four plant samples from each plot was measured.

• Leaf number: Leaf number was manually counted. All the leaves in each plant were considered for counting leaf number.

• Leaf length (cm): Two mature leaves from each plant were selected to measure leaf length. So, total eight leaf length from each plot was measured with the help of a meter scale to measure the leaf length.

• Leaf width (cm): The leaves that were considered for measuring leaf length were also considered for leaf width measurement and were measured with the help of a meter scale.

• Stem diameter (cm): The plant samples that were considered for plant height was also selected for the measurement of stem diameter. The circle sphere of the stem was measured at the point of 1m above from the ground with the help of a meter scale. Then the plant diameter was calculated.

• Plant biomass (kg): After measuring all the agronomic attributes of the plants, the plants were cut into small pieces and fresh weight was weighed with the help of an electric balance.

Measurement of electrical conductivity (EC)
The electrical conductivity of the soil was measured at a soil: water ratio of 1:5 with the help of EC meter (USDA, 2004) whereas the EC of water sample was measured by using the EC meter after filtering the sample.
Statistical analysis
The results were expressed as the averages of four replications. The data was subjected to ANOVA using computer built-in statistical software program Minitab-16. Differences between means were statistically analyzed following one-way analysis of variance and using Fisher’s one way multiple comparison method (p=0.05). The association between water salinity and plant attributes was statistically tested by linear regression analysis. Graphs were prepared by using computer built-in Microsoft Excel-2010 program.

RESULTS
Morphological parameters
Plant height (m), Leaf number, Leaf length (cm), Leaf width (cm), Stem diameter (cm)
All the morphological parameters of the fodder sorghum plants measured after harvest are presented in table 2. The average plant height (n=4) varied from 1.83±0.19 m to 2.51±0.38 m among different concentrations of salinity in irrigation water. The tallest plant (2.93 m) was observed in 0.78 dS m\(^{-1}\) irrigated plot whereas the shortest plant (1.64 m) was observed in 14.04 dS m\(^{-1}\) irrigated plot. Plant height was significantly (p=0.045) reduced only at the highest degree of irrigation water salinity at 14.04 dS m\(^{-1}\) (Table 2). The association between plant height and water salinity was statistically tested by linear regression analysis and it was found that the plant height was significantly (p=0.002, R-Sq=54.5%) reduced with increasing rate of water salinity. Increasing salinity levels from 0.78 to 14.04 dS m\(^{-1}\) reduced plant height by 3.98, 14.34, 14.34, and 27.09\%, respectively, as compared with the control treatment.

The average leaf number varied from 11±1.0 to 13±2.7 (Table 2). There was no significant (p=0.51) difference in leaf number among the plants grown in different degrees of salinity in irrigation water (Table 2). While increasing salinity levels from 0.78 to 14.04 dS m\(^{-1}\) reduced leaf number by 0, 0, 7.69, and 15.38\%, respectively as the salinity levels order compared with the control treatment.

The mean leaf length of the plant varied from 89.2±11.0 cm to 107.2±8.9 cm (Table 2). The longest plant leaf (114.5 cm) was observed when the plants were irrigated with water containing EC value of 4.19 dS m\(^{-1}\) whereas the shortest plant leaf (76.5 cm) was observed in treatment of 14.04 dS m\(^{-1}\). There was no significant (p=0.18) change in leaf length among the treatments up to 10.79 dS m\(^{-1}\). But the average plant leaf length was significantly reduced at 14.04 dSm\(^{-1}\) irrigated plot. Increasing salinity levels from 0.78 to 14.04 dS m\(^{-1}\) reduced leaf length by 0, 4.10, 3.64, and 16.79\%, respectively as the salinity levels order compared with the control treatment.
Table 2. Response of water salinity on morphological parameters of sorghum

<table>
<thead>
<tr>
<th>EC (dS m⁻¹) in irrigation water</th>
<th>Plant height (m)</th>
<th>Leaf number</th>
<th>Leaf length (cm)</th>
<th>Leaf width (cm)</th>
<th>Stem diameter (cm)</th>
<th>Plant biomass (kg plant⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.78</td>
<td>2.51±0.38</td>
<td>13±2.7</td>
<td>107.2±8.9</td>
<td>8.02±0.53</td>
<td>2.25±0.23</td>
<td>0.74±0.16</td>
</tr>
<tr>
<td>4.19</td>
<td>2.41±0.22</td>
<td>13±0.6</td>
<td>108.7±5.4</td>
<td>6.88±0.55</td>
<td>2.11±0.21</td>
<td>0.54±0.11</td>
</tr>
<tr>
<td>7.18</td>
<td>2.15±0.05</td>
<td>13±1.0</td>
<td>102.8±2.4</td>
<td>7.12±0.63</td>
<td>2.19±0.15</td>
<td>0.51±0.05</td>
</tr>
<tr>
<td>10.79</td>
<td>2.15±0.24</td>
<td>12±1.5</td>
<td>103.3±1.0</td>
<td>6.33±0.36</td>
<td>1.80±0.10</td>
<td>0.34±0.07</td>
</tr>
<tr>
<td>14.04</td>
<td>1.83±0.19</td>
<td>11±1.0</td>
<td>89.2±11.0</td>
<td>5.55±0.69</td>
<td>1.72±0.25</td>
<td>0.32±0.08</td>
</tr>
</tbody>
</table>

Data indicates the average value ± standard deviation of four replications. Different letters indicate the significant differences.

The average leaf width of the fodder sorghum plant varied from 5.55±0.69 cm to 8.02 ± 0.53 cm. The widest leaf (8.6 cm) was observed in 0.78 dS m⁻¹ irrigated plot whereas the narrowest leaf (4.75 cm) was observed in 14.04 dS m⁻¹ irrigated plot. The leaf width was significantly (p=0.015) reduced only at 14.04 dS m⁻¹. Increasing salinity levels from 0.78 to 14.04 dSm⁻¹ reduced leaf width by 14.21, 11.22, 21.07, and 30.80%, respectively as the salinity levels order compared with the control treatment.

The mean value of stem diameter of fodder sorghum plant varied from 1.72±0.25 cm to 2.25±0.23 cm. No significant (p=0.07) differences on stem diameter were observed among the plants irrigated with different levels of salinity. Increasing salinity levels from 0.78 to 14.04 dS m⁻¹ reduced stem diameter by 6.22, 2.67, 20.0, and 23.56%, respectively as the salinity levels order compared with the control treatment.

Plant biomass (kg plant⁻¹)

The maximum plant biomass (0.91 kg) was observed in 0.78 dS m⁻¹ irrigated plot whereas the minimum plant biomass (0.26 kg) was observed in 14.04 dSm⁻¹ irrigated plot. The plant biomass was significantly (p=0.003) reduced at 10.79 and 14.04 dS m⁻¹ irrigated plot. Increasing salinity levels from 0.78 to 14.04 dS m⁻¹ reduced plant biomass by 27.03, 31.08, 54.05, and 56.76%, respectively as the salinity levels order compared with the control treatment. The association between plant biomass and water salinity was statistically tested by linear regression analysis and it was found that the plant height was significantly (p<0.001, R-Sq=71.2%) reduced with increasing rate of water salinity.

Soil EC (dS m⁻¹) after harvest

The initial soil EC value was 0.30±0.01 dS m⁻¹. The soil EC values after plant harvest is shown in figure 1. It was found that soil EC value was lower than the initial EC value up to 10.79 dS m⁻¹ irrigated plot. The soil EC value was higher only in case of where the irrigation water contained EC value of 14.04 dS m⁻¹.
RESULTS in table 2 revealed that salinity concentrations had a significant effect on averages of plant height (m), leaf number, leaf length (cm), leaf width (cm), stem diameter (cm) and plant biomass (kg). Increasing salinity levels decreased all these characters. These findings are in agreement with other works (Bashir et al., 2011, El Naim et al., 2012, Haghighat et al., 2012) reported on sorghum. It was reported that salinity decreased leaf area of sorghum (Bashir et al., 2011; Jafari et al., 2009). Sadeghi & Shourijeh (2012) measured the number of leaves and found that the number of leaves was decreased. Increasing salinity levels decreased sorghum growth which is directly related to the amount of absorbed water by the roots and the toxic effects of Na+ at high salt concentrations might have caused physical damage to roots thereby decreasing their ability to absorb water and nutrient, which may resulted in poor growth (Iqbal et al., 2000). Excess of salt in growth medium restricts the availability of water to plant. This restriction causes in dehydration of cytoplasm which in turn affects the metabolism of the cells and ultimately reduces the growth of plant. Sorghum was classified as moderately salt tolerant plant (Krishnamurthy et al., 2007, Niu et al., 2012) which is confirmed in the present study. In the present study, sorghum was grown up to 7.18 dSm\(^{-1}\) EC without affecting its total biomass (Table 2). Some studies also reported variation in salinity tolerance between sorghum cultivars (Asfaw, 2011, El Naim et al., 2012). Salts in soil and water can reduce water availability to crops at all stages of plant development and affect physiological and biochemical processes via ion toxicity, osmotic stress and mineral deficiencies to such an extent that yields can be affected (Hasegawa et al., 2000; Munns, 2002).

Average salinity concentrations at the coast are higher in the dry season than in the monsoon, due to the lack of freshwater flows from upstream. The salinity normally builds up from October to the late May, and it remains higher during the dry season,
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usually from February to May. At the end of May, salinity level drops sharply due to upstream flows and rainfall. Regarding the soil EC values after harvest, in the present study the authors stated that during the monsoon, due to rain water, soil salinity was decreased up to 10.79 dS m\(^{-1}\) from its initial value (Figure 1). Increasing salinity levels from 0.78 to 10.79 dS m\(^{-1}\) reduced soil EC by 53.33, 20, 10, and 3.33%, respectively as the salinity levels order compared with the control treatment whereas at 14.04 dS m\(^{-1}\) soil EC was increased by 20%.

CONCLUSION

The results of the present study revealed that the growth of the fodder sorghum was harmfully affected by the irrigation water salinity at higher salt concentrations. It was found that the plant height and plant biomass was significantly decreased when the plants were grown irrigating with water containing EC of 10.79 dS m\(^{-1}\). So, the sorghum plants might be cultivated in the coastal regions of Bangladesh where fresh water is limited due to salinity problem as well as might be grown irrigating with saline water up to 10.79 dS m\(^{-1}\). Moreover, it was found that the soil irrigating with saline water up to 10.79 dS m\(^{-1}\) did not show any increase of soil salinity after harvest that might be due to heavy rainfall at the later stage of the plant’s growth period during the monsoon. Further study on actual soil salinity with fodder sorghum should be necessary.

ACKNOWLEDGEMENT

The authors would like to acknowledge Dr. S. Kawai, Professor, Plant Nutrition and Physiology Laboratory, Iwate University, Morioka, Japan for providing fodder sorghum seeds.

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PROFITABILITY AND RESOURCE USE EFFICIENCY OF MAIZE SEED PRODUCTION IN PALPA DISTRICT OF NEPAL

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ABSTRACT

The study aimed to analyze profitability and resource use efficiency of maize seed production in Palpa district of Nepal. Raosoft Inc. software was used to determine the sample size of 182 maize seed producers from the total 260 maize seed producers in the district. Data was collected using a pre-tested semi-structured questionnaire survey administered to the randomly selected samples. Results showed that the uses as well as cost of major inputs such as seed, labor, farmyard manure (FYM), and management/other cost including tillage were higher among small scale farmers compared to the large scale farmers. The average cost of production among small scale farmers was NRs. 94,195 per hectare compared to NRs. 64,145 among large scale farmers. A benefit cost ratio of maize seed production was higher for large scale farmers (1.12), which in case of small scale farmers was less than 1, i.e. 0.9. Hence, maize seed production was found profitable only for large scale farmers. Resource use efficiency analysis showed FYM, tillage, and labor were overused. This suggests that the use of FYM, tillage and labor should be decreased by 665, 456 and 68 percent respectively. Similarly, cost on seed, chemical fertilizer and management/other were underused, hence, need to increase by 92, 69 and 97 percent respectively for the optimum allocation of resources. Overall, maize seed production is profitable but resources should be optimally utilized and should be carried on larger scale.

Keywords: Maize seed, profitability, resources, resource use efficiency.

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INTRODUCTION

Production and profitability of any crop depends upon the inputs used in the production process. Seed is very essential input in any agricultural production system. Seed is the most important, strategic and relatively inexpensive input that determines the crop yield (Langyintuo, 2005; Maredia & Howard, 1998). It should be made easily available and should be efficiently used for an improvement of the livelihood and income of rural people. The yield of maize production in Nepal is 2.43 metric tons per hectare (AICC, 2016) which is less with compared to global yield records. The use of low quality/less potential seed and lack of proper crop management technologies along with low soil fertility are attributed to the low yield of maize in Nepal (Karki et al., 2015). Similarly, disease infestation at different stages of crop (Subedi, 2015) labor shortage (Joshi et al., 2012) and water stress condition due to drought (Khalili et al., 2013) are responsible for the lower yield. The haphazard and inefficient use of inputs or resources leads to wastage of time, money and effort which ultimately declines the yield and profitability of farm which leads to the weak agriculture economic growth.

There is a growing practice of using hybrid seed for maize production to meet the demand of poultry feed industry, which is annually increasing at 11 percent (IFPRI, 2010; KC et al., 2015). However, there is very limited amount of hybrid seed available in Nepal which is unable to meet the growing demand of maize seed. This provides an opportunity for Nepali farmer to produce hybrid maize seed, which usually has higher return than the non-seed production. For instance, the net return from hybrid maize seed production in Bangladesh was 50 percent higher compared to the non-seed producers (Haque et al., 2012). In response to this increasing demand of hybrid maize seed and the higher return from it, growing number of Nepali farmers are pursuing hybrid maize seed production in Nepal. However, there is a lack of study on its profitability and resource use efficiency. This type of study is crucial for promoting the hybrid maize seed production, by providing the relevant suggestions needed to improve the efficiency of inputs for better production. Hence, this study aimed to assess the profitability, level of resources used and its efficiency in the maize seed production.

MATERIALS AND METHODS

The production of maize seed in Palpa district is practiced in consultation with District Agriculture Development Office (DADO) and Nepal Agricultural Research Council (NARC) which are responsible government entities working for the improvement of agriculture sector in Nepal. The production of maize seed was highest in Palpa district which was the main reason to select it for the study. Altogether there were 260 farmers involved in maize seed production through eight registered farmer groups and three cooperatives from nine Village Development Committees (VDCs) i.e. Pokharathok (N=91), Chirtungdaha (N=21), Bhairavsthан
(N=32), Khasauli (N=38), Deurali (N=33), Rampur (N=9), Thimure (N=8), Kusumkhola (N=7) and Chidipani (N=6) and one municipality i.e. Tansen (N=15). So the total population size for this study was 260. The required sample size was determined using Raosoft. Inc. which is considered as a scientific and standard technique for the determination of sample size (Raosoft, 2014). It recommended sample size to be 182 at 95 percent confidence level and four percent margin of error with about 50 percent response expected. The simple random sampling technique was used to select sample in order to avoid possible bias. The simple random sampling is considered as the best way as this provides an equal chance for a selection of the element from the sampling frame (Scheaffer, 1979). The sample mainly comprised the maize seed producers from Pokharathok (n=75), Chirtungdharma (n=15), Bhairavsthan (n=28), Khasauli (n=31), Deurali (n=27) and Tansen (n=6).

Pre-testing of questionnaire was done in Madanpokhara VDC with 10 respondents (5.5 percent of the sample size), which is common in pre-test of questionnaire (Perneger et al., 2015) and more relevant in our case constrained by the resources. The pre-tested questionnaire was administered to collect primary data in June, 2016. It is believed that the more and reliable information can be collected from the interactions between the participants. Focus group discussions (FGD) was done to gather collective information and also to verify the responses obtained in questionnaire survey. A total of four FGDs were conducted and a key informant interview (KII) was done with the crop development officer of DADO, Palpa; chairperson of cooperatives and farmer groups; lead and progressive farmers. Secondary data were collected from governmental and non-governmental organizations, cooperative and journals.

The average area under maize seed production was 0.32 hectare (i.e. 6.202 ropani). Those farmers who were involved in the production of maize seed above the average maize seed area (>0.32 hectare) were categorized as large scale farmers and others (<0.32 hectare) as small scale farmers.

**Benefit cost analysis**

Benefit cost ratio (BCR) is a widely used technique to evaluate the economic performance of any firm including agricultural farms. It is regarded as a quick and the easiest technique. BCR was calculated using the following formula:

\[
BCR = \frac{\text{Total return (NRs.)}}{\text{Total cost of production (NRs.)}}
\]

Total return = Price of maize seed × Total amount of maize seed produced

Total cost of production = Summation of cost incurred for all inputs

Inputs considered seed, farmyard manure (FYM), chemical fertilizer, labor, tillage and management/other. Almost all sampled farmers are producing maize seed in their own farm without incurring any cost in land rent. Moreover, the value of land is more
or less uniform across the study area. Hence, land cost is not included in this study.

**Estimation of efficiency ratios using Cobb-Douglas production function**

Cobb-Douglas production function was used to compute marginal value product (MVP) in order to determine the optimum, over and underuse of resources following Gujarati (2009).

\[
Y = aX_1^{b_1}X_2^{b_2}X_3^{b_3}X_4^{b_4}X_5^{b_5}X_6^{b_6}e^u
\]

Transformed to linear form for ease in computation

\[
\ln Y = \ln a + b_1\ln X_1 + b_2\ln X_2 + b_3\ln X_3 + b_4\ln X_4 + b_5\ln X_5 + b_6\ln X_6 + u
\]

Where,

- \(Y\) = Total income from maize seed production (NRs. per hectare)
- \(X_1\) = Seed (NRs. per hectare)
- \(X_2\) = FYM (NRs. per hectare)
- \(X_3\) = Chemical fertilizer (NRs. per hectare)
- \(X_4\) = Labor (NRs. per hectare)
- \(X_5\) = Tillage (NRs. per hectare)
- \(X_6\) = Management/other (NRs. per hectare)
- \(u\) = Error term
- \(a\) = Intercept
- \(\ln\) = Natural logarithm

The efficiency ratio \((r)\) was computed using the formula

\[
r = \frac{\text{MVP}}{\text{MFC}}
\]

Where,

- \(\text{MFC}\) = Marginal factor cost

The MVP was estimated using the formula:

\[
\text{MVP}_i = b_i \times \frac{Y}{X_i}
\]

Where,

- \(b_i\) = Estimated regression coefficients
- \(Y\) and \(X_i\) are the values from geometric mean.

**Decision criteria:**

- \(r = 1\) indicate the efficient use of resource
- \(r > 1\) indicate underused of resource
r < 1 indicate overused of resource

The relative percentage change in MVP of each resource was estimated as:

\[ D = (1 - \frac{\text{MFC}}{\text{MVP}}) \times 100 \]

Or, \[ D = (1 - \frac{1}{r}) \times 100 \]

Where, \( D \) = Absolute value of percentage change in MVP of each resource

**Return to scale analysis (RTS)**

The return to scale was calculated as follow:

\[ \text{RTS} = \sum b_i \]

**Decision rule:**

- RTS<1: Decreasing return to scale; percentage change in output is less than percentage change in input
- RTS = 1: Constant return to scale; percentage change in output is equal to percentage change in input
- RTS> 1: Increasing return to scale; percentage change in output is more than percentage change in input

**RESULTS AND DISCUSSION**

**Inputs used in maize seed production**

The major inputs used in maize seed production were seed, labor, FYM, chemical fertilizer and tillage operation. The average amount of seed, labor, FYM, chemical fertilizer, tillage by tractor and tillage by bullock was found 23.08 kg, 184.47 man-days, 16145.13 kg, 56.94 kg, 2.33 hours and 5.24 days per hectare, respectively. Small scale farmers used higher amount of seed (24.97 kg ha\(^{-1}\)) in comparison to large scale farmers (20.11 kg) and the difference was highly significant at one percent level. The reason of using higher amount of seed by small scale farmers was to replant the plant where there is no germination as they were found reluctant to take unnecessary burden of sowing seed again and also to minimize risk of germination. The labor used for maize seed production in large scale was almost half (133.58 man-days) to small scale (217.01 man-days). Farmers were found to care their farm more precisely in small area as it becomes easy to manage farm in a smaller area. The difference in the labor used was found statistically significant at one percent level.

Similarly, the FYM used by large scale farmers was significantly less (13058.07 kg) than small scale farmers (18119.74 kg). Whereas, higher use of chemical fertilizer (60.38 kg) by large scale farmers than that of small scale farmers (54.74 kg) was observed. However, the difference was statistically non-significant. Tractor usage (in hours) for tillage by large scale farmers was 2.17 hours which was less compared to small scale farmers (2.43 hours), the difference however, was statistically non-significant. The use of bullock for tillage in farm was found less among large scale
farmers. The number of days required to till the farm of large scale farmers was 3.73 days and that of the small scale was 6.21 days and the difference was found statistically significant at one percent level.

Table 1. Inputs used for maize seed production (per hectare)

<table>
<thead>
<tr>
<th>Variable cost items</th>
<th>Overall Mean</th>
<th>Farm category</th>
<th>Large scale</th>
<th>Small scale</th>
<th>Mean difference</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed (kg)</td>
<td>23.08 (8.57)</td>
<td>20.11 (4.05)</td>
<td>24.97 (10.06)</td>
<td>-4.86***</td>
<td>-3.873</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>Labor (man-days)</td>
<td>184.47 (79.68)</td>
<td>133.58 (49.01)</td>
<td>217.01 (78.60)</td>
<td>-83.43***</td>
<td>-8.00</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>FYM(^1) (kg)</td>
<td>16,145.13 (14,732.44)</td>
<td>13,058.07 (8,023.92)</td>
<td>18,119.74 (17,495.15)</td>
<td>-5,061.66**</td>
<td>-2.287</td>
<td>0.023</td>
<td></td>
</tr>
<tr>
<td>Chemical fertilizer (kg)</td>
<td>56.94 (43.47)</td>
<td>60.38 (42.43)</td>
<td>54.74 (44.18)</td>
<td>5.64</td>
<td>0.853</td>
<td>0.395</td>
<td></td>
</tr>
<tr>
<td>Tillage tractor (hour)</td>
<td>2.33 (2.89)</td>
<td>2.17 (2.17)</td>
<td>2.43 (3.27)</td>
<td>-0.26</td>
<td>-0.595</td>
<td>0.552</td>
<td></td>
</tr>
<tr>
<td>Tillage bullock (days)</td>
<td>5.24 (5.68)</td>
<td>3.73 (3.79)</td>
<td>6.21 (6.44)</td>
<td>-2.49***</td>
<td>-2.944</td>
<td>0.004</td>
<td></td>
</tr>
</tbody>
</table>

Notes: Figures in parentheses indicate percent. ***, ** indicate significant at 1% and 5% levels respectively.

\(^1\)FYM in kg was computed as: 1 doko cow/buffalo dung = 30 kg, 1 doko goat manure = 20 kg and 1 bag poultry manure = 30 kg (source: FGD and KII with DADO)

Maize seed production cost

Table 2, shows that the total cost of production for small scale farmers was more than that of large scale farmers. The average cost of seed, labor, FYM, chemical fertilizer, tillage and management/other was found to be NRs. 1,839.92, 45,048.04, 23,368.44, 1,657.70, 10,021.96 and 482.45 per hectare, respectively. The average seed cost under large scale farms was significantly less (NRs. 1,653.21) as compared to small scale farms (NRs. 2,047.89). The average seed cost for small scale was more because small scale farmers were found using more amount of seed per hectare to avoid the risk of no germination. The labor cost for maize seed production was found significantly higher in small scale farms NRs. 52,781.51 as compared to large scale farms NRs. 32,957.67 per hectare. Man-days required for carrying whole deposited FYM to field and manual shelling cost was found more for small scale farmers which had increased the labor cost. The majority of large scale farmers used shelling machine which decreased the labor cost highly as compared to small scale farmers. The average FYM cost of small scale farmers was found to be NRs. 25,799.67 per hectare and that of large scale farmers was NRs. 19,567.50 and the difference was significant at five percent level. All large and small scale farmers were found applying all the deposited FYM to the field which has increase the FYM cost for small scale farmers.
Table 2. Comparative cost of maize seed production (NRs. per hectare)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Overall</th>
<th>Farm category</th>
<th>Mean difference</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Large scale</td>
<td>Small scale</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seed</td>
<td>1,893.92</td>
<td>1,653.21</td>
<td>2,047.89</td>
<td>-394.67</td>
<td>-3.711</td>
</tr>
<tr>
<td></td>
<td>(724.09)</td>
<td>(342.86)</td>
<td>(852.41)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Labor</td>
<td>45,048.04</td>
<td>32,957.67</td>
<td>25,799.67</td>
<td>-19,823.84</td>
<td>-7.392</td>
</tr>
<tr>
<td></td>
<td>(20,092.25)</td>
<td>(12,852.65)</td>
<td>(22,815.47)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FYM</td>
<td>23,368.44</td>
<td>19,567.50</td>
<td>11,389.53</td>
<td>-5,200.91</td>
<td>-2.083</td>
</tr>
<tr>
<td></td>
<td>(19,869.21)</td>
<td>(13,370.68)</td>
<td>(7,184.40)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemical fertilizer</td>
<td>1,657.70</td>
<td>1,717.39</td>
<td>1,619.53</td>
<td>97.86</td>
<td>0.449</td>
</tr>
<tr>
<td></td>
<td>(1,430.81)</td>
<td>(1,332.40)</td>
<td>(1,495.03)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tillage</td>
<td>10,021.96</td>
<td>7,883.94</td>
<td>11,389.53</td>
<td>-3,505.59</td>
<td>-3.868</td>
</tr>
<tr>
<td></td>
<td>(6,189.28)</td>
<td>(3,215.43)</td>
<td>(7,184.40)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Management/other</td>
<td>482.45</td>
<td>365.42</td>
<td>557.30</td>
<td>-191.88*</td>
<td>-1.813</td>
</tr>
<tr>
<td></td>
<td>(700.98)</td>
<td>(404.99)</td>
<td>(830.46)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cost of</td>
<td>82,472.51</td>
<td>64,145.13</td>
<td>94,195.42</td>
<td>-30,050.29</td>
<td>-5.490</td>
</tr>
<tr>
<td>production</td>
<td>(38,808.03)</td>
<td>(24,230.93)</td>
<td>(41,821.59)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Notes: Figures in parentheses indicate standard deviation. ***, ** and * indicate significance at 1%, 5% and 10% levels respectively.

The average cost of chemical fertilizer for large scale and small scale farmers was NRs. 1,717.39 and 1,619.53 per hectare, respectively, however, the difference was statistically non-significant. The cost on tillage for large scale and small scale farmers was NRs. 7,883.94 and 11,389.53 per hectare respectively. The difference on tillage cost was found statistically significant at one percent level. The large scale farmers generally use tractor for land preparation which lowered the cost. The management/other cost of large scale and small scale farmers was NRs. 365.42 and 557.30 per hectare and the difference was statistically significant at 10 percent level. The total cost of maize seed production was found NRs. 82,472.51. The total cost of production for small scale farmer was NRs. 30,050.3 more than that of the large scale farmers. The difference in cost of production was statistically significant at one percent level. The share of labor cost (55%) was high followed by FYM (28%), tillage (12%), seed (2%), chemical fertilizer (2%) and management/other (1%) (Figure 1). This revealed that the huge amount of money (83%) was spent on labor and FYM. Both these inputs are usually supplied from the household itself. Hence, it can be implied that maize seed production remains an important enterprise for the use of FYM produced by their own livestock as well as household labor.
Bhandari et al. (2015) stated the total cost of maize production per hectare was NRs. 65,112.80 in the nine hilly district of Nepal. A study by Bhandari et al. (2014) found the average cost of cultivation of maize production was NRs. 59,763.20 in the Palpa district. The total cost of production calculated in this study was higher because maize seed production requires intensive care and management than maize for grain cultivation. Moreover, it involves the cost of inspection, rouging, certification, sorting, grading and packaging.

**Yield and profitability of maize seed**

The average maize production per household (both as a seed and grain) for large scale and small scale farmers was 755.28 kg and 342.90 kg respectively. The difference in production per household was found statistically significant at one percent level. The average yield of maize seed production was 1,636.11 kg in the study area. The yield of maize seed production was 1,503.18 kg and 1,721.14 kg for large scale and small scale farmers respectively and the difference was statistically significant at 10 percent level. More FYM application and the better management practice (weeding, thinning and rouging) has increased the yield of small scale farmers.

The returns from maize seed was the summation of the returns from quality seed, grain used in feed and consumption, stovers and cone of maize. The returns and profitability from maize seed production was NRs. 75,733.07 and -6,739.44 (loss) in the study area. The returns from the maize seed production was found higher in small scale i.e. NRs. 79,384.44 than that of large scale i.e. NRs. 70,024.60. It might be due to significantly higher yield achieved by the small scale farmers. There was loss of
NRs. 14,810.99 in small scale farming and profit of NRs. 5,879.47 in large scale farming. This loss in small scale was due to the high cost involved in production, specifically labor and FYM. Thus, despite the loss they perceive maize seed production to have a lower opportunity cost compared to the maize production for grain. The benefit cost ratio of large scale farmers was found higher (1.12) than that of small scale farmers (0.90). This indicated that, in large scale farmers, one rupee spent on production yields benefit of NRs. 0.12 but there was a loss of NRs. 0.1 in the case of small scale farmers. The difference was found statistically significant at one percent level. The low BCR was due to high cost of FYM and labor (mainly the family labor). The FYM was applied at the time of land preparation of maize seed which is also utilized by other crops in a year. Family members were more engaged in agricultural operations which had increased the share of labor cost.

Estimation of efficiency ratios using Cobb-Douglas production function

The overall F value was 18.13 and it was statistically significant at one percent level which implies that the explanatory variables included in the model are important for the explanation of variation in dependent variable. The R² value was 0.383 which indicates that about 38 percent of variation in the maize income was explained by the explanatory variables. The multicollinearity was checked using Variance inflation factor (VIF) and there was no problem of multicollinearity. The efficiency ratio less than one indicated that the FYM, tillage and labor were overused in the study area whereas seed, chemical fertilizer and management/other were underused resources. The value of efficiency ratios indicated that the inputs were not allocatively efficient. For optimum allocation of resources, cost on FYM, tillage and labor is to be decreased by 665, 456 and 68 percent respectively and cost on seed, chemical fertilizer and management/other cost should be increased by 92, 69 and 97 percent respectively (Table 4).

Table 3. Yield and profitability of maize seed production

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Overall</th>
<th>Mean difference</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Large scale</td>
<td>Small scale</td>
<td></td>
</tr>
<tr>
<td>Production in household (kg)</td>
<td>503.77 (398.89)</td>
<td>755.28 (516.25)</td>
<td>342.90 (158.97)</td>
<td>412.39***</td>
</tr>
<tr>
<td>Yield (kg/ha)</td>
<td>1,636.11 (749.97)</td>
<td>1,503.18 (702.47)</td>
<td>1,721.14 (769.91)</td>
<td>-217.96*</td>
</tr>
<tr>
<td>Returns (NRs/ha)</td>
<td>75,733.07 (38,151.58)</td>
<td>70,024.60 (36,125.38)</td>
<td>79,384.44 (39,115.99)</td>
<td>-9,359.84</td>
</tr>
<tr>
<td>Profit (NRs/ha)</td>
<td>-6,739.44 (38,505.01)</td>
<td>5,879.47 (29,178.83)</td>
<td>-14,810.99 (41,584.67)</td>
<td>20,690.45***</td>
</tr>
<tr>
<td>BCR</td>
<td>0.98 (0.43)</td>
<td>1.12 (0.48)</td>
<td>0.90 (0.38)</td>
<td>0.22***</td>
</tr>
</tbody>
</table>

Notes: Figures in parentheses indicate standard deviation. *** and * indicate significant at 1% and 10% levels, respectively. p-values are result of t-test.
Table 4. Estimation of elasticity, MVP and efficiency ratios

<table>
<thead>
<tr>
<th>Variables</th>
<th>Coefficient</th>
<th>Standard error</th>
<th>t value</th>
<th>MVP</th>
<th>MFC</th>
<th>r</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed cost</td>
<td>0.338***</td>
<td>0.109</td>
<td>3.112</td>
<td>12.650</td>
<td>1</td>
<td>12.650</td>
<td>92.095</td>
</tr>
<tr>
<td>FYM Cost</td>
<td>0.032</td>
<td>0.022</td>
<td>1.453</td>
<td>0.131</td>
<td>1</td>
<td>0.131</td>
<td>664.698</td>
</tr>
<tr>
<td>Chemical Fertilizer cost</td>
<td>0.012*</td>
<td>0.006</td>
<td>1.838</td>
<td>3.245</td>
<td>1</td>
<td>3.245</td>
<td>69.179</td>
</tr>
<tr>
<td>Labor cost</td>
<td>0.364***</td>
<td>0.091</td>
<td>4.016</td>
<td>0.595</td>
<td>1</td>
<td>0.595</td>
<td>68.083</td>
</tr>
<tr>
<td>Tillage Cost</td>
<td>-0.036</td>
<td>0.064</td>
<td>-0.561</td>
<td>-0.281</td>
<td>1</td>
<td>-0.281</td>
<td>456.250</td>
</tr>
<tr>
<td>Management/other cost</td>
<td>0.151***</td>
<td>0.033</td>
<td>4.619</td>
<td>33.195</td>
<td>1</td>
<td>33.195</td>
<td>96.987</td>
</tr>
<tr>
<td>Constant</td>
<td>3.805***</td>
<td>0.862</td>
<td>4.416</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Observations 182
F value (6, 175) 18.13
Prob>F 0.000
R-Squared 0.383
Adj. R-Squared 0.362
Return to scale 0.861

Note: ***, * indicate significant at 1% and 10% level respectively.

Increase in cost of seed and chemical fertilizer was in accordance with the findings of Dhakal et al. (2015); Ghimire and Dhakal (2014); Sharma (2009); Gani and Omomona (2009); Ojo, Salami and Mohammed (2008) and in contrary with Chapke, Mondal and Mishra, (2011). Similarly, decreasing cost of FYM is supported by results of Ojo et al. (2008). Danso-Abbeam, Dahamani and Bawa (2015) and Ghimire and Dhakal (2014) has figured similar results of reducing labor cost for optimum allocation. Return to scale analysis showed value of 0.861 which indicates decreasing return to scale in the study area and this finding was in line with the findings of Gani and Omomona (2009) who found decreasing return to scale (0.961) on small scale irrigated maize producers in Nigeria and it was contrary to the findings of Olarinde (2011) who found increasing return to scale among the maize farmers in Nigeria.

CONCLUSION

From this study, we conclude that maize seed production is profitable in case of large scale farms whereas small scale farms have to bear loss. Therefore, maize seed production is recommended in larger land area so that there is minimization in costs of inputs and thus higher profitability can be obtained. Yield was observed higher in small scale farms due to better managerial activities. Hence, despite suffering a loss, the small scale farmers still find it as a better choice considering the lower opportunity cost of maize seed production. This shows that the district is potential for
maize seed production. Application of better cultivation practices (manures and fertilizers, rouging, weeding) and using quality seed for seed production can increase yield of maize seed production in the study area. This study also identified that inputs used in maize seed production were inefficiently utilized. Cost on seed, chemical fertilizer and management activities need to be increased whereas cost on FYM, labor and tillage should be decreased in the study area. This will lead to optimal allocation of the resources resulting in the increased profitability.

ACKNOWLEDGEMENTS

The authors would like to acknowledge Directorate of Research and Extension, Agriculture and Forestry University, Chitwan for partial funding to accomplish this study.

REFERENCES


GROWTH, SURVIVAL AND INTACTNESS OF GREEN MUD CRAB (Scylla Paramamosain) BROODSTOCK UNDER DIFFERENT CAPTIVE GROW OUT PROTOCOLS

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ABSTRACT

Development of broodstock of the green mud crab (Scylla paramamosain) was assessed under three different captive growout protocols viz. in the open fibre glass tanks (T1), in plastic boxes (T2) floating on fibre glass tanks and in plastic drawer/compartment (T3) for a period of 5 months under the Centre for Marine and Coastal Studies (CEMACS), University Sains Malaysia (USM). The male (M) and female (F) mud crab were cultured separately to maintain virginity. Suitability of both the sexes were evaluated considering the weight gain (size), survival and intactness of limbs during harvesting. Result of the present study revealed that, irrespectively of growout protocols, growth of mud crab happened following the sigmoid pattern. A noticeable intersexual weight attainment was observed with significantly higher (p<0.05) weight gain for the males. Meanwhile, growth was influenced by the culture protocols for both the sexes with significant (p<0.05) weight gain in outdoor tanks (M= 319.75 g, F= 246.17 g) followed by outdoor floating boxes (M= 250.50 g, F= 198.70 g) and indoor compartment (M= 246.40 g, F= 178.50 g). Survival and the proportion of intact crabs under indoor compartment and outdoor floating box were significantly higher (p<0.05) than out door tank culture system. The result of the present study suggested that, outdoor growout protocol could be followed for faster broodstock development purposes to reduce the dependence on natural broodstock for hatchery operations.

Keywords: Captive condition, growth, survival, intactness, mud crab, brood stock.

INTRODUCTION

For successful aquaculture of any commercial species, a comprehensive understanding about the growth under different culture system is essential. Concepts
on growth and survival are considered as useful tools in aquaculture for projecting culture cycle and deciding the optimum harvesting time and size; thus ultimately minimizes the production cost and maximizes the farm output in terms of yield and revenues (Agbayani, 2001; Moksnes et al., 2014). On the other hand, growth, age and size are considered as necessary basic elements in determining the level of maturity for breeding purposes. In most crustaceans including mud crab, growth occurs through a moulting process (Shelley, 2008).

Mud crab is an aggressive and cannibalistic animal (Moksnes et al., 1998), which often lead a lowered survival and harvesting of limbless animals. For breeding purposes, an optimum sized and intact animal is the first desirable to achieve better reproductive performance and larvae quality (Quinitio and Parado-estepa, 2008; Thache, 2009). To understand the growth features of mud crab, several studies have been conducted (Le Vay et al., 2007) through tagging and recapture method on wild animals. Besides that, some of the information is also found on aquacultural growth of mud crab under pond culture (Ut et al., 2007), growth under drive-in cage culture (Shimpton and Hecht, 2007; Mirera, 2011). But all these results have been expressed from the aquaculture point of views. However, various aquaculture practices (viz. pond culture, cage culture, drive-in cage culture, pen culture) have been developed for mud crab to meet up the growing global demand. But specific documented information on broodstock development under captive condition is therefore scanty yet, except the findings of Quinitio et al. (2010) on Scylla serrata broodstock under pond condition.

The green mud crab, Scylla paramamosain providing the lion share of mud crab aquaculture in the South-east Asian countries, including Malaysia. Scarcity of suitable broodstock often hinder the seed production operations (Islam et al., 2017). Development of captive brood stock is the emerging issue for successful seed production in hatchery condition (Shelley, 2008). But information on broodstock development under captive condition is therefore nil for this species. Considering the above, this experiment was aimed to find out the growth, survival and morphological features (intactness) of the green mud crab S. paramamosain under different indoor and outdoor grow out protocols for broodstock development.

**MATERIALS AND METHODS**

**Experimental sites**

The experiment was conducted at the Centre for Marine and Coastal Studies (CEMACS) under UniversitiSains Malaysia (USM), Penang, Malaysia during 2012 to 2015. Location of the site was at North-East part of Penang Island under 5° 28' 2.3664” N and 100° 12' 2.8728” E in Global Positioning System (GPS).
Experimental design
The experiment was designed with three grow out protocols for each sexes. The grow out treatments were: crabs reared in outdoor fibre glass tank bottom (T1), crabs grown individually in outdoor floating plastic boxes (T2) and crabs were individually grown under indoor plastic drawers/compartment (T3). Treatment T1 had three replicated tanks. Whilst, 40 boxes were set up in each tank and they were considered as replication for T2. On the other hand, three different layers of the compartment were considered as different replications for T3. Stocking density for T1 was 4 crabs/m², and for those of the box and compartment; it was single crab per box and compartment, separately.

Description and preparation of grow out protocols
A multi storied fabricated plastic compartments comprising 324 drawers were used for the T3 growout protocol (Plate 1: A). Each of the drawers had an area of 30cm × 25cm × 15cm. The drawers were arranged in such a way that one drawer was set upon another and formed 9 layers (row), 18 columns with two back to back stacks. All drawers were set up onto a base table holding a recirculation water tray in beneath with the volume of total volume of all drawers. On the other hand, soft shell crab shedding plastic boxes of 30cm × 25cm × 15cm each were used for the second treatment (T2; Plate 1: B). The boxes together with the floating plastic frame were set up in the fibre glass tanks with an area of 7 m² each (4.6 m × 1.52 m). For the T1 growout protocol, fibre glass tanks with the bottom surface area of 7m² (4.6 m × 1.52 m) were used (Plate 1: C). The tanks were prepared with inlet and outlet facilities, and provided a sand bed of 1m² (with a height of 12-15 cm). Seaweed (Ulvalactuca) was stocked in each tank at a rate of 150 g/m² (wet weight basis) before 1 month of crablet stocking to provide shelter and for water cleaning (Plate 1: D).

Stocking of crabs, feeding and management
For the first two months, crablets were reared in communal basis to grow to the juvenile stage. After that, all the juvenile crabs were harvested, sorted (male, female) and measured (individual total weight, carapace width). Then the intact crabs of both sexes were randomly selected for different treatments by ignoring the size to minimize the initial error among the treatments. Crabs were fed with chopped trash fish at the rate of 3 to 10% of body weight once a day at 9:30 am. For the boxes and compartments, single piece of trash fish was given to each chamber. Whereas, for the tank culture, total feed biomass was equally spread for the entire tank. Uneaten feeds and residuals were removed daily prior to feeding. Water salinity was maintained between 18 to 24 ppt.
Monitoring and measurement of growth and water quality variables

The growth parameters of crab were monitored at monthly intervals. Five crabs of each sex were randomly taken from each grow out protocols and total weight (TW), and carapace width (CW) was measured following the standard method (Jantrarotai et al., 2006). Water quality variables were monitored on a monthly basis following standard methods (APHA, 1992). The water temperature ranged between 28.5-34.0 °C, salinity ranged between 18.0 to 24.0 ppt, water pH ranged between 7.7 to 8.9 and dissolved oxygen between 4.8 to 8.6 mg l⁻¹ in all the treatments. All the water quality variables were within the acceptable range for grow out of crustaceans like mud crab (Cholik and Hanafi, 1992; Baliao et al., 1981; Baliao et al. 1999; Trino et al., 2001). The grow out experiment was continued up to five months (seven months from crablet). After that crabs were harvested, measured and data were processed.

Estimation and calculation of survival and SGR (specific growth rate)

Survival and SGR for each treatment replication for both the sexes (male and female
crabs) were calculated using the following formula:

\[
\text{Survival} \, (\%) = \frac{\text{Initial number} - \text{final number}}{\text{Initial number}} \times 100 \quad ------ (1)
\]

\[
\text{SGR} \, (\%/\text{day}) = 100 \times \frac{\ln(W_f) - \ln(W_i)}{\text{Time (total days of culture)}} \quad ------ (2)
\]

Where, \( W_f \) = Final weight of crab, \( W_i \) = Initial weight of crab, \( \ln \) = Natural logarithm

**Data analysis**

Data was computed in MS Excel according to the treatment and sex. Data were analyzed through SPSS, version 22. ANOVA was performed to observe the differences between treatments and sexes. DMRT (Duncan’s Multiple Range Test) was performed for ranking the differences. A confidence level of 95\% (p<0.05) was considered as significant between the treatment for a specific variable.

**RESULTS**

**Growth pattern of crabs**

Irrespective of culture protocols, the increase in weight of both the sexes of mud crab seemed slower for the first two months, then it started to increase at a higher pace from the third month onwards (Figure 1&2). In the case of carapace width, initially the growth was faster and eventually it slowed down for the sixth and seventh month (Figure 3&4). The growth performance of both sexes of mud crab was faster in T1 (outdoor free culture) than that of T2 (outdoor box culture) and T3 (indoor compartment culture). Both weight and carapace width increment for both sexes showed a slow sigmoid pattern (s-shaped) against culture days in all the culture protocols (Figure1&4). Increment in weight (Figure1&2) and carapace width (Figure3 &4) started to vary under different culture protocols from first month when assigned into the treatment and continued up to sixth months for both the sexes.

![Figure 1. Growth pattern of male mud crab cultured under different captive growout protocols](image1)

![Figure 2. Growth pattern of female mud crab grown under different captive growout protocols](image2)
Initial weight of female mud crab (21.32 g) and respective carapace width (2.80 cm) was similar in all the treatments. After five months of culture, female mud crab attained an average weight of 246.17 g, 198.70 g and 178.50 g in T1 (outdoor tank), T2 (outdoor box) and T3 (indoor compartment), respectively (Table 1). Weight gain was significantly higher (p<0.05) in T1 than T2 and T3. Similarly, SGR also differed statistically (p<0.05) among the treatments with the highest value (3.46%/day) in T1, but that was statistically similar (0>0.05) in T2 (3.25%/day) and T3 (3.14%/day). The lowest survival of female crab was 42.85% in T1 and it differed statistically (p<0.05) with other two treatments (65% in T2 and 60% in T3). During harvest, 16% and 2.5% of the female crabs were observed with broken appendages (limbless) in T1 and T2, respectively, whereas, none was observed in T3 (Table 1).

Weight gain, survival and intactness of male mud crab

As shown in table 2, final weight of male mud crab under outdoor free tank was 319.75 g and it was significantly higher (p<0.05) than outdoor boxes (250.50 g) and indoor compartment (246.4 g). Similarly, specific growth rate (SGR) of 3.63%/day was also statistically significant (p<0.05) followed by outdoor boxes (3.39%/day) and indoor compartments (3.37%/day). Survival rate of 67% under outdoor boxes and indoor compartment (64%) was statistically significant (p<0.05) than outdoor tanks (29%). Among the harvested male crabs 25.26%, 4.50% and 2.00% was limblost in outdoor free tank, outdoor boxes and indoor compartment system, respectively (Table 2).
Table 1. Growth, survival and intactness of female mud crab cultured under different captive grow out protocols

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Female grow out protocols</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
</tr>
<tr>
<td>Initial wt. (g)</td>
<td>21.32±3.18&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Initial CW (cm)</td>
<td>2.80±0.20&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Final wt. (g)</td>
<td>246.17±21.53&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Final CW (cm)</td>
<td>10.20±0.51&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Survival rate (%)</td>
<td>42.85±7.15&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>SGR (%/day)</td>
<td>3.46±0.09&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Limb lost (%)</td>
<td>16.03±5.74&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Different superscripts in the same row indicates significant differences (p<0.05), shared superscript indicate similarity, and a>b>c; CW: carapace width. Data are presented as Mean±SD.

Table 2. Growth, survival and intactness of male mud crab cultured under different grow out protocols in captive condition

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Male grow out protocols</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
</tr>
<tr>
<td>Initial wt. (g)</td>
<td>24.50±3.20&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Initial CW (cm)</td>
<td>4.60±0.20&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Final wt. (g)</td>
<td>319.75±24.20&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Final CW (cm)</td>
<td>10.63±0.50&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Survival rate (%)</td>
<td>29.00±3.57&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>SGR (%/day)</td>
<td>3.63±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Limb lost (%)</td>
<td>25.26±3.18&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Different superscripts in the same row indicate significant differences (p<0.05), shared superscript indicates similarity, and a>b>c; CW: carapace width. Data are presented as Mean±SD.

Comparison of bio-parameters between male and female crabs

Comparison of bio-parameters among male and female mud crab was done only for outdoor free tank culture system for both sexes. As presented in table 3, weight increment of male crab was 29.89% and SGR was 4.9% higher over the female, indicated faster growth of male. On the other hand, survival of male crab seemed 32.32% lower and appendages broken was 57.58% higher over the female mud crab (Table 3) designated the males as more aggressive and canabalistic than the female crabs.
Table 3. Comparison of bio-parameters (growth, survival and intactness) among different sexes of mud crab cultured under outdoor tank system

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Female</th>
<th>Male</th>
<th>% of increase or decrease over female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial wt. (g)</td>
<td>21.32±3.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.50±3.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(+) 14.91</td>
</tr>
<tr>
<td>Initial CW (cm)</td>
<td>2.80±0.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.60±0.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(+) 64.28</td>
</tr>
<tr>
<td>Final wt. (g)</td>
<td>246.17±21.53&lt;sup&gt;b&lt;/sup&gt;</td>
<td>319.75±24.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(+) 29.89</td>
</tr>
<tr>
<td>Final CW (cm)</td>
<td>10.20±0.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.63±0.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(+) 4.22</td>
</tr>
<tr>
<td>Survival rate (%)</td>
<td>42.85±7.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.00±3.57&lt;sup&gt;b&lt;/sup&gt;</td>
<td>(-) 32.32</td>
</tr>
<tr>
<td>SGR (%/day)</td>
<td>3.46±0.094&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.63±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(+) 4.91</td>
</tr>
<tr>
<td>Appendages broken (%)</td>
<td>16.03±5.74&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25.26±3.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(+) 57.58</td>
</tr>
</tbody>
</table>

(+ ) indicates increase and (- ) indicates decrease over the opposite sex; Different superscript in the same row indicates significant differences (p<0.05), shared superscripts indicate similarity, and a>b. CW: carapace width. Data are presented as Mean±SD.

**DISCUSSION**

Like other crustacean species, mud crab also grows through moult ing and moult ing might be affected by many factors (like, temperature, stress and scares from predator, lack of shedding/hiding places, inadequate nutritional feeding, hydrology) and any interruption in moult ing might slower the growth (Kulmiye and Mavuti, 2004), thus longer time is needed to attain desirable size and even cause death to the victim. In this experiment, both male and female crab showed a sigmoid growth form (Figure 1-4). A discrete growth system of sharp changes in external size through moult ing and slow growth in muscle content during the entire intermoult period might be the reason of such type of growth in crustaceans like mud crab (Shelley and Lovatelli, 2011). In crustaceans, moult ing in juvenile stages is frequent and the moult ing duration increases as the size grows bigger (Ehrhard, 2008; Shelley and Lovatelli, 2011) and in many crustaceans, growth attainment rate per moult reduces with age, especially after pubertal moult ing or maturity, resulted complex growth patterns (Ehrhardt, 2008). Meanwhile, a sigmoid growth pattern was modeled for *S. serrata* from mark-recapture methods, pond cultures and laboratory experiment (Moksnes et al., 2014). The growth pattern observed from this experiment on *S. paramamosain* is thus supported by the theme of the above mentioned authors.

In this experiment higher growth and SGR of both sexes of mud crab was achieved from the outdoor culture tank (T1) than outdoor box and indoor compartment (Table 1&2). The reasons behind this might be due to the provisions of semi-natural culture conditions in the outdoor tanks that might facilitate some diversified food choices like periphyton, aquatic insects and larvae of insectsthose grew onto the tanks and substrates (*Ulva*). In addition, it is also probably due to cannibalism on their siblings, which added extra nutrition to grow faster. Similar faster growth in pond culture than
BROODSTOCK UNDER DIFFERENT CAPTIVE GROW OUT PROTOCOLS

Cages and indoor tanks was previously reported for *S. serrata* (Srinivasagam and Kathirvel, 1992). On the contrary, a higher growth of mud crab under drive-in cages set into mangrove than the pen culture was reported (David, 2009). Meanwhile, a 40% less growth of *S. serrata* in cage system than pond culture or natural growth was stated (Moksnes et al., 2014).

The survival of mud crab in this experiment showed wide variation between different culture protocols. Under outdoor boxes and indoor compartment, the survival seemed high (60-65%) and in tank system, it was 42.85% for female (Table 1) and 29% for male crabs (Table 2). Crabs restricted in a box and compartment might minimized the cannibalism in this study. Cannibalism in coupled with natural death (moult death) lowered the survival in the tank culture system. Moult death is the main fact of mortality in crustaceans (Cholik and Hanafi, 1992) and coupled with post moult cannibalism reportedly reduce the survival and pointed as the main problem in mud crab aquaculture (David, 2009). In a study on *S. serrata*, the survival rate was 53.2% under drive-in cages and 31.25% under pen culture (David, 2009). Whereas, survival rate of *S. serrata* was 45-57% under the drive-in cage culture system (Mirera, 2011), and that for pond culture was 40.2-51.6% (Trino et al., 2001).

Specific growth rate (SGR) observed in this trial ranged between 3.46-3.14%/day for female crabs (Table 1) and 3.63-3.37%/day for males (Table 2) with highest values under outdoor tanks for both sexes. SGR value of 1.25g/day for the drive-in cage culture condition and 0.68 g/day for pen culture system for mixed sex culture of adult *S. serrata* was previously reported in Kenya (David, 2009). In another reporton *S. serrate* mentioned the SGR of 10 g/month in tanks, 19 g/month in cages, and 29 g/month in ponds (Srinivasagam and Kathirvel, 1992). On the other hand, SGR value of 70 g/month (2.33 g/day) for *S. serrata* monoculture by starting with juveniles of 7 g size (Marichamy et al., 1986) seemed quite consistent with this study.

This study observed significantly higher (p<0.05) body weight and SGR values for the male than female for same culture protocols (Table 3). The SGR values from 1.8 - 1.9 g/day for *S. serrata* did not differ between sexes (Trino et al., 2001). While, a distinct difference in growth between sexes was noticed for *S. Oceania* and for *S. serrata* (Marichamy and Rajapachiam, 2001). However, daily weight gain of 1-4 g was reported for mud crab that varies with species and sex, with males having a faster growth than females (Christensen et al., 2004); size of experimental animal (Ehrhardt, 2008); and location of experiment (Moksnes et al., 2014). Thus growth and SGR values obtained in this experiment for *S. paramamosain* are supported by the above mentioned authors.

Despite the higher growth of male over female, a lowered survival and higher appendages broken was noticed in male mud crab (Table 3) which indicated aggressiveness of the male. Male mud crab was reported as aggressive to secure territory (Shelley, 2008). All these are negative sides in point of production, market price, breeding and reproductive performance as cheliped lost is reported more
vulnerable to predation and cannibalism (Shelley, 2008). In addition, it caused lowered production because chelipod consisted about 40% of the total weight of male and 22% of the total weight of the female; of those has had a weight of 668 g (Heasman, 1980). Intact crabs of marketable size commands better prices than the injured or limb lost ones (Agbayani, 2001). Each of the appendages has had specific activities like feeding, escaping, defense or offense mechanism, mating and egg hatching for female. A suitable brood might be intact and has had a minimum weight of 450 g for *S. serrata* and 350 g for *S. Olivacea* and *S. tranqubarica* (Quinitio and Parado-Estepa, 2008). Intact brood provided better reproductive performance (Thache, 2009) and broods with injured appendages are not suitable due to low hatching rate and unsuitable larvae quality due to utilization of nutrients for regeneration of lost appendages (Zainoddin, 2001; Quinitio et al., 2010).

CONCLUSION
This experiment on growth of mud crab under the different protocols in captive condition has shown that growth in outdoor open tank is better than outdoor boxes or indoor compartments, but outdoor boxes and indoor compartment produced more intact animals. Both size and morphological features (intactness) of broodstock are regarded as pre-recusite for satisfactory reproductive performance and larvae quality. However, among the tested grow out protocols, outdoor culture system could be considered as superior option for quickest broodstock development of mud crab in captive condition. Extending the initial communal culture duration for a further one month and then arresting into boxes or compartment might be another option to achieve suitable sized and intact broodstock and that needs to be incorporated in future studies.

ACKNOWLEDGEMENT
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REFERENCES
BROODSTOCK UNDER DIFFERENT CAPTIVE GROW OUT PROTOCOLS


GENETIC VARIABILITY AND CHARACTER ASSOCIATION OF QUANTITATIVE TRAITS IN JHUM RICE GENOTYPES

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M.A.A. Mamun² and M.A.Z. Chowdhury³

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ABSTRACT

Thirty jhum rice genotypes of hilly origin were studied for genetic variability, correlation and path analysis under medium high land of Bangladesh Rice Research Institute, Gazipur, Bangladesh. The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications for the period of March to July (Aus season), 2016. Analysis of variance revealed significant difference among the genotypes for all the characters studied. The PCV values were greater than GCV, revealing little influence of environment in character expression. High values of heritability along with high genetic advance were observed for filled grain and plant height. Such outcomes suggested predominance of additive gene action in gene expression for these characters. Grain yield showed positive association with number of effective tiller and thousand grain weight at genotypic in conjunction with phenotypic level. Most of the traits had significant genetic variability besides, plant height and panicle length exhibited positive direct effect together with positive correlation with yield. Thousand grain weight possessed negative direct effect but highest positive significant correlation with yield.

Keywords: Correlation, variability, genetic advance, heritability, path coefficients, rice, yield, yield components.

INTRODUCTION

Rice is a food grain crop of global importance with special preference in Asian countries (Sahu et al., 2017). Rice is life for majority of the residents of this sub-continent. Rice landraces play a vital role for food and nutritional security besides resistance to diseases and pests and resilience to climate changes which is essential for the survival of mankind facing the unpredictable climate change on earth. Jhum

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rice is a unique kind of plant genetic resource which is cultivated through hilly areas mostly by tribal people of Bangladesh. The local farmers usually grow low yielding local landraces. More than 300 local jhum rice landraces have been collected from various hilly districts of Bangladesh and conserved in Bangladesh Rice Research Institute (BRRI) genebank (Source: BRRI genebank accession book). This collection is an invaluable genetic resource that can be used for varietal development.

Breaking yield ceiling is the key breeding objective of rice breeding program with the noble intend to feed the ever increasing population. For that reason, knowledge on the nature and magnitude of the genetic variation leading the inheritance of quantitative characters like yield and its components is crucial for effective genetic improvement. The success of breeding program depends upon the quantum of genetic variability available for exploitation and the extent to which the desirable characters are heritable (Tiwari et al., 2011).

Genetic variability of promising genotypes of certain crop is like a directory of their genetic vitality. Variability has two components such as additive and non-additive. To obtain a clear understanding of the pattern of variations, the phenotypic variance has been partitioned into genotypic and phenotypic variance. A critical analysis of the genetic variability parameters, namely, Genotypic Coefficient of Variability (GCV), Phenotypic Coefficient of Variability (PCV), heritability and genetic advance for different traits of economic importance is a major pre-requisite for any plant breeder to work with crop improvement programmes. Further, information on correlation coefficients between grain yield and its component characters is essential for yield improvement, since grain yield in rice is a complex entity and is highly influenced by several component characters (Kishore et al., 2015). Role of environmental attributes on the expression any genotype and reliability of characters can be determined precisely by high broad sense heritability united with high genetic advance (Babu et al., 2012).

Moreover, knowledge of heritability is essential for selection based improvement as it indicates the extent of transmissibility of a character into future generations. Knowledge of correlation between yield and its contributing characters are basic and for most endeavor to find out guidelines for plant selection. Partitioning of total correlation into direct and indirect effect by path analysis helps in making the selection more effective. Comprehending the above facts, the present investigation was undertaken to know variability and correlation among yield and its contributing characters using 30 jhum rice genotypes under direct seeded condition in a medium high land.

**MATERIALS AND METHODS**

A total of 30 jhum rice landraces (Table 1) were collected from Rangamati district of Bangladesh. The collected landraces (code: J1-J30) have been conserved at short term storage of the BRRI Gene bank for safe keeping. Local names and place of collection of the studied jhum rice are listed in table1.
Table 1. Information on local name and place of collection of the Jhum rice landraces

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>local name</th>
<th>Code</th>
<th>Upazilla</th>
<th>Sl. No</th>
<th>name</th>
<th>Code</th>
<th>Upazilla</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Kobrok-1</td>
<td>J1</td>
<td>Rangamati Sadar</td>
<td>16</td>
<td>Chorui</td>
<td>J16</td>
<td>Rangamati Sadar</td>
</tr>
<tr>
<td>02</td>
<td>VanguriJhum</td>
<td>J2</td>
<td>..</td>
<td>17</td>
<td>Pattiki</td>
<td>J17</td>
<td>..</td>
</tr>
<tr>
<td>03</td>
<td>Amey-1</td>
<td>J3</td>
<td>..</td>
<td>18</td>
<td>Amey-2</td>
<td>J18</td>
<td>..</td>
</tr>
<tr>
<td>04</td>
<td>Horinbinni</td>
<td>J4</td>
<td>..</td>
<td>19</td>
<td>Guri</td>
<td>J19</td>
<td>..</td>
</tr>
<tr>
<td>05</td>
<td>Bai</td>
<td>J5</td>
<td>..</td>
<td>20</td>
<td>Kamarang-2</td>
<td>J20</td>
<td>..</td>
</tr>
<tr>
<td>06</td>
<td>Lonkaparabinni</td>
<td>J6</td>
<td>..</td>
<td>21</td>
<td>Suri</td>
<td>J21</td>
<td>..</td>
</tr>
<tr>
<td>07</td>
<td>Turni</td>
<td>J7</td>
<td>..</td>
<td>22</td>
<td>Kamarang-3</td>
<td>J22</td>
<td>..</td>
</tr>
<tr>
<td>08</td>
<td>Vangurivalo</td>
<td>J8</td>
<td>..</td>
<td>23</td>
<td>Amey-3</td>
<td>J23</td>
<td>..</td>
</tr>
<tr>
<td>09</td>
<td>Bandornokbinni</td>
<td>J9</td>
<td>..</td>
<td>24</td>
<td>Badoi</td>
<td>J24</td>
<td>..</td>
</tr>
<tr>
<td>10</td>
<td>Kamarang-1</td>
<td>J10</td>
<td>..</td>
<td>25</td>
<td>Turki</td>
<td>J25</td>
<td>Kaptai</td>
</tr>
<tr>
<td>11</td>
<td>Lokkhibinni</td>
<td>J11</td>
<td>..</td>
<td>26</td>
<td>Kangbui</td>
<td>J26</td>
<td>..</td>
</tr>
<tr>
<td>12</td>
<td>Gonda</td>
<td>J12</td>
<td>..</td>
<td>27</td>
<td>Kongcho</td>
<td>J27</td>
<td>..</td>
</tr>
<tr>
<td>13</td>
<td>Kangbui</td>
<td>J13</td>
<td>..</td>
<td>28</td>
<td>Bidi</td>
<td>J28</td>
<td>..</td>
</tr>
<tr>
<td>14</td>
<td>Koborok-2</td>
<td>J14</td>
<td>..</td>
<td>29</td>
<td>Kobrok</td>
<td>J29</td>
<td>..</td>
</tr>
<tr>
<td>15</td>
<td>Galong</td>
<td>J15</td>
<td>..</td>
<td>30</td>
<td>Mongkhoi</td>
<td>J30</td>
<td>..</td>
</tr>
</tbody>
</table>

The experiment was conducted following a Randomized Complete Block design with three replicates for each treatment at the experimental field of BRRI, Gazipur, during March to July (Aus season), 2016. Geographically, the place is located at about 24.00° N latitude and 90.25°E longitude with an elevation of 8.4 meters from the sea level and is characterized by subtropical climate. The soil of the experimental site was clay loam in texture.

Twenty days-old seedlings from each entry were transplanted using single seedling per hill in 2.4 m² plot with 25cm and 20cm space between rows and plants, respectively.

Fertilizers were applied @ 80:60:40:12kg N: P: K: S per hectare. However, except N, the other fertilizers were applied at final land preparation. Nitrogen was applied in three equal splits, at 15 days after transplanting (DAT), at 35 DAT, and just before flowering. Intercultural operations and pest control measures were done as and when necessary.

Data were collected on culm diameter (mm), flag leaf angle, plant height (cm), days to flowering, days to maturity, effective tiller number (ET), panicle length (cm), number of filled grains per panicle, grain length (mm), grain breadth(mm), grain length breadth ratio, 1000 grain weight (g) and grain yield per hill (g). Kernels were
classified on the basis of length (size) and for LB ratio (shape) following classification described by Cruz and Khush (2004).

**Statistical analysis**

Genotypic and phenotypic co-efficient of variation were calculated following the methodology delineated by Burton (1952), while the estimates of heritability and genetic advance were computed as per the procedures elaborated by Burton and Devane (1953), and Johnson et al. (1955), respectively. Normal Pearson’s correlation and path coefficient analysis was undertaken using R software (version 3.2.1). Furthermore, Genotypic and phenotypic correlation coefficients were calculated with META-R software.

**RESULTS AND DISCUSSION**

Agronomic traits are quantitative in nature, and interact with the environment under study, so partitioning the traits into genotypic, phenotypic, and environmental effects is essential to find out the additive or heritable portion of variability. The mean, range, genotypic and phenotypic variance (Vg, Vp) and coefficient of variation (GCV, PCV), \( h^2_b \), genetic advance (GA) and genetic advance in percent of mean (GAPM) are presented in table 2.

**Table 2.** Estimation of genetic parameters of different quantitative characters in 30 Jhum rice landraces

<table>
<thead>
<tr>
<th>Character</th>
<th>Mean</th>
<th>Range</th>
<th>( V_p )</th>
<th>( V_g )</th>
<th>PCV</th>
<th>GCV</th>
<th>( h^2_b )</th>
<th>GA (5%)</th>
<th>GAPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flag leaf angle (cm)</td>
<td>50.10</td>
<td>30.62-67.40</td>
<td>75.60</td>
<td>72.72</td>
<td>17.36</td>
<td>17.02</td>
<td>96.18</td>
<td>13.21</td>
<td>26.38</td>
</tr>
<tr>
<td>Culm Diameter(mm)</td>
<td>4.80</td>
<td>3.48-6.52</td>
<td>0.44</td>
<td>0.31</td>
<td>13.76</td>
<td>11.68</td>
<td>72.12</td>
<td>0.75</td>
<td>15.68</td>
</tr>
<tr>
<td>Plant Height (cm)</td>
<td>119.65</td>
<td>90.59-144.37</td>
<td>186.91</td>
<td>186.17</td>
<td>11.42</td>
<td>11.40</td>
<td>99.60</td>
<td>21.52</td>
<td>17.98</td>
</tr>
<tr>
<td>Effective Tiller</td>
<td>6.97</td>
<td>4.00-10.00</td>
<td>3.34</td>
<td>2.97</td>
<td>19.78</td>
<td>18.65</td>
<td>88.95</td>
<td>2.57</td>
<td>27.79</td>
</tr>
<tr>
<td>Panicle Length (cm)</td>
<td>28.81</td>
<td>23.69-33.87</td>
<td>6.31</td>
<td>5.86</td>
<td>8.72</td>
<td>8.40</td>
<td>82.85</td>
<td>3.69</td>
<td>12.79</td>
</tr>
<tr>
<td>Days to Flowering</td>
<td>91.33</td>
<td>74.00-113.00</td>
<td>65.52</td>
<td>65.08</td>
<td>8.89</td>
<td>8.86</td>
<td>99.32</td>
<td>12.70</td>
<td>13.95</td>
</tr>
<tr>
<td>Days to Maturity</td>
<td>118.00</td>
<td>104.00-141.00</td>
<td>62.53</td>
<td>62.06</td>
<td>6.70</td>
<td>6.67</td>
<td>99.26</td>
<td>12.40</td>
<td>10.50</td>
</tr>
<tr>
<td>Filled Grain</td>
<td>105.40</td>
<td>81.71-137.25</td>
<td>221.26</td>
<td>220.38</td>
<td>14.11</td>
<td>14.08</td>
<td>99.60</td>
<td>23.41</td>
<td>22.21</td>
</tr>
<tr>
<td>Grain Length (mm)</td>
<td>9.07</td>
<td>5.89-12.69</td>
<td>2.20</td>
<td>1.79</td>
<td>16.32</td>
<td>14.75</td>
<td>81.67</td>
<td>1.91</td>
<td>21.06</td>
</tr>
<tr>
<td>Grain Breadth (mm)</td>
<td>3.37</td>
<td>2.05-4.68</td>
<td>0.38</td>
<td>0.06</td>
<td>18.19</td>
<td>7.08</td>
<td>15.16</td>
<td>0.15</td>
<td>4.36</td>
</tr>
<tr>
<td>LB ratio</td>
<td>3.15</td>
<td>1.65-4.48</td>
<td>0.47</td>
<td>0.24</td>
<td>21.90</td>
<td>15.43</td>
<td>49.63</td>
<td>0.54</td>
<td>17.18</td>
</tr>
<tr>
<td>1000 grain wt.(g)</td>
<td>23.30</td>
<td>13.88-31.54</td>
<td>16.33</td>
<td>15.99</td>
<td>17.34</td>
<td>17.16</td>
<td>97.92</td>
<td>6.25</td>
<td>26.83</td>
</tr>
<tr>
<td>Yield (g)</td>
<td>5.14</td>
<td>2.52-9.28</td>
<td>2.47</td>
<td>2.31</td>
<td>30.57</td>
<td>29.54</td>
<td>93.33</td>
<td>2.32</td>
<td>45.08</td>
</tr>
</tbody>
</table>

\( V_p \) = Phenotypic variance, \( V_g \) = Genotypic variance, PCV = Phenotypic Coefficient of variation, GCV = Genotypic coefficient of variation, \( h^2_b \) = Heritability (Broad sense), GA = Genetic advance, GAPM = Genetic advance in percent of mean
The range of variation was much pronounced for all the traits in the study. The highest genotypic and phenotypic variances were observed for filled grain. Plant height and flag leaf angle exhibited high genotypic and phenotypic variances indicating the presence of the wide range of variability for the traits under study and had greater scope of selection for the improvement of jhum rice. Moderate genotypic and phenotypic variances were obtained from days to flowering and days to maturity, hence these traits might be considered for the improvement of jhum rice genotypes.

In this study, most of the growth traits showed higher PCV compared to yield and yield component traits. However, lower PCV belonged to days to maturity (6.70%) while yield (30.57%) was recorded with higher value. Length breadth ratio (21.99%), effective tiller (19.78%) and grain breadth (18.19%) were recorded with higher values of PCV. Panicle length (8.72%) and days to flowering (8.89%) were found with lower values. The higher GCV was associated with yield (29.54%) and the value was fairly low in case of days to maturity (6.67%). Heritability ranged from 15.16 to 99.60%. The highest amount of heritability was recorded at filled grain and plant height whereas, the lowest value was found at grain breadth. The results were in conformity with that of Anjaneyulu et al. (2010), Idris et al. (2012) and Sandhya (2014). Flag leaf angle, panicle length, days to flowering, days to maturity, 1000 grain wt. and yield were highly heritable all with an estimated $H^2 > 0.90$ whereas other characters showed relatively low heritability. Characters with high values of GCV and heritability indicating that they might transmit to their progenies and therefore, phenotypic selection based on these characters would be effective. Similar results have been reported by Akand et al. (1997), Sabesan et al. (2009) and Jayasudha and Sharma (2010). Genetic advance (GA) ranged from 0.15% for grain breadth to 23.41% for filled grain. The genetic advance as percent of mean (GAPM) ranged from 4.36% for grain breadth to 45.08% for yield.

Pearson’s correlation coefficient was computed among 13 quantitative traits of 30 accessions of jhum rice genotypes (Table 3).
Table 3. Pearson correlation analysis among yield and its contributing characters in jhum rice genotypes

<table>
<thead>
<tr>
<th></th>
<th>Culm Diameter</th>
<th>Plant Height</th>
<th>Effective Tiller</th>
<th>Panicle Length</th>
<th>Days to Flowering</th>
<th>Days to Maturity</th>
<th>Filled Grain</th>
<th>Grain Length</th>
<th>Grain Breadth</th>
<th>LB ratio</th>
<th>1000 grain wt.</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flag leaf angle</td>
<td>0.255</td>
<td>0.225</td>
<td>-0.429**</td>
<td>0.426**</td>
<td>0.310*</td>
<td>0.264</td>
<td>0.136</td>
<td>-0.266</td>
<td>-0.092</td>
<td>-0.099</td>
<td>-0.098</td>
<td>-0.135</td>
</tr>
<tr>
<td>Culm Diameter</td>
<td>0.075</td>
<td>-0.37*</td>
<td>0.080</td>
<td>0.531**</td>
<td>0.519**</td>
<td>0.420**</td>
<td>0.191</td>
<td>-0.207</td>
<td>0.303</td>
<td>-0.105</td>
<td>-0.017</td>
<td></td>
</tr>
<tr>
<td>Plant Height</td>
<td>0.053</td>
<td>0.696***</td>
<td>0.204</td>
<td>0.221</td>
<td>0.146</td>
<td>-0.307</td>
<td>-0.145</td>
<td>-0.158</td>
<td>-0.522</td>
<td>0.245</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Effective Tiller</td>
<td>-0.130</td>
<td>-0.154</td>
<td>-0.148</td>
<td>0.135</td>
<td>-0.291</td>
<td>-0.145</td>
<td>-0.130</td>
<td>-0.364*</td>
<td>0.446**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Panicle Length</td>
<td>0.236</td>
<td>0.234</td>
<td>0.030</td>
<td>-0.244</td>
<td>-0.109</td>
<td>-0.115</td>
<td>-0.416**</td>
<td>0.119</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days to Flowering</td>
<td>0.992***</td>
<td>0.036</td>
<td>0.173</td>
<td>-0.486**</td>
<td>0.441**</td>
<td>-0.331*</td>
<td>-0.055</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days to Maturity</td>
<td>0.015</td>
<td>0.191</td>
<td>-0.457**</td>
<td>0.434**</td>
<td>-0.321*</td>
<td>-0.063</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Filled Grain</td>
<td>-0.390*</td>
<td>-0.077</td>
<td>-0.189</td>
<td>-0.443**</td>
<td>0.209</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grain Length</td>
<td>-0.185</td>
<td>0.793***</td>
<td>0.565***</td>
<td>-0.476**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grain Breadth</td>
<td>-0.735***</td>
<td>0.387*</td>
<td>-0.337*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LB ratio</td>
<td>0.133</td>
<td>-0.123</td>
<td>-0.542**</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

*, **, ***Significant at 5%, 1% and 0.1% levels, respectively
Flag leaf angle was significantly and positively correlated with panicle length but negatively correlated with effective tiller. Culm diameter showed significant positive correlation with days to flowering (0.531**), days to maturity (0.519**) and filled grain (0.420**). On the other hand, panicle length and filled grain showed negative association with 1000 grain wt. Effective tiller was positively correlated with yield. Mirza et al. (1992) reported positive correlation of number of panicles/m2 and grain yield with number of tillers/plant. However, plant height was found significantly correlated with panicle length (0.696**). Days to flowering was highly significant and positively correlated with days to maturity (0.992**) and LB ratio (0.441**). Again, grain length showed positive and highly significant correlation with LB ratio (0.793**) and 1000 grain wt. (0.565**).

The genotypic and phenotypic correlations for yield and yield components are showed in table 4. Effective tiller possessed highly negative association with flag leaf angle followed by culm diameter. Panicle length showed highly significant and positive correlation with plant height (Rangare et al., 2012) and significant positive correlation with flag leaf area both at genotypic and phenotypic level. Days to maturity showed significant positive correlation with culm diameter and days to flowering. Moreover, days to flowering and filled grain also has positive association with culm diameter at genotypic as well as phenotypic level.

Grain breadth has negative but significant association with both days to flowering and maturity.

Effective tiller and 1000 grain weight showed significant positive correlation with yield, but negatively significant association with grain length at genotypic along with phenotypic level. Hence, effective tiller and 1000 grain weight should be given prior attention in rice improvement program because of their major influence on yield. This finding was in accordance with Hasan et al. (2010), Manikyaminnie et al. (2013) and Adilakshmi and Girijarani (2012).

Path coefficient analysis was done to partition the direct and indirect effects of different yield contributing traits on yield of rice. Path coefficient analysis (Table 5) revealed that effective tiller (-0.238) possessed negative direct effect on yield but made the indirect effect positive and significant. This indicates that more number of effective tiller is the highly reliable component of grain yield (Garg et al., 2010). Another important character with high direct effect is length-breadth ratio, which showed the highest positive direct effect (0.352) on yield but had negative indirect effect on total correlation. Hence, direct selection based on length-breadth ratio would not be effective for improvement of yield. Moreover, negative direct effect was observed for flag leaf angle, days to flowering, grain length and grain breadth.

On the other hand, plant height and panicle length exhibited positive direct effect along with positive correlation with yield. Furthermore, 1000 grain weight possessed negative direct effect and highest positive significant correlation with yield.

The residual effect of the present study was 0.368, indicated that 63.2% of the variability was accounted for 13 yield contributing traits included in the present study. The rest amount of variability might be controlled by other yield contributed traits that was not included in the present investigation.
Table 4. Genotypic (G) and phenotypic (P) correlations among yield and yield contributing characters in jhum rice genotypes

<table>
<thead>
<tr>
<th>Traits</th>
<th>Flag leaf angle</th>
<th>Culm Diameter</th>
<th>Plant Height</th>
<th>Effective Tiller</th>
<th>Panicle Length</th>
<th>Days to Flowering</th>
<th>Days to Maturity</th>
<th>Filled Grain</th>
<th>Grain Length</th>
<th>Grain Breadth</th>
<th>LB ratio</th>
<th>1000 grain wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culm diameter</td>
<td>G 0.235</td>
<td>P 0.231</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G 0.225</td>
<td>P 0.227</td>
<td>G -0.462**</td>
<td>-0.390*</td>
<td>0.024</td>
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<tr>
<td>Effective Tiller</td>
<td>P -0.454**</td>
<td>-0.382*</td>
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<tr>
<td>G 0.376*</td>
<td>P 0.009</td>
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<tr>
<td>Panicle Length</td>
<td>P 0.378*</td>
<td>-0.014</td>
<td>0.675***</td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>Days to Flowering</td>
<td>G 0.336</td>
<td>0.520**</td>
<td>0.210</td>
<td>-0.251</td>
<td>0.241</td>
<td></td>
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<tr>
<td>P 0.337</td>
<td>0.499**</td>
<td>0.189</td>
<td>-0.258</td>
<td>0.216</td>
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<tr>
<td>G 0.279</td>
<td>0.502**</td>
<td>0.202</td>
<td>-0.234</td>
<td>0.224</td>
<td>0.987***</td>
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</tr>
<tr>
<td>Days to Maturity</td>
<td>P 0.280</td>
<td>0.477**</td>
<td>0.174</td>
<td>-0.244</td>
<td>0.194</td>
<td>0.984***</td>
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<tr>
<td>G 0.099</td>
<td>0.492**</td>
<td>0.272</td>
<td>0.074</td>
<td>0.293</td>
<td>0.207</td>
<td>0.168</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Filled Grain</td>
<td>P 0.101</td>
<td>0.466**</td>
<td>0.228</td>
<td>0.060</td>
<td>0.245</td>
<td>0.177</td>
<td>0.127</td>
<td></td>
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<tr>
<td>G -0.322</td>
<td>0.184</td>
<td>-0.205</td>
<td>-0.294</td>
<td>-0.234</td>
<td>0.118</td>
<td>0.158</td>
<td>-0.267</td>
<td></td>
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<tr>
<td>Grain Length</td>
<td>P -0.329</td>
<td>0.123</td>
<td>-0.280</td>
<td>-0.304</td>
<td>-0.312</td>
<td>0.072</td>
<td>0.103</td>
<td>-0.386*</td>
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<tr>
<td>G -0.464**</td>
<td>-0.692***</td>
<td>-0.210</td>
<td>0.131</td>
<td>-0.318</td>
<td>-0.817***</td>
<td>-0.753***</td>
<td>-0.249</td>
<td>-0.318</td>
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<tr>
<td>Grain Breadth</td>
<td>P -0.289</td>
<td>-0.406*</td>
<td>-0.102</td>
<td>0.060</td>
<td>-0.167</td>
<td>-0.493***</td>
<td>-0.448**</td>
<td>-0.104</td>
<td>-0.163</td>
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<tr>
<td>LB ratio</td>
<td>G -0.227</td>
<td>0.245</td>
<td>-0.244</td>
<td>-0.109</td>
<td>-0.127</td>
<td>0.381*</td>
<td>0.380*</td>
<td>0.053</td>
<td>0.715***</td>
<td>-1.000***</td>
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<tr>
<td>P -0.228</td>
<td>0.196</td>
<td>-0.329</td>
<td>-0.122</td>
<td>-0.201</td>
<td>0.321</td>
<td>0.307</td>
<td>-0.074</td>
<td>0.560***</td>
<td>-0.614***</td>
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</tr>
<tr>
<td>G 0.021</td>
<td>-0.063</td>
<td>-0.391*</td>
<td>-0.158</td>
<td>-0.409*</td>
<td>-0.254</td>
<td>-0.459**</td>
<td>0.304</td>
<td>0.352*</td>
<td>0.050</td>
<td></td>
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</tr>
<tr>
<td>1000 grain wt.</td>
<td>P 0.022</td>
<td>-0.040</td>
<td>-0.369*</td>
<td>-0.147</td>
<td>-0.387*</td>
<td>-0.237</td>
<td>-0.223</td>
<td>-0.434**</td>
<td>0.371*</td>
<td>0.191</td>
<td>0.028</td>
<td></td>
</tr>
<tr>
<td>Yield</td>
<td>G -0.086</td>
<td>0.040</td>
<td>0.289</td>
<td>0.431***</td>
<td>0.163</td>
<td>-0.045</td>
<td>-0.068</td>
<td>0.276</td>
<td>-0.592***</td>
<td>0.036</td>
<td>-0.112</td>
<td>0.425**</td>
</tr>
<tr>
<td>P -0.084</td>
<td>0.042</td>
<td>0.290</td>
<td>0.431***</td>
<td>0.161</td>
<td>-0.046</td>
<td>-0.069</td>
<td>0.287</td>
<td>-0.592***</td>
<td>0.022</td>
<td>-0.115</td>
<td>0.426**</td>
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</tr>
</tbody>
</table>

*, **, ***Significant at 5%, 1% and 0.1% levels, respectively
Table 5. Partitioning of genotypic correlation into direct (bold phase) and indirect components of 30 genotypes of jhum rice

<table>
<thead>
<tr>
<th></th>
<th>Flag leaf angle</th>
<th>Calm Diameter</th>
<th>Plant Height</th>
<th>Effective Tiller</th>
<th>Panicle Length</th>
<th>Days to Flowering</th>
<th>Days to Maturity</th>
<th>Filled Grain</th>
<th>Grain Length</th>
<th>Grain Breadth</th>
<th>LB ratio</th>
<th>1000 grain wt.</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flag leaf angle</td>
<td><strong>-0.471</strong></td>
<td>0.064</td>
<td>0.010</td>
<td>0.102</td>
<td>0.002</td>
<td>-0.117</td>
<td>0.020</td>
<td>-0.029</td>
<td>0.268</td>
<td>0.036</td>
<td>-0.035</td>
<td>0.014</td>
<td>-0.135</td>
</tr>
<tr>
<td>Calm Diameter</td>
<td>-0.120</td>
<td><strong>0.252</strong></td>
<td>0.003</td>
<td>0.088</td>
<td>0.000</td>
<td>-0.201</td>
<td>0.039</td>
<td>-0.091</td>
<td>-0.193</td>
<td>0.081</td>
<td>0.107</td>
<td>0.015</td>
<td>-0.018</td>
</tr>
<tr>
<td>Plant Height</td>
<td>-0.106</td>
<td>0.019</td>
<td><strong>0.046</strong></td>
<td>-0.013</td>
<td>0.004</td>
<td>-0.077</td>
<td>0.017</td>
<td>-0.031</td>
<td>0.309</td>
<td>0.097</td>
<td>-0.056</td>
<td>0.076</td>
<td>0.245</td>
</tr>
<tr>
<td>Effective Tiller</td>
<td>0.202</td>
<td>-0.093</td>
<td>0.002</td>
<td>-0.238</td>
<td>-0.001</td>
<td>0.058</td>
<td>-0.011</td>
<td>-0.029</td>
<td>0.292</td>
<td>0.157</td>
<td>-0.046</td>
<td>0.053</td>
<td>0.347**</td>
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<tr>
<td>Panicle Length</td>
<td>-0.201</td>
<td>0.020</td>
<td>0.032</td>
<td>0.031</td>
<td>0.006</td>
<td>-0.089</td>
<td>0.018</td>
<td>-0.007</td>
<td>0.246</td>
<td>0.043</td>
<td>-0.041</td>
<td>0.061</td>
<td>0.119</td>
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<tr>
<td>Days to Flowering</td>
<td>-0.146</td>
<td>0.134</td>
<td>0.009</td>
<td>0.037</td>
<td>0.001</td>
<td>-0.378</td>
<td>0.074</td>
<td>-0.008</td>
<td>-0.174</td>
<td>0.190</td>
<td>0.155</td>
<td>0.048</td>
<td>-0.056</td>
</tr>
<tr>
<td>Days to Maturity</td>
<td>-0.125</td>
<td>0.131</td>
<td>0.010</td>
<td>0.035</td>
<td>0.001</td>
<td>-0.375</td>
<td><strong>0.075</strong></td>
<td>-0.003</td>
<td>-0.192</td>
<td>0.179</td>
<td>0.153</td>
<td>0.047</td>
<td>-0.063</td>
</tr>
<tr>
<td>Filled Grain</td>
<td>-0.064</td>
<td>0.106</td>
<td>0.007</td>
<td>-0.032</td>
<td>0.000</td>
<td>-0.013</td>
<td>0.001</td>
<td><strong>-0.216</strong></td>
<td>0.392</td>
<td>0.030</td>
<td>-0.067</td>
<td>0.065</td>
<td>0.209</td>
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<tr>
<td>Grain Length</td>
<td>0.125</td>
<td>0.048</td>
<td>-0.014</td>
<td>0.069</td>
<td>-0.001</td>
<td>-0.065</td>
<td>0.014</td>
<td>0.084</td>
<td><strong>-1.006</strong></td>
<td>0.072</td>
<td>0.279</td>
<td>-0.083</td>
<td>-0.477**</td>
</tr>
<tr>
<td>Grain Breadth</td>
<td>0.043</td>
<td>-0.052</td>
<td>-0.007</td>
<td>0.035</td>
<td>-0.001</td>
<td>0.183</td>
<td>-0.034</td>
<td>0.017</td>
<td>0.186</td>
<td><strong>-0.392</strong></td>
<td>-0.259</td>
<td>-0.057</td>
<td>-0.337*</td>
</tr>
<tr>
<td>LB ratio</td>
<td>0.047</td>
<td>0.076</td>
<td>-0.007</td>
<td>0.031</td>
<td>-0.001</td>
<td>-0.167</td>
<td>0.033</td>
<td>0.041</td>
<td>-0.797</td>
<td>0.288</td>
<td><strong>0.352</strong></td>
<td>-0.020</td>
<td>-0.123</td>
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<tr>
<td>1000 grain wt.</td>
<td>0.046</td>
<td>-0.026</td>
<td>-0.024</td>
<td>0.087</td>
<td>-0.002</td>
<td>0.125</td>
<td>-0.024</td>
<td>0.096</td>
<td>-0.568</td>
<td>-0.152</td>
<td>0.047</td>
<td><strong>-0.146</strong></td>
<td>0.543**</td>
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</table>

*, **, *** Significant at 5% and 1% levels, respectively

Residual Effect, \( R = 0.3685925 \)
CONCLUSION
The experiment reveals the significant genetic variability among the yield contributing traits of jhum rice genotypes. Higher mean values of plant height and days to maturity are evidence of their superiority in contribution of variation. Moreover, considering the results of character association and path analysis it is well understood that 1000-grain weight and the number of effective tiller works as a selection criteria for yield improvement. Such kind of characters could be used in rice breeding strategy and biotechnological research for further yield and quality improvement.

ACKNOWLEDGMENTS
The authors are grateful to project on “Collection, characterization and promotion of rice, chili, cucumber and melon in Bangladesh” supported by AFACI (Asian Food and Agriculture Cooperation Initiative) for providing funds for this research.

REFERENCES


PATHOLOGICAL INVESTIGATION OF MAREK’S DISEASE IN A PULLET FARM OF BANGLADESH AND DETECTION OF MAREK’S DISEASE VIRUS BY PCR

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ABSTRACT

Marek’s disease (MD) is a lymph proliferative disease of chickens, characterized by progressive emaciation, morbidity and mortality. The causative agent is a cell associated oncogenic alpha-herpes virus. This study investigated an outbreak of MD in a pullet farm (N=2200) of Ramu Upazilla, Cox’s bazaar during May 2016 vaccinated against MD. The infectivity was reported on day 45 of age. Birds (N=10) submitted to diagnose disease at necropsy in the Department of Pathology, Bangladesh Agricultural University showed prominent keel bone, asymmetric progressive paralysis of one or both of the legs and wings. At necropsy, the skeletal muscle appeared thinner and there was enlargement of liver, spleen, kidney and sciatic nerve. Impression smears prepared from the liver showed huge infiltration of lymphocytes. Sections of heart, lungs, liver, kidney, nerve, skin and spleen were stained with hematoxylin and eosin showed wide spread infiltrations and accumulation of lymphocytes. Lymphocytic infiltration was seen in the skin, nerves and all visceral organs and showed combined infectivity due to visceral and classical forms. The etiology of MD was confirmed by using polymerase chain reaction (PCR) targeting fragment of Meq gene of very virulent plus or very virulent MDV1. Results of PCR showed amplification of 317bp fragment of Meq gene suggestive for infectivity due to MDV1GA (Md/5) strain. It requires isolating viruses in culture to test further for its virulence and pathotype in vivo. Sequencing and phylogenetic analysis of Meq gene may unveil the pathotype of the virus involved.

Keywords: Marek’s disease, pullet, PCR, Meq gene, MDV1GA strain

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INTRODUCTION

Marek’s disease (MD) in adult cockerels with leg paralysis was first described in 1907 by Jozsef Marek in Hungary (Marek, 1907). The name “Marek’s disease” was first proposed by Biggs in 1961 and was regarded as synonymous with “fowl paralysis” or “neurolymphomatosis” (Pappenheimer et al., 1929). Due to association of lymphoid tumors in the peripheral nerves and visceral organs, the disease was named as “neuro-lymphomatosis gallinarum” (Pappenheimer et al., 1929). The disease is caused by viruses, lymphoproliferative in nature, widely prevalent in commercial flock and causes variable rate of morbidity and mortality. Confirmatory diagnosis of lymphoproliferative diseases in poultry is often difficult. Three main classes of virus causing neoplasms of lymphatic tissues in poultry are a) Marek’s disease virus (MDV), a herpes virus b) Avian leukemia virus (ALV) and a retrovirus c) Reticuloendotheliosis virus (REV). Commercial chickens are the species most commonly affected with these viruses and neoplasms. Turkeys and quails can suffer from neoplasms caused by MDV and REV. Reticuloendotheliosis virus also causes neoplasms in geese and Muscovy ducks, pheasants and partridges (Payne and Venugopal, 2000). Marek’s disease in chickens is a common malady in layer or elderly chickens and is characterized by the formation of lymphoma in different visceral organs and infiltration and proliferation of lymphoid cells into peripheral nerves (Quere, 1992).

Marek’s disease virus induces T-cell lymphoma in chickens and is a member of the Alphaherpes virinae sub family of Herpes viridae. There are three serotypes of MDV: a) serotype 1 includes all pathogenic or oncogenic strains; b) serotype 2 includes naturally occurring non-pathogenic strains; and c) serotype 3 consists of turkey herpes virus (HVT), an oncogenic, MDV-related virus isolated from turkeys that has been widely used for immunization against Marek’s disease (Zhang et al., 2014). B-lymphocytes are the principal cells first targeted by the virus in a lytic fashion (Haq et al., 2013). The early cytolytic events contributed in atrophy of the bursa of Fabricius and thymus, leading to severe debilitation of the immune system and marked immunosuppression. The systemic infectivity spread viruses to the feather follicle epithelium, from where viruses shed in the environment and infect other birds (Heidari et al., 2016).

Before the introduction of vaccination of commercial flocks in 1971, MD was a major global issue in chickens. Vaccination dramatically reduced losses, but the disease remained one of the significant burdens for poultry industry, particularly because of the periodic appearance of new strains of MDV against which existing vaccines provide suboptimal protection. This has required the continued development of new vaccines and vaccination strategies (Payne and Venugopal, 2000). The diagnosis of infection with MDV is usually made by observing clinical signs (OIE 2010), isolation and identification of MDV from the infected tissues in cell culture or identification of the infected cells by immune histochemistry (De Laney et al., 1998).
However, recently the polymerase chain reaction (PCR) has appeared as a quick and sensitive method of diagnosis of MDV in a variety of tissues and materials (Handberg et al., 2001). Real-time polymerase chain reaction (RT-PCR) assays were developed simultaneously to measure MDV genome number in chicken tissues and environmental samples (Baigent et al., 2006; Islam et al., 2004). The pullet flock investigated in this study was vaccinated earlier against MD but the flock had lymphoproliferative lesions and suspected as a case of MD. The extensive clinical signs, gross and histopathology and detection of Meq gene of MDV by polymerase chain reaction was, therefore, carried out to identify the cause of illness and death of infected chicken.

**MATERIALS AND METHODS**

**Disease investigation**

This investigation was carried out in the Department of Pathology, Faculty of Veterinary Science, Bangladesh Agricultural University (BAU), Mymensingh. A pullet farm of Cox’s bazar district had 2200 chickens that showed progressive weight loss, higher rate of morbidity and mortality. There was paralysis of legs and wings and the disease was suspected as a case of MD. Extensive investigation was, therefore, carried out for confirmatory detection of the specific cause of illness. A total of 10 pullets were investigated and samples were collected at necropsy during May 2016. Systemic dissection and analysis were carried out on to the liver, sciatic nerve, feather follicles, intestine, skeletal muscles, kidneys, spleen, heart, lungs, and skin to unveil the cause of illness.

**Histology of tissue sections**

The collected tissue samples were preserved in 10% buffered neutral formalin and processed for histopathological investigation. Thin sections of liver, spleen, heart, kidney, skeletal muscle, feather follicles, sciatic nerve, intestine and lungs were embedded in paraffin, sectioned at 4-5µm using a microtome, deparaffinized in xylene and stained with H&E (Luna, 1968). The stained sections on to the slides were mounted using DPX, air dried and studied under low and high power microscopic fields.

**Giemsa staining of smears**

The liver of sick and dead pullets was cut into fine pieces, soaked with paper towel and impression smears were made onto the clean slides. The slides were air dried, fixed in ice cool absolute methanol for 45 mins. Smears were also taken from the venous blood onto clean slides, fixed for 45mins in ice cool methanol and air dried. The slides were stained with Giemsa’s for 45 mins (Luna, 1968), washed in distilled water, air dried and examined under 100x microscopic field. Special emphasis was given to identify the type of mononuclear cells distributed in the smears.
Detection of Meq gene of MDV by PCR

Sections of liver, spleen, heart, kidney, skeletal muscle, feather follicles, sciatic nerve, intestine and lungs were preserved at -20°C. The tissues were used to extract genomic DNA and PCR detection of Meq gene of MD viruses. Traditional method was used to extract DNA (N=10) from tissues of suspected pullets. Briefly, about 200 mg of individual tissue was macerated on sterile mortar and pestle while still in frozen. The crushed samples were transferred into the eppendorf tube containing 600μl cell lysis buffer and incubated at 56°C for an hour. The tissue suspension was then centrifuged at 5000g for 10mins. The supernatant was collected in a fresh tube, 3μl of RNase solution was added to the nuclear lysate and mixed by inverting the tube 2-5 times. The mixture was incubated for 25mins at 37°C. Equal volume of phenol chloroform isoamyl alcohol (25:24:1) was added and vortexed vigorously for 20 seconds. The mixture was centrifuged at 10000g for 2mins and supernatant was collected. Then 1/10th volume of 0.5N NaCl and 2.5 times ice cool absolute ethanol was added and incubated on ice for 30mins. The solution was centrifuged at 13000g for 15 mins at 4°C and the supernatant was carefully discarded. The pellet was desalted twice with 70% ethanol by centrifugation at 13000g for 15 mins at room temperature. The ethanol was carefully aspirated, the DNA pellet was air dried and 50μl nuclease free water was added. The purity and concentration of extracted DNA was measured by using agarose gel (1.5%) electrophoresis and spectrophotometry ($A_{260}/A_{280}$). The concentration of genomic DNA (100ng/μl) was adjusted by adding nuclease free water. Ratio of $A_{260}$ and $A_{280}$ greater than 1.8 was considered as high purity and used in PCR. Appropriate primer sequences for the PCR detection of Meq gene of MD viruses (Table 1) were used as described previously.

Table 1. Primers and their sequences used to identify the Meq gene of MD viruses

<table>
<thead>
<tr>
<th>Primers name</th>
<th>Primer sequence (5'-3')</th>
<th>Amplicon size (bp) specific for MD viral strains</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oligo1F</td>
<td>tgcatgaaagttgag</td>
<td>185bp= JM/102W</td>
<td>(Silva et al., 1992)</td>
</tr>
<tr>
<td>Oligo1R</td>
<td>gagaatcctgagaaacg</td>
<td>317bp= GA and 449bp= Mdl8 strain</td>
<td></td>
</tr>
</tbody>
</table>

PCR reactions was performed on each DNA sample in a 25μl volume consisting of reaction mixture (12.5μl), primers (forwards and reverse, 25 pmol/1μl each), template DNA (5μl) and nuclease free H2O (5.5μl). The thermal profile consisted of an initial denaturation for 2 mins at 94°C followed by 35 cycles of DNA amplification reaction in a Master Cycler (Master Cycler Gradient, Eppendorf, Germany). The condition of PCR amplifications were denaturation for 60secs at 94°C, annealing for 60secs at 55°C and extension for 3mins at 72°C followed by a final extension for 5mins at
72°C. The PCR reactions were held at 4°C and the reaction was terminated by adding 3µl 50mM EDTA. The PCR amplicons were analyzed by electrophoresis in 1.5% agarose gel, stained with ethidium bromide and examined under UV light using an image documentation system (Cell Biosciences, Alphalmager HP, USA).

RESULTS

A pullet farm at Ramu Upazilla, Cox’s bazar, Bangladesh showed progressive weight loss, wing and leg paralysis. Out of 2200 pullet in the farm, more than 35% chickens appeared ill during the age of 45-58 days, with a morbidity to mortality rate of 3-4% per day. About 1000 birds were fallen into chronic sickness during the investigation and 10 sick birds submitted to the Department of Pathology were examined for the clinical signs, pathology, impression smear staining and PCR detection of the specific cause of illness.

Examination of clinical signs

All of the pullet submitted for necropsy showed either wing or leg paralysis or both (Figure 1). Clinical signs typically observed were depression or unwilling to move, leg dragging/knuckling, drooping of wings, mild opisthotonous and wing and leg paralysis. The characteristics leg paralysis (one leg stretched forward and the other backward) typically involved in Marek’s diseases (MD) of chickens was not seen but the leg of infected pullet showed paralysis of its toes (Figure 1a). The flaccid paralysis or paresis of necks and wings were commonly observed. Upon examination of body condition, the birds appeared much thinner with a prominent keel bone (Figure 1b).

Figure 1. Infected pullets showed paralysis of toe and wings (a), severe atrophy of muscle (b, prominent kill bone) and enlargement of liver (c).

Necropsy findings

The pullet at necropsy showed very thin musculature and prominent keel bone (Figure 1b). The sciatic (Figure 2a) and brachial nerves were enlarged, the nerve appeared two or three times larger than normal thickness. The infected sciatic nerve
lost their striation and dull in appearance. Sciatic nerve enlargement was seen in five out of eight chickens necropsied. The liver (Figure 1c and 2d), kidney (Figure 2b) and spleen (Figure 2c) were enlarged with grayish discoloration. Lymphomas as seen in the classical form of MD, was seen as small, soft, grey tumors in the kidney and liver. The feather follicles of infected chickens were severely congested and appeared prominent. Characteristics nodular lesions and enlargement of liver, kidney and spleen of infected chickens were variably noted.

![Figure 2](image)

**Figure 2.** Sciatic nerve of infected pullet appeared distended (a, arrow). There was nodular outgrowth in kidney (b). The splenomegaly (c) and hepatomegaly (d) were observed in 50-60% of infected chickens.

**Histopathologic investigation of affected tissues**

Following histopathological examination, lymphocytic infiltration was seen in all of the tissues examined. The feather follicles (Figure 3a), subcutaneous tissues (Figure 3b) and cutaneous muscle (Figure 3c) found infiltrating with mononuclear cells. The sciatic nerve showed demyelination and infiltration of lymphocytes (Figure 5b). Lymphocytic infiltration and accumulation was commonly seen in the lungs (4a), kidney (4b), liver (Figure 4c), gizzard, feather follicles, and heart muscle. The proliferating and infiltrating cells were consisted mostly of lymphoblasts (large, medium and smaller type) with fewer macrophages. The nuclei of lymphocytes were much darker and appeared to be neoplastic. The lympho proliferative nature of the disease was further examined by studying smears of blood and liver smears stained with Giemsa’s.

![Figure 3](image)

**Figure 3.** Section of skin from infected pullet stained with H&E staining. Lymphocytic infiltration and accumulation were seen in feather follicles (a, circle, 10x), subcutaneous tissues (b, circle, 10x) and in cutaneous muscle (c, circle, 40x).
Figure 4. Sections of lungs (a), kidney (b) and liver (c) obtained from infected pullet and stained with H&E (10x). There was infiltration and aggregation of lymphocytes in the sections of lungs (a, circle), kidney (b, circle) and liver (c, arrow) as seen under microscopic fields.

**Smears staining from the liver and blood**

Giemsa’s staining is a classic blood film and impression smear staining technique, identify blood cells distinctly. Giemsa’s staining of impression smear from liver (Figure 5b) and blood showed infiltration of polymorphic mononuclear cells predominantly lymphocytes. The nuclei of proliferating lymphocytes appeared much darker and thought to be neoplastic in nature but the actual etiology was not confirmed. The lymphoproliferative nature of the disease was, further confirmed by using polymerase chain reaction (PCR).

Figure 5. Section of nerve (a) stained with H&E showed demyelination and infiltration of lymphocytes (10x). Impression smears prepared from the liver (b, 40x) and stained with Giemsa’s stain showed huge infiltration of lymphocytes (b, black arrow). DNA extracted from the liver, spleen, heart, kidney, skeletal muscle, feather follicles, sciatic nerve, intestine and lungs were used in PCR amplification of Meq gene of MD viruses. The lane L is for 100bp ladder, PC is for positive control, NC is for negative control and lane 1 to 10 are for organ specific detection of MD. A single band of PCR amplicon (317bp) was found to generate with the DNA extracted from the known positive liver (PC), infected liver (lane 10), spleen (lane 9), kidney (lane 7), feather follicles (lane 5) and lungs (lane 1).

**PCR detection of MD virus**

PCR amplification of the Meq gene of serotype 1 MDV was performed with the LAMP F3 and B3 primers and generated a 317bp amplicon in gel electrophoresis (Figure 5c). DNA from the liver, spleen, heart, kidney, skeletal muscle, feather
follicles, sciatic nerve, intestine and lungs were used in PCR. The fragment of Meq gene was amplified from the liver, spleen, kidney, feather follicles, and lungs suggested the infectivity due to MDV-1.

**DISCUSSION**

The causative agent of MD is MDV, which is ubiquitous, globally distributed and very common in commercial poultry flocks with an estimated cost to the industry more than US$ 1-2 billion (Baigent et al., 2007; Morrow and Fehler 2004). Domestic fowls are the natural host of the MDV, but quail, geese and turkey are also affected. In this study infectivity of pullet due to Marek’s disease was investigated by clinical, necropsy, histopathology and PCR means and identifies causes of illness and death.

**Investigation of outbreak**

The infected birds at early age showed progressive emaciation, in-appetence, leg and wing paralysis. Marek’s disease (MD) is a chronic infection and elderly chickens are mostly affected. The nature of MD has changed since the disease was first described. The classical form of MD was seen in elder chickens and was known as “fowl paralysis”. It involved leg and wing paralysis often with a typical appearance of one leg stretched forward and another leg backward but this was absent in this study. The toes appeared curling; the legs were unable to carry body weight and put down on to the ground by taking support from her ventral surface of the body. The incomplete unilateral or bilateral wing paralysis was also observed. The flaccid paralysis of neck was observed. The paralysis of neck, legs and wings could be due to lymphocytic infiltration of peripheral nerves, spinal cord and associated ganglia (Marek, 1907) with myelin degeneration. Mortality seen was generally low, about 3-5% birds (20-25 birds/ day) found dying every day in the pullet farm. The nature of infectivity as seen in the young and infected pullet indicated the infectivity due to very virulent or very virulent plus MD viruses (Afonso et al., 2001). The flocks were vaccinated against MD viruses but still the pullet had severe forms of MD.

**Necropsy and histopathology**

Marek's disease is characterized by T cell lymphomas and infiltration of nerves and visceral organs by lymphocytes (Angamuthu et al., 2012). Following natural infection, microscopic lesions were observed after one to two weeks, but gross lesions were observed after three to four weeks of infection (Abreu et al., 2016). The pullet showed signs of infectivity at the age of 45 and onwards, hence they may catch the infection at early age (25-30 days of age). The birds at necropsy showed thin musculature, prominent keel bone, congested and inflamed feather follicles, enlarged kidneys, hepatomegaly, splenomegaly, enlargement of sciatic and brachial nerve. The cross striation was absence in the enlarged nerve. The lungs appeared congested and consolidated. Out of 10pullet necropsied tumor growth was seen in the liver (N=06), spleen (N=04), kidney (N=05) and proventriculous (N=02). Combined splenomegaly and hepatomegaly was seen in three cases. Enlargement of sciatic nerve was seen in
five cases. A congested and edematous feather follicle was seen in three cases. Out of 10 birds examined, typical lesions suggestive for lympho-proliferative disease were observed in four cases. However, the lesions observed at necropsy such as enlargement of liver, spleen, kidney, nerve were not consistent in all cases. This disease was suspected as a case of MD and the nature of cells infiltrated in visceral organs were further evaluated by using H&E staining of tissues sections and Giemsa’s staining of smears.

**Staining of sections and smears**

Staining of tissue sections and smears contribute better information about the cellular type involved in lympho-proliferative disease of chicken and to support diagnosis. Sections of visceral organs stained with H&E and yielded specific morphology of infiltrated cells. Massive infiltration and accumulation of lymphocytes was seen in lungs, liver, kidney, spleen, nerves, skins and muscles etc. Smears from the liver and blood stained with Giemsa’s showed highest densities of lymphocytes followed by macrophages and plasma cells.

Lymphomas in the visceral organs and nerve as seen cytologically were mixed cell types; consisting of small and medium lymphocytes, but in some lesions large lymphocytes and lymphoblasts was also seen. The heterogeneous population of lymphoid cells as seen in H&E stained sections and in impression smears stained with Giemsa’s, showed an important feature in differentiating the disease from lymphoid leukosis; in which the lymphomatous infiltrations are composed of uniform lymphoblasts. In lymphoid leukosis, gross lymphomas occur in the bursa of Fabricius, and the tumor has an intra-follicular in origin (Barrow et al., 1999). In MD, although the bursa is sometimes involved in the lympho-proliferation, the tumor is less apparent, diffuse and inter-follicular in location. Peripheral nerve lesions are not a feature of lymphoid leukosis but the lesion is predominated in MD (Renz et al., 2012).

**Specific detection of MD by using PCR**

Genes which are unique for MDV-1 strains have been used in attempt to identify and pathotype MDV-1 strains by Using PCR. One major gene of interest is the Meq (MDV EcoRI-Q) gene. The Meq gene is constantly expressed through latency and in all tumors (Kung et al., 2001; Liu et al., 1999). Meq gene has got an anti-apoptotic function and the potential to stimulate cell growth and transformation by MDV-1 (Liu et al., 1999; Renz et al., 2012). In this study PCR amplification was carried out targeting fragment of Meq gene with the DNA extracted from liver, spleen, heart, kidney, skeletal muscle, feather follicles, sciatic nerve, intestine and lungs of infected pullets. DNA extracted from the liver, spleen, kidney, feather follicles and lungs found to generate expected (317bp) amplicon.

In this study a PCR was adapted using published primers (Silva et al., 1992). The PCR primers were designed from the flanking region of MDV1. The amplified band
(132bp repeat) generated from the flanking region and 185bp, 317bp and 449bp were specific for pathogenic strain of JM/102W, Md/5 (attenuated MDV1 GA strain) and Mdll/8 viruses, respectively. In this study the amplicon generated was 317bp, suggestive for infectivity with Md/5 (MDV1 GA strain) viruses. The Marek’s disease virus involved with the disease processes was belonging to very virulent strain (OIE 2010) as the PCR detected only 317bp fragment. The attenuated or avirulent strains of MDV1 usually generated more than one band (185bp repeat) in PCR but which was not seen in this study, so the viruses detected were belonging to virulent group. The Md/5 or MDV1 (GA strain) is a very virulent strain of MD virus (OIE, 2010) the viruses also causes higher rate of morbidity and mortality. To enable systematic virulence classification of MDV isolates on the basis of the pathology (Renz et al., 2012) they induce, and their ability to overcome the effects of vaccination, a standardized MDV pathotyping scheme was developed by the USDA ADOL may be used. Virulent MDV1 challenge strains were graded according to the protection induced by HVT and HVT/ MDV2 bivalent vaccines (Witter 1997; Witter et al., 2005). While MD vaccines reduce virus replication and prevent tumor formation, they do not induce sterilizing immunity; vaccinated chickens are still susceptible to infection with pathogenic strains of MDV that replicate in the host and shed into the environment in the absence of clinical disease. The inability of MDV vaccines to prevent virus replication following field challenge (‘‘imperfect vaccination’’) is thought to have contributed to the worldwide increase in the virulence of MDV over the last 40 to 50 years, as has been postulated as a probable cause of change for other pathogens (Gandon et al., 2001; Renz et al., 2012). This is for the first time in Bangladesh reported the occurrence of very virulent Md/5 (MDV1 GA strain) infectivity in pullet with higher rate of morbidity and mortality. Until 2010 (OIE 2010), the viruses were not approved by the USDA to use commercially in poultry farms. The Md/5 strain of viral vaccine may be used in our poultry industry somewhere in the globe and thus the virus may have introduced in the pullet farms. Due to limitation of research resources such virulent or pathotyping (Witter et al., 2005) was not carried out in this study.

CONCLUSION

The pullet in the farm was vaccinated against MDV but still had the clinical outbreaks and mortality due to MD. The etiology of the disease was thought to be due to MDV1GA (Md/5) strain of MDV. This PCR protocol used can be a suitable tool to selectively identify various strains of MSV in a reaction. It needs to isolate the viruses in culture, identify pathotype in vivo and evaluate protective efficacy of the existing viral vaccines against circulating viruses.

ACKNOWLEDGEMENT

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REFERENCES


FORMULATION OF VALUE ADDED CHICKEN MEATBALL WITH DIFFERENT LEVEL OF WHEAT FLOUR

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ABSTRACT

The present study was undertaken to evaluate the effect of different levels of wheat flour on the quality characteristics of chicken meatball. Wheat flour which acts as a binding agent of meatball except for control group T₁. The meatballs were formulated having 0%, 5%, 10% and 15% wheat flour. The sensory (colour, flavour, texture, juiciness, tenderness, overall acceptability), physicochemical (proximate analysis, pH, cooking loss), biochemical (TBARs, POV, FFA) were analyzed. Treatments were analyzed in a 4×3 factorial experiment in CRD replicated three times per cell. Wheat flour inclusion in meatballs increased cooking yield by reducing weight loss from 27.06 to 26.49%. Among four treatments most preferable colour, odour, tenderness, juiciness was observed significantly (p<0.05) at 15% wheat flour group and the less preferable colour was observed from the control group. The preferable colour was observed at 0 days and less preferable colour at 30 day. Meatballs made with the addition of 15% wheat flour had the highest tenderness, overall acceptability, raw pH, cooked pH and lower DM, ash, PV and TBA & showed significant value (p<0.05) The cooked pH was decreased with the increased storage period. Meatballs with wheat flour inclusions at 15% were most acceptable. It is recommended that further studies of the wheat flour inclusion in meatballs production be carried out to ensure the availability of cheaper, nutritious and acceptable convenience food in the Bangladeshi market.

Keywords: Meatballs, chicken, wheat flour, fibre and prebiotics.
INTRODUCTION

Chicken meat has gained much popularity among consumers and the consumption rate of chicken meat and chicken meat products is increasing day by day throughout the world. Among the different meat products, the meatball is one of the tasty and popular food's item. Meatballs can be made with beef, lamb, veal, pork, turkey, chicken and even offal. Meatball is a small ball of chopped or ground meat often mixed with bread crumbs and spices. For economic benefits, the substitution of beef in meatball with the meat of lower price such as chicken takes place frequently. The addition of fat replacers may lead to decreased energy value and cholesterol contents. Fat contributes key sensory benefits to foods and is perceived through mouthfeel, taste, and aroma/odour (Sampaio et al., 2004). Therefore, the reduction of fat content may have a large effect on the quality attributes of meat products such as colour, flavour, texture and binding properties. For this reason, ideal sources of fat replacers are needed to improve the functional value of meat products. Non-meat ingredients play a significant role in the modification of functional properties such as emulsification, and water and fat binding capacity, which may impact the textural properties. In the past, starch was added as a source of carbohydrates and to thicken the texture of meatballs by Huda et al. (2009). Today, starch is extensively used as a stabilizer, texturizer, water or fat binder and emulsifier. Apart from these functions, starch can also increase the gel strength and freeze-thaw stability of meatballs if added to appropriate levels by Serdaroglu et al. (2005). The aims of this study were to investigate the suitable level of wheat flour adding and its effects on the proximate composition, physicochemical properties and sensory qualities of chicken meatballs.

MATERIALS AND METHODS

Sample preparation

Boneless broiler meat of 2.5 kg from the freshly slaughtered chicken was collected from Bangladesh Agricultural University Poultry Farm, Mymensingh. The meat was ground properly and the spices, garam masala, salt, Ice flakes, refined vegetable oil, refined wheat flour, the sauce was mixed with the ground meat properly as per experimental design. There were four treatment groups are treated as a T_1-control group (no flour), T_2- 5% flour, T_3-10% flour, and T_4-15% flour. Then meatball of proper shape was prepared separately. It was then boiled in hot water for 2-3 minutes. Then the water was removed from the meatball properly and was fried in hot oil until the reddish brown colour was obtained.

Sensory evaluation

Sensory evaluation was carried out in individual booths under controlled conditions of light, temperature and humidity. Prior to sample evaluation, all panellists participated in orientation sessions to familiarize with the scale attributes (colour, smell, juiciness, tenderness, overall acceptability) of meatball using an intensity scale. Sensory qualities of the samples were evaluated after thawing of before cook.
and after cook using a 5-point scoring method. Sensory scores were 5 for excellent, 4 for very good, 3 for good, 2 for fair and 1 for poor. Sensory evaluation was accomplished at 0-day and repeated at 15, 30 and 60 day; up to the end of refrigerated storage at -20±1°C.

**Proximate composition**

A proximate composition such as dry matter (DM), ether extract (EE), crude protein (CP) and ash was carried out according to the methods (AOAC, 2005).

**Biochemical analysis**

Three types of biochemical analysis were practised in this research. The free fatty acid value was determined according to Rukunudin et al. (1998). Peroxide value (POV) was determined according to Sallam et al. (2004). Lipid oxidation was assessed in triplicate using the 2-thiobarbituric acid (TBA) method described by Schmedes and Holmer (1989).

**Physicochemical properties measurement**

pH value of raw and cooked meatball was measured using pH meter from raw meatball homogenate. The homogenate was prepared by blending 5g of meat with 10 ml distilled water. Cooking loss of chicken meatball also determined.

**Statistical model and analysis**

The proposed model for the planned experiment was a factorial experiment with two factors A(Treatments) and B(Days of Intervals)
is:

\[ y_{ijk} = \mu + A_i + B_j + (AB)_{ij} + \varepsilon_{ijkl} = 1, \ldots, a; j = 1, \ldots, b; k = 1, \ldots, n \]

Where,

- \( y_{ijk} \) = observation \( k \) in level \( i \) of factor A and level \( j \) of factor B
- \( \mu \) = the overall mean
- \( A_i \) = the effect of level \( i \) of factor A
- \( B_j \) = the effect of level \( j \) of factor B

Data were statistically analyzed using SAS Statistical Discovery software, NC, USA. DMRT test was used to determine the significance of differences among treatments means.

**RESULTS AND DISCUSSION**

**Sensory evaluation**

Mean scores for different sensory attributes obtained from the sensory evaluation are shown in table 1. The preferable colour was observed at 0-day. The decreased colour test scores during storage resulted from the denaturation of proteins, particularly the myofibrillar protein that affects gel formation. Among four treatments significantly (p<0.05) higher colour score was observed in 15% wheat flour group than other
treatments which are collaborating with the finding of Naveena et al. (2008). Preferable good flavour was observed in 15% wheat flour group and the quality was deteriorated with increased storage period. Among these four treatments most preferable tenderness was observed at 15% wheat flour group at 0-day which is similar with the findings of Ali and Zahran (2010) also reported that supplementation improved chicken meat tenderness during storage. Ngapo et al. (2004) stated that juiciness as an indicator of meatballs freshness or even eating quality. The range of overall observed juiciness score at different treatments was 4.11 to 4.56. The preferable acceptability was observed at 0 day. The data show that the lowest test score was reduced to 3.83 in all treatments after 30 days of storage.

Table 1. Effect of wheat flour on sensory parameters in chicken meatballs

<table>
<thead>
<tr>
<th>Parameters</th>
<th>DI</th>
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<td>T3</td>
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Column means having different superscript varies significantly at values p<0.05. Again, mean values having the same superscript in each row did not differ significantly at p>0.05. T1=Control group, T2=05% wheat flour group, T3= 10% wheat flour group, T4=15% wheat flour group, DI=Day Intervals, Treat= Treatment, TxDI=Interaction of Treatment and Day Intervals.
Proximate analysis

The dry matter content was increased with the increased storage period because moisture loss was decreased with the storage period (Table 2). Similar results were reported for Indonesian traditional meatballs with a dry matter content ranged from 56.17 to 60.32% mentioned by Purnomo and Rahardiyan (2008). The data showed that the highest amount of CP content was 20.70 in all treatments at 0 days of storage and the lowest amount of CP content was found after 30 days of storage. The protein result was lower compared to the protein content of Indonesian beef meatballs which ranged from 13.38 to 14.44% reported by Purnomo and Rahardiyan et.al. (2008). The highest amount of EE content was increased to 8.52% in all treatments after 0 days of storage. The Malaysian Food Regulation of 1985 stated that manufactured meat should not contain more than 30% fat. Malaysian beef meatballs can be classified as low-fat meatballs since the fat content ranges from 1.69 to 11.09%. The ash content was significantly changed with the increased storage period. The data show that the highest amount of Ash content was increased to 1.35% in all treatments after 60 days of storage.

Table 2. Effects of wheat flour on proximate components in chicken meatballs

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<td>T₃</td>
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Column means having different superscripts varies significantly at values p<0.05. Again, mean values having the same superscript in each row did not differ significantly at p>0.05. T₁=Control group, T₂= 05% wheat flour group, T₃= 10% wheat flour group, T₄= 15% wheat flour group, DI=Day Intervals, Treat= Treatment, T×DI=Interaction of Treatment and Day Intervals.
Physicochemical properties

The raw and cooked meat mean pH was decreased with the increased storage period. Table 3 showed a slight decrease in the raw meat pH values and an increase in the acidity values for all samples along with storage time during the 60 days of storage as a result of the increase of free fatty acids due to rancidity. The preferable cooked meat pH content was observed from 0-day and less preferable cooked pH was observed from 60 days observation. These results are similar to those of Sallam et al. (2004) who reported that storage time had a significant (p<0.05) effect on pH values, which tended to increase with storage time. The range of overall observed cooking loss at different treatments was 27.42 to 26.45%. The cooking loss was decreased with the increased storage period. Major components of cooking losses are thawing, dripping and evaporation. The cooking loss in meat cuts is important for maintaining an attractive retail display of meat. The values of cooking yield were similar to the results in high-fat Kung-wan meatballs reported by Huang et al. (2005).

Table 3. Effect of wheat flour on physicochemical parameters in chicken meatballs

<table>
<thead>
<tr>
<th>Parameters</th>
<th>DI</th>
<th>Treatments</th>
<th>Mean</th>
<th>Level of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>T1</td>
<td>T2</td>
<td>T3</td>
</tr>
<tr>
<td>Raw pH</td>
<td>0</td>
<td>5.95±0.04</td>
<td>5.94±0.04</td>
<td>5.89±0.07</td>
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<tr>
<td></td>
<td>30</td>
<td>5.70±0.14</td>
<td>5.89±0.17</td>
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<tr>
<td></td>
<td>60</td>
<td>5.84±0.17</td>
<td>5.41±0.18</td>
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<tr>
<td>Mean</td>
<td>0</td>
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<td>6.06±0.03</td>
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<tr>
<td></td>
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<td>6.02±0.01</td>
<td>6.11±0.06</td>
<td>6.02±0.01</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>6.05±0.05</td>
<td>6.02±0.01</td>
<td>6.07±0.02</td>
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<tr>
<td>Cooked pH</td>
<td>0</td>
<td>6.04±0.02</td>
<td>6.06±0.03</td>
<td>6.04±0.02</td>
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<tr>
<td></td>
<td>30</td>
<td>6.24±0.15</td>
<td>6.37±0.09</td>
<td>28.04±0.13</td>
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<tr>
<td></td>
<td>60</td>
<td>27.10±0.29</td>
<td>27.10±0.40</td>
<td>27.02±0.06</td>
</tr>
<tr>
<td>Cooking Loss (%)</td>
<td>0</td>
<td>26.37±0.47</td>
<td>26.15±0.06</td>
<td>27.20±0.16</td>
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<tr>
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<td>30</td>
<td>26.10±0.29</td>
<td>27.10±0.40</td>
<td>27.02±0.06</td>
</tr>
<tr>
<td>Mean</td>
<td>26.90±0.30</td>
<td>26.45±0.18</td>
<td>27.42±0.12</td>
<td>26.63±0.12</td>
</tr>
</tbody>
</table>

Column mean value in each row having different superscript varies significantly at values p<0.05. Again, mean values having the same superscript in each row did not differ significantly at p>0.05. T1=Control group, T2= 05% wheat flour group, T3= 10% wheat flour group, T4= 15% wheat flour group, DI=Day Intervals, Treat= Treatment, T×DI=Interaction of Treatment and Day Intervals.

Biochemical properties

The number of FFA increased with storage time (Table 4). The range of overall observed of different days of intervals of FFA was 0.33 to 0.34. The FFA value was increased with storage period. The range of overall observed of different days of
intervals of peroxide value was 3.56 to 3.70. Rhee and Myers (2003) examined peroxide values in plain meatloaf made from ground goat meat and reported a similar trend in peroxide value during storage. The number of peroxide values detected in the samples increased. Generally, TBARs levels significantly (p<0.05) increased with storage time, showing decreasing shelf life. The range of overall observed of different days of intervals of TBARs value was 0.10 to 0.12. The control sample, without any added antioxidants, showed a higher level of TBA than samples treated with 05%, 10%, 15% wheat flour. The TBA value on day 60 was 0.10 for the control samples, 0.11, 0.11, 0.11 for those treated with 05%, 10%, and 15% wheat flour. The different superscript was observed from four treatment groups indicates there were significant differences of TBA value among these four treatments. Racanici et al. (2004) also found increasing thiobarbituric acid reactive substances (TBARS) as storage time increased to 8 days.

Table 4. Effect of wheat flour on biochemical parameters in chicken meatballs

<table>
<thead>
<tr>
<th>Parameters</th>
<th>DI</th>
<th>Treatments</th>
<th>Mean</th>
<th>Level of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>T1</td>
<td>T2</td>
<td>T3</td>
</tr>
<tr>
<td>FFA (%)</td>
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<td>0.33±0.01</td>
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<tr>
<td>Mean</td>
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<tr>
<td></td>
<td>30</td>
<td>3.70±0.10</td>
<td>3.66±0.16</td>
<td>3.08±0.08</td>
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<tr>
<td></td>
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<td>3.84±0.07</td>
<td>3.58±0.20</td>
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<tr>
<td>Mean</td>
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<td>3.69±0.17</td>
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<td>0.11±0.00</td>
</tr>
<tr>
<td></td>
<td>60</td>
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<tr>
<td>Mean</td>
<td>0</td>
<td>0.10±0.00</td>
<td>0.11±0.00</td>
<td>0.11±0.00</td>
</tr>
</tbody>
</table>

Column mean value having different superscript varies significantly at values p<0.05. Again, mean values having the same superscript in each row did not differ significantly at p>0.05. T1=Control group, T2=05% wheat flour group, T3=10% wheat flour group, T4=15% wheat flour group, DI=Day Intervals, Treat=Treatment, TxDI=Interaction of Treatment and Day Intervals.

CONCLUSION

The highest amount of dry matter content indicates this product is less preferable but the highest amount of CP content indicates this product is more preferable. Among four treatments most preferable colour, odour, tenderness, juiciness was observed at 15% wheat flour group. Therefore, it can be concluded that the 15% of wheat flour could be used for the production of meatball.
ACKNOWLEDGEMENT

We would like to express our appreciation to the Bangladesh Agricultural University Research System (BAURES) for funding this research and their encouragement.

REFERENCES


NUTRITIONAL VALUES OF MINOR CARPS

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ABSTRACT

The nutrient profile of five carps, viz. Labeo bata, Labeo calbasu, Labeo fimbriatus, Cirrhinus reba and Puntius javanicus were studied. The samples were collected from different geographical locations of West Bengal, Odisha and Karnataka states of India. The data on proximate composition reveal that the moisture and fat content differed significantly (P<0.01) among the carp species. The fat content is significantly (P<0.01) higher in P. javanicus, L. bata and L. calbasu compared to L. fimbriatus. However, the protein and ash content did not differ significantly among the carp species. The potassium and copper contents differed significantly (P<0.01) among the fish species. Both potassium and copper contents were significantly higher in L. bata. The calcium content was maximum in L. fimbriatus. The saturated fatty acid (SFA), mono unsaturated fatty acid (MUFA) and poly unsaturated fatty acid (PUFA) differed significantly (P<0.01) among all the carp species. The palmitic acid was significantly higher in L. fimbriatus, which is the predominant SFA. Among MUFA, the oleic acid was significantly higher in P. javanicus. The total MUFA was significantly (P<0.05) higher in C. reba. eicosapentaenoic acid (EPA) and total PUFA is significantly higher in L. bata. Among the essential amino acids, methionine was maximum in L. fimbriatus, P. javanicus and L. bata whereas in case of non-essential amino acids, the glutamic acid and aspartic acid were high in C. reba and L. calbasu. The gross energy content of the fish was higher in L. fimbriatus followed by C. reba and L. bata. The nutrient profile of these fish species reveal that they were rich in essential nutrients required for human health.

Keywords: Proximate composition, fatty acid profile, vitamin, mineral, amino acid composition, freshwater carps.

INTRODUCTION

Balanced human diet should meet the requirements for energy and nutritive components including essentials fatty acids, amino acids, proteins, fat, minerals and

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vitamins. Fish is an excellent food for human beings for centuries (Ayoola, 2010) and is preferred as a balanced diet not only due to its taste and quick digestibility rate but also because of having good proportions of essential amino acids, vitamins and essential fatty acids for the formation of functional and structural proteins (Kumar, 1992). The high nutritional value of fish is mainly related to their readily digestible proteins which are an excellent source of EAA (Sanchez-Alonso et al., 2007, helping in protein synthesis in human beings (Limin et al., 2006). Meat has been accepted as a good source of protein in almost all parts of the world, specially the Western countries. But this leads to some major human health problems regarding overweight and cardiovascular diseases in the developed countries (Das et al., 2009; Giri et al., 2010 and Mohanty et al., 2016). Hence, for the last couple of decades, people have become more aware of fish as a health food alternative to meat. Intake of the saturated fat in red meat is one of the main causes of cardiovascular diseases, while the unsaturated fat of fish and vegetables does not have this type of health hazards.

Consumption of fish also provides a range of essential nutrients besides energy. Flesh texture, protein and fat composition are usually the main factors that determine consumer acceptance (Pal and Ghosh, 2013). Fish has got a particular role as a source of the long-chain omega-3 fatty acids viz. eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which is important for optimal brain and neural system development in children (Giri et al., 2010; Paul et al., 2015a and Mohanty et al., 2016). The n-3 PUFAs, especially the eicosapentaenoic acid (EPA and docosahexaenoic acid (DHA) are found in high concentrations in the phosphoglycerides of cellular membranes, and DHA is particularly abundant in the retina and brain, where it has a crucial role in maintaining the structure and function of the excitable membranes of these tissues (Lauritzen et al., 2001). Arachidonic acid (AA), a PUFA of the n-6 series, is a precursor of biologically important products, such as epoxides, AA-ethanolamide, anandamide and iso-prostanoids, an isomer of prostaglandins (Galli and Marangoni, 1997).

Fish protein occupies an important position in human nutrition (Nargis, 2006). Fish accounts for 16.7% of the global population’s intake of animal protein and it accounts for 6.5% of total protein intake. A portion of 150g of fish can provide about 50-60 percent of an adult’s daily protein requirement (FAO, 2014). Awareness about the importance of diet in human health is increasing day by day. Through research over the years, it is now proved that many of the diseases and health problems of people today are due to wrong lifestyle, characterized by wrong diet. When we think about a balanced diet, fish along with cereals is a good combination. The nutrient profile of freshwater fish is very important because it provides useful information to the nutritionists with readily available sources of low fat and high protein content with finest quality of flavour and texture and safety for the consumers. The diversity in nutrient content of fish species and in particular the rich nutrient composition of small indigenous species would guide the nutritional security (Jabeen and Chaudhary, 2011).
Though some information is available on nutrient compositor of Indian Major Carp (Paul et al., 2015a, and 2016), Catfish (Paul et al., 2015) and air breathing fish (Paul et al., 2017). Keeping in view of the importance of eating fish as health food; the nutrient profile of five freshwater carp species viz. Labeobata, Labeocalbasu, Labeofimbriatus, Cirrhinus reba and Pantius javanicus have been studied to document the information of protein, fat, minerals, amino acids, fatty acids and along with some vitamins. These indigenous fish species are particularly available in the eastern and North Eastern states of India. Thus, a database can be developed on the basis of nutrient profile of these fish that would help the dieticians, nutritionists, researchers, fish farmers and related stake holders, policy makers to take decision, not only on manufacturing and value addition of fish food products but also for consumer guidance and to promote fish as health food.

**MATERIALS AND METHODS**

**Collection of samples**

The samples were collected from various places of different states viz. West Bengal, Odisha and Karnataka. The collection points were mainly at the place of harvest and fish market. From West Bengal the places of collection were Rahara Fish Farm of ICAR-CIFA; Barackpore; Doperia (Khardah), Malancha, Barasat, Bongaon, Basirhat, Kharibari, Sasan, Baranagar, Nilgaunge, Naihati are from the North 24 Paraganas district; Bali form Howrah district; Taratala, Kankipara for South 24 Paraganas district; Behrampur, Lalgola from Murshidabad district; Pandua, Sererampur, Sheorahuli, Chanditala from Hooghly district; Kalyani. Chakdah, Krishnanagar from Nadia district; Mecheda, Kolaghat from East Midnapore district and Budbud from Burdwan district. From Odisha, ICAR-CIFA, Kausalyaganga and Karnataka, Hubli and ICAR-CIFA, RRC Bangalore. The length and weight ranges of collected species were i.e. 15.0-185g and 14.8-45.0 cm for L. bata, 30.0-600.0 g and 12.5-45.0 cm for L. calbasu, 250-550g and 29.8-36.5 cm for L. fimbriatus, 20-400g and 12.5-50.0 cm for C. reba and 75-350g and 18.5-40 cm for P. javanicus. The number of fish samples collected were viz. L.bata (n=52), L. calbasu (n=54), L. fimbriatus (n=11), C. reba (n=51) and P. javanicus (n=53). The present work is part outreach Activity on Nutrient Profiling of Fish, which is an ICAR network project, wherein 7 ICAR institutes are involved viz. ICAR-CIFRI, Barrackpore, ICAR-CIFA-Bhubaneswar, ICAR-DCFRR, Bhimtal, ICAR-CIBA, Chennai, ICAR-CIFT, Kochi and ICAR-CMFRI, Kochi. Under this network project there is a common methodology for sample collection, preparation and analysis; which is prepared by the partners of the Project (Sankar et al., 2010).

**Proximate and mineral composition analysis**

Proximate composition of fish tissue samples were done as per AOAC (1995). The mineral assay was done as per AOAC (2005) and Paul et al. (2014) using Atomic Absorption spectrophotometer (AAS) (Thermofisher, M Series). The data were
statistically analyzed as per Snedecor and Cochran (1968) by one-way ANOVA and the least significance difference (LSD) was used for comparison of the mean values. The energy content of fish species samples were analysed by Bomb Calorimetric Method as per the AOAC (2005).

**Fatty acid analysis**

Pooled samples were extracted for fatty acid analysis following the method of Folch et al. (1957) using chloroform: methanol (2:1, v/v) solvent system that contained 0.01% butylated hydroxyl anisole as an antioxidant. Fatty acid methyl esters (FAMEs) were prepared by the transmethylation with boron trifluoride (BF₃, Hi Media, Mumbai, India) in methanol from lipids fraction according to Metcalfe et al. (1996). The fatty acid methyl esters were quantified by injecting 1µL (50:1 split ratio) into a Gas Chromatograph (GC) (Perkin Elmer; CLARUS 480). The oven temperature was programmed from an initial temperature at 30°C rising to 140°C (hold time 4 min.) and up to 200°C. Nitrogen gas was used as a carrier gas. The injection port and the flame ionization detector were maintained at 260°C and 300°C. GC operating software “Total Chrome” was followed. Identification of individual FA was identified by comparing with retention times to those of standards (SUPELCO, Cat. No. 47885-U) and quantified by comparing with respective areas. The data are presented as Mean± S.E.

**Amino acid analysis**

The amino acid analysis was done as per the method of Ishida et al. (1981). The amino acid samples were analysed from Edward Food Research and Analysis Centre Limited, Nilgunge, Kolkata- 700121 (www.efrac.org)

**Vitamin analysis**

The fat soluble vitamins Retinol (Vitamin A) and Cholecalciferol (Vitamin D) were assayed by High Performances Liquid Chromatography. Fish tissue (30g) was grinded with anhydrous sodium sulfate and extracted the oil using 2:1 chloroform: methanol after adding BHA as antioxidants (Folch et al., 1957).The sample preparation was done as per Sankar et al. (2010) and vitamin samples were analysed from Edward Food Research and Analysis Centre Limited, Nilgunge, Kolkata- 700121 (www.efrac.org).

**RESULTS AND DISCUSSION**

The proximate composition of the five freshwater fish species viz. *L. bata*, *L. calbasu*, *L. fimbriatus*, *C. reba* and *P. javanicus* are presented in table 1. Perusal of data reveals that the moisture content was significantly (P<0.01) higher in *L. fimbriatus* and followed by *L. calbasu*. The protein content of the fish species ranges from 14.38 to 15.77 and the means did not differ significantly among the treatments. However, the fat content of the fish species differed significantly (P<0.01) among the treatments. The fat content was significantly (P<0.01) higher in *P. javanicus* and *C.*
reba; followed by L. bata and L. calbasu. The ash content of the fish species did not differ significantly among the treatment groups.

The moisture content of the fish as studied are in agreement with the moisture content of IMC reported Joseph et al. (1990) and Paul et al. (2016). The protein content of these five fish did not differ significantly among the species. The fat content of these species ranges from 1.05 to 4.71 (%). The finding of our result on fat content are higher than the fat content of IMC as reported earlier (Paul et al., 2016). The ash content in the species was similar to the earlier report of Sankar et al. (2001) and Paul et al. (2015 and 2016). The proximate composition data of this study are also in agreement with Sharma et al. (2009) and Mazumdar et al. (2008).

Table 1. Proximate composition (% as such basis) of five freshwater fish species

<table>
<thead>
<tr>
<th>Particulars</th>
<th>L. bata</th>
<th>L. calbasu</th>
<th>L. fimbriatus</th>
<th>C. reba</th>
<th>P. javanicus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture**</td>
<td>73.45±0.32^a</td>
<td>74.94±0.18^ab</td>
<td>78.51±0.27^b</td>
<td>73.40±0.23^a</td>
<td>73.61±0.40^a</td>
</tr>
<tr>
<td>Protein</td>
<td>15.64±0.27</td>
<td>14.38±0.14</td>
<td>15.77±0.31</td>
<td>15.15±0.22</td>
<td>14.85±0.21</td>
</tr>
<tr>
<td>Fat**</td>
<td>3.74±0.13^bc</td>
<td>2.92±0.12^abc</td>
<td>1.05±0.14^a</td>
<td>4.5±0.22^c</td>
<td>4.71±0.25^c</td>
</tr>
<tr>
<td>Ash</td>
<td>2.55±0.07</td>
<td>2.23±0.03</td>
<td>2.28±0.08</td>
<td>2.37±0.04</td>
<td>2.41±0.05</td>
</tr>
</tbody>
</table>

^a, ^b, ^c Means bearing different superscripts in a row differ significantly **(P<0.01)
The mineral contents of the five freshwater fish species are presented in Table 2. The sodium content did not differ significantly among the treatments. The low sodium containing fish are *L. calbasu* and *L. bata*. On the other hand potassium content differed significantly (P<0.05) among the fish species. The potassium and copper contents were significantly (P<0.01) higher in *L. bata* compared to four other carp species. The trace minerals viz. iron, zinc and manganese did not differ significantly among the fish species. Calcium contents of the carp species are presented in Figure 2. The calcium content (mg/100g) ranges from 197.00 to 325.00. The calcium content was maximum in *L. fimbriatus* and followed by *C. reba*, *L. bata* and *P. javanicus*. The calcium level reported in these fish are similar to carp as reported earlier (Shekhar et al., 2004 and Paul et al., 2016) and higher than Indian catfishes (Paul et al., 2015).

Table 2. Mineral content (ppm) of five freshwater fish species

<table>
<thead>
<tr>
<th>Particulars</th>
<th><em>L. bata</em></th>
<th><em>L. calbasu</em></th>
<th><em>L. fimbriatus</em></th>
<th><em>C. reba</em></th>
<th><em>P. javanicus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>42.30±1.45</td>
<td>39.09±5.46</td>
<td>61.29±1.84</td>
<td>47.15±6.89</td>
<td>56.47±3.23</td>
</tr>
<tr>
<td>Potassium**</td>
<td>145.08±6.57</td>
<td>118.16±3.47</td>
<td>127.30±4.86</td>
<td>116.41±3.30</td>
<td>128.33±4.79</td>
</tr>
<tr>
<td>Iron</td>
<td>0.66±0.06</td>
<td>0.46±0.04</td>
<td>0.43±0.04</td>
<td>0.64±0.07</td>
<td>0.45±0.03</td>
</tr>
<tr>
<td>Copper**</td>
<td>0.59±0.07</td>
<td>0.21±0.03</td>
<td>0.38±0.08</td>
<td>0.18±0.03</td>
<td>0.41±0.04</td>
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<tr>
<td>Zinc</td>
<td>0.71±0.06</td>
<td>0.70±0.04</td>
<td>0.80±0.12</td>
<td>0.57±0.03</td>
<td>0.70±0.06</td>
</tr>
<tr>
<td>Manganese</td>
<td>0.17±0.02</td>
<td>0.13±0.01</td>
<td>0.15±0.01</td>
<td>0.16±0.02</td>
<td>0.11±0.02</td>
</tr>
</tbody>
</table>

*a, b, c* Means bearing different superscripts in a row differ significantly **(P<0.01)**

Figure 2. Calcium content (mg/100g) of five freshwater carp species
The potassium (K) level is usually higher than sodium (Na) level in both marine water and freshwater fishes (Otitologbon, 1997). The potassium (116.41-145.08 ppm) content was higher than sodium (39.09-61.29 ppm) content in our present study which is in agreement with the above findings. The present data on sodium and potassium are lower than the earlier report by Paul et al. (2016). In the context of fish as health food, an optimum balance between the K (high) and Na (low) levels is required which is present in these carp species.

The iron level of these fish species ranges from (0.45 to 0.66 ppm) which is lower in rohu, catla and mrigal as studied earlier (Paul et al., 2016) as well as in catfish (Paul et al., 2015). Iron plays an important role in oxidation-reduction reaction and electron transport associated with cellular respiration (Paul and Mukhopadhyay, 2001). The manganese content of the present study is also lower than the earlier report by Ozyurt et al. (2009) and Paul et al. (2015 and 2016). Manganese is responsible for normal functioning of brain and proper metabolism of lipid and carbohydrate as reported earlier (Chanda et al., 2015). Zinc has a structural role in nucleoproteins and involved in prostaglandin metabolism (Lall, 2002). The zinc and copper content of these fish species ranges from 0.57 to 0.80 and 0.21 to 0.59 (mg/100g) respectively. The zinc content is lower than the earlier report in Indian Major Carp (Paul et al., 2015)

The fatty acid profile of five fish species are presented in table 3. The data reveals that among the saturated fatty acid (SFA), Myristic acid, Stearic acid and Palmitic acid differed significantly (P<0.01) among the fish species. The Myristic acid was significantly (P<0.01) higher in L. bata and P. javanicus. The Stearic acid was significantly (P<0.01) higher in L. Calbasu and followed by C. reba and L. bata. The predominant saturated fatty acid (SFA) i.e. Palmitic acid is significantly (P<0.01) higher in L. fimbriatus vis-à-vis other fish species. The SFA differed significantly (P<0.01) among the fish species; where the level was significantly higher in L. fimbriatus.

Table 3. Fatty acid profile (% of total fatty acid) of five freshwater fish species

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>L. bata</th>
<th>L. calbasu</th>
<th>L. fimbriatus</th>
<th>C. reba</th>
<th>P. javanicus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butyric acid (C4:0)</td>
<td>0.15±0.05</td>
<td>0.07±0.02</td>
<td>ND</td>
<td>0.05±0.04</td>
<td>0.18±0.08</td>
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<tr>
<td>Caproic acid (C6:0)</td>
<td>ND</td>
<td>0.06±0.02</td>
<td>0.19±0.04</td>
<td>0.03±0.02</td>
<td>0.12±0.05</td>
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<td>Caprylic acid (C8:0)</td>
<td>0.05±0.24</td>
<td>0.03±0.02</td>
<td>0.05±0.01</td>
<td>0.05±0.01</td>
<td>0.04±0.01</td>
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<tr>
<td>Capric acid (C10:0)</td>
<td>ND</td>
<td>0.06±0.04</td>
<td>ND</td>
<td>ND</td>
<td>0.03±0.02</td>
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<tr>
<td>Undecanoic acid (C11:0)</td>
<td>0.07±0.03</td>
<td>0.57±0.54</td>
<td>0.02±0.01</td>
<td>0.06±0.04</td>
<td>ND</td>
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<tr>
<td>Lauric acid (C12:0)</td>
<td>0.28±0.12</td>
<td>0.43±0.32</td>
<td>0.03±0.01</td>
<td>0.16±0.01</td>
<td>0.10±0.02</td>
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<tr>
<td>Tridecanoic acid (C13:0)</td>
<td>0.67±0.05</td>
<td>0.60±0.51</td>
<td>0.02±0.01</td>
<td>0.31±0.01</td>
<td>0.06±0.02</td>
</tr>
<tr>
<td>Myristic acid* (C14:0)</td>
<td>5.73±0.31</td>
<td>2.60±0.16</td>
<td>0.26±0.01</td>
<td>3.42±0.17</td>
<td>4.26±1.73</td>
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<tr>
<td>Pentadecanoic acid (C15:0)</td>
<td>2.88±1.44</td>
<td>0.88±0.24</td>
<td>0.24±0.01</td>
<td>1.88±0.09</td>
<td>1.10±0.67</td>
</tr>
<tr>
<td>Palmitic acid** (C16:0)</td>
<td>39.91±6.18</td>
<td>30.95±1.14</td>
<td>81.17±0.30</td>
<td>41.61±4.58</td>
<td>43.54±3.24</td>
</tr>
<tr>
<td>Fatty acid</td>
<td>L. bata</td>
<td>L. calbasu</td>
<td>L. fimbriatus</td>
<td>C. reba</td>
<td>P. javanicus</td>
</tr>
<tr>
<td>------------------------------------------------</td>
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<td>--------------</td>
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<tr>
<td>Heptadecanoic acid (C17:0)</td>
<td>0.49±0.11</td>
<td>1.73±0.48</td>
<td>0.46±0.01</td>
<td>0.45±0.03</td>
<td>1.43±0.72</td>
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<td>Stearic acid** (C18:0)</td>
<td>5.04±1.16</td>
<td>10.44±0.82</td>
<td>2.52±0.03</td>
<td>5.20±0.25</td>
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<tr>
<td>Arachidic acid (C20:0)</td>
<td>0.20±0.15</td>
<td>0.53±0.05</td>
<td>0.12±0.01</td>
<td>0.21±0.16</td>
<td>0.45±0.30</td>
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<tr>
<td>Heneicosanoic acid (C21:0)</td>
<td>ND</td>
<td>0.77±0.36</td>
<td>2.03±0.03</td>
<td>0.36±0.23</td>
<td>2.66±0.67</td>
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<tr>
<td>Behenic acid (C22:0)</td>
<td>0.28±0.22</td>
<td>0.99±0.04</td>
<td>ND</td>
<td>0.62±0.36</td>
<td>0.30±0.24</td>
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<tr>
<td>Tricosanoic acid (C23:0)</td>
<td>1.80±0.50</td>
<td>0.26±0.09</td>
<td>0.32±0.10</td>
<td>2.49±1.99</td>
<td>0.32±0.05</td>
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<tr>
<td>∑SFA**</td>
<td>57.45±3.95</td>
<td>50.71±2.88</td>
<td>87.41±0.10</td>
<td>56.87±1.65</td>
<td>52.61±1.23</td>
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<tr>
<td>Myristoleic acid (C14:1)</td>
<td>6.05±0.04</td>
<td>0.07±0.04</td>
<td>ND</td>
<td>0.07±0.01</td>
<td>0.08±0.03</td>
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<tr>
<td>Pentadecenoic acid** (C15:1)</td>
<td>0.26±0.06</td>
<td>0.12±0.09</td>
<td>0.03±0.01</td>
<td>0.04±0.00</td>
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<td>Palmitoleic acid (C16:1)</td>
<td>ND</td>
<td>5.75±1.60</td>
<td>0.31±0.03</td>
<td>3.12±0.83</td>
<td>1.81±0.25</td>
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<tr>
<td>Heptadecanoic acid (C17:1)</td>
<td>0.04±0.01</td>
<td>0.17±0.10</td>
<td>0.03±0.01</td>
<td>0.04±0.00</td>
<td>0.50±0.24</td>
</tr>
<tr>
<td>Oleic acid** (C18:1n9c)</td>
<td>18.51±0.93</td>
<td>23.57±3.77</td>
<td>4.99±0.05</td>
<td>20.04±1.44</td>
<td>32.07±2.35</td>
</tr>
<tr>
<td>Elaidic acid (C18:1n9)</td>
<td>0.11±0.07</td>
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<td>ND</td>
<td>0.18±0.03</td>
<td>15.30±2.80</td>
</tr>
<tr>
<td>Eicosanoic acid (C20:1n9c)</td>
<td>0.10±0.05</td>
<td>0.91±0.38</td>
<td>0.21±0.02</td>
<td>0.08±0.02</td>
<td>1.18±0.26</td>
</tr>
<tr>
<td>Erucic acid (C22:1n9)</td>
<td>0.32±0.09</td>
<td>ND</td>
<td>ND</td>
<td>0.61±0.03</td>
<td>0.56±0.32</td>
</tr>
<tr>
<td>∑MUFA**</td>
<td>19.19±0.96</td>
<td>30.72±4.04</td>
<td>6.19±0.01</td>
<td>24.58±0.74</td>
<td>36.27±1.78</td>
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<tr>
<td>Linolelaidic acid (C18:2n6c)</td>
<td>0.11±0.03</td>
<td>1.01±0.80</td>
<td>1.00±0.01</td>
<td>0.10±0.01</td>
<td>0.12±0.03</td>
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<tr>
<td>Linoleic acid (C18:2n6c)</td>
<td>6.82±0.32</td>
<td>9.22±2.72</td>
<td>3.44±0.04</td>
<td>8.94±0.32</td>
<td>2.77±1.69</td>
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<tr>
<td>γ-Linolenic acid (C18:3n6)</td>
<td>0.44±0.21</td>
<td>0.59±0.38</td>
<td>0.13±0.01</td>
<td>0.04±0.01</td>
<td>0.24±0.11</td>
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<tr>
<td>α-Linolenic acid (C18:3n3)</td>
<td>4.08±2.65</td>
<td>3.96±0.79</td>
<td>0.10±0.01</td>
<td>5.68±0.27</td>
<td>1.66±1.18</td>
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<td>Eicosadienoic acid (C20:2)</td>
<td>0.71±0.24</td>
<td>1.12±0.39</td>
<td>0.18±0.09</td>
<td>ND</td>
<td>0.36±0.04</td>
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<tr>
<td>Eicosaatrienoic acid (C20:3n6)</td>
<td>2.51±0.28</td>
<td>1.35±0.22</td>
<td>0.46±0.05</td>
<td>1.02±0.06</td>
<td>1.83±0.59</td>
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<tr>
<td>Eicosaatrienoic acid (C20:3n3)</td>
<td>0.34±0.15</td>
<td>0.35±0.22</td>
<td>ND</td>
<td>0.26±0.01</td>
<td>0.09±0.01</td>
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<tr>
<td>Arachidonic acid** (C20:4n6)</td>
<td>1.89±0.22</td>
<td>0.59±0.01</td>
<td>ND</td>
<td>0.76±0.04</td>
<td>0.17±0.06</td>
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<tr>
<td>Eicosapentaenoic acid or EPA** (C20:5n3)</td>
<td>3.75±0.52</td>
<td>1.33±0.63</td>
<td>0.29±0.01</td>
<td>1.08±0.06</td>
<td>0.59±0.20</td>
</tr>
<tr>
<td>Docosahexaenoic acid or DHA (C22:6n3)</td>
<td>3.21±0.84</td>
<td>1.44±0.70</td>
<td>0.82±0.08</td>
<td>0.72±0.16</td>
<td>2.62±1.40</td>
</tr>
<tr>
<td>∑PUFA*</td>
<td>23.36±3.31</td>
<td>18.58±4.68</td>
<td>6.40±0.09</td>
<td>18.56±0.91</td>
<td>10.29±0.98</td>
</tr>
<tr>
<td>o3: o6</td>
<td>0.88±0.32</td>
<td>0.69±0.27</td>
<td>0.28±0.01</td>
<td>0.72±0.02</td>
<td>1.22±0.58</td>
</tr>
<tr>
<td>∑o3</td>
<td>10.88±3.61</td>
<td>6.87±2.79</td>
<td>1.37±0.01</td>
<td>7.73±0.49</td>
<td>4.93±1.40</td>
</tr>
<tr>
<td>∑o6*</td>
<td>12.48±3.03</td>
<td>11.72±3.12</td>
<td>5.03±0.09</td>
<td>10.83±0.42</td>
<td>5.36±1.40</td>
</tr>
</tbody>
</table>

*a, b, c, d Means bearing different superscripts in a row differ significantly* (P<0.05); **(P<0.01), ND: Not detected

The total monounsaturated fatty acid (MUFA) differed significantly (P<0.01) among all the five fish species. The pentadecanoic acid was significantly (P<0.01) higher in
L. fimbriatus and followed by C. reba. The predominant MUFA is Oleic acid and it is found significantly (P<0.01) higher in P. javanicus and followed by L. calbasu, C. reba and L. bata. The other monounsaturated fatty acids like myristoleic acid, palmitoleic acid, heptadecenoic acid, Elaidic acid, Eicosaenoic acid and Erucic acid did not differ significantly among the carp species.

The polyunsaturated fatty acids (PUFA) are the most important fatty acid so far the human health is concerned. Linoleic acid is significantly (P<0.05) higher in L. calbasu and L. bata. The Arachidonic acid was significantly (P<0.01) higher in L. bata followed by L. calbasu and C. reba. The Eicosapentaenoic acid (EPA) is significantly (P<0.01) higher in L. bata followed by L. calbasu and C. reba. The Docosahexaenoic acid (DHA) did not differ among the carp species as studied. The PUFA differed significantly (P<0.01) among the carp species; where PUFA content was significantly higher in L. bata, C. reba and L. calbasu. The ω3:ω6 ratio and sum total of ω3 did not differ significantly among the treatment groups. The ω3:ω6 ratio is 1.22±0.5 in P. javanicus which is near to ideal ratio 1.0 compared to others. The summation of ω6 differed significantly (P<0.05) among the carp species and it is significantly higher in L. bata, L. calbasu and C. reba.

Fatty acid composition of aquatic animals was influenced by intrinsic variables, such as species, sex, age and size; as well as extrinsic factors, such as diet, salinity, temperature, geographical regions, and the general rearing conditions (Abd Rahman et al., 1995; Sener et al., 2005). Fatty acids in fishes are derived from two main sources, viz. biosynthesis and diet (Kamler et al., 2001). The chain length varies from C_{14}-C_{24} of varying degree of unsaturation, from saturated to polyunsaturated (Swapna et al., 2010). Palmitic acid content among the SFA was maximum in these fish species which is in agreement with earlier report (Kaya et al., 2008; Jakhar et. al., 2012 and Paul et al., 2015a). Palmitic acid is considered to be a key to many metabolic processes in fish and other aquatic animals (Ackman and Eaton, 1966). Nath and Banerjee (2012) reported that the abundant quantity of SFAs shows that the less efficiency of fish species in utilizing the SFAs as core energy source. Regost et al. (2003) noticed that the two main sources of fatty acids in the muscles are diet and de novo synthesis. Level of saturated fatty acids in the body rises if the fish mostly feed on the diet containing insects and other aquatic organisms but if the fish diet mainly depends on the feed containing plant and algae sources then the level of unsaturated fatty acids become higher in the body. Continual recycling of fatty acids in feed, habitat and food web is the main reason of variation of fatty acids in the body.

Among monounsaturated fatty acid (MUFA), the oleic acid is predominant fatty acid as reported in these fish species which is in agreement with the results of other freshwater fish species (Chedoloh et al., 2011 and Paul et al., 2015a). Memon et al. (2011) and Paul et al. (2015a) reported that oleic acid was the main MUFA in L. rohita, C. mrigala and C. catla. 60-68% of the MUFA in freshwater carps was C18:1
(oleic acid) as reported by Sankar and Ramachandran (2001). Oleic acid has exogenous origin and usually reflects the type of diet of the fish (Ackman, 1989). The PUFA content ranges from 6.40 to 23.36 in the studied fish species. Vlieg and Body (1988) reported that freshwater fish have lower content of PUFAs as the freshwater fish feed is largely based on plant materials. Fish oils are known to be rich source of essential PUFA of the omega-3 family (Kenari et al., 2009). Memon et al. (2011) also reported that Indian major carp contains good amount of long chain PUFA, which was in agreement with our present study. Several studies have reported that consumption of fish and fish oils containing ω-3 fatty acids are favorable for a number of biological factors associated with cardiovascular diseases, rheumatoid arthritis, psoriasis, etc. (Kris-Etherton and Harris, 2002).

The gross energy content of the studied carp species are presented in figure 5. The energy content is maximum in *L. fimbriatus* and followed by *L. bata* and *C. reba*. The gross energy content of the carp species of the present study are higher than energy content of eels (*Anguilla Anguilla*) as reported by Schreckenbach et al. (2001). Chrisolite et al. (2015) reported the gross energy content of fifteen freshwater species wherein the gross energy content is lower than our report in the present study. The vitamin A and D content are presented in figure 1, wherein the vitamin A content is maximum in *L. bata, C. reba* and *P. javanicus*. The vitamin D content is maximum in *C. reba, L. bata* and *L. fimbriatus* as studied in the present experiment. Fish acts as a good source of fat solubles vitamins, viz. A, D, E and K. Liu (2003) reported that vitamin A content from fish is easily utilized by our body than from plant source. Vitamin A is responsible for normal vision, bone growth and its derivative retinoic acid which helps in the regulation of gene expression in developmental epithelial tissue (Roos et al., 2003). Fat soluble vitamin content in fish flesh is affected by the level of fat (Ozyurt et al., 2009). The vitamin A content is maximum *L. bata, C. reba* and *P. javanicus*. Vitamin A content in *L. bata* is 207.00 (I.U./100g). The vitamin D content is higher in *C. reba, L. bata* and *L. fimbriatus*. Vitamin D plays a major role in activation of the innate and the adaptive immune systems (Hewison, 2011).
Figure 3. Essential Amino Acid composition (g/100N) of five freshwater carp species

Figure 4. Non-Essential amino acid composition (g/100N) of five freshwater carp species
The figure 3 represents the essential amino acid content of all the five carp species. Perusal of figure 3 depicts that the methionine content is maximum among all the essential amino acids, wherein the methionine content is maximum in *L. fimbriatus*, *P. javanicus* and *L. bata*. Threonine content is high in *L. bata* and *L. fimbriatus*. Histidine content is more in *L. calbasu* and *P. javanicus*. The tryptophan content is high in *L. calbasu* and *C. reba*. The non-essential amino acids (NEAA) content of the five fish species are presented in figure 4. Among the NEAA, glutamic acid is predominant and it is followed by aspartic acid, asparagine and serine. Aspartic acid, glutamic acid and asparagine are maximum in *L. calbasu* and *C. reba*. Iwasaki and Harada (1985) reported that the main amino acids of fish are aspartate, glutamate and lysine. Over the last 20 years, increasing evidence suggests the importance of glutamine for the proper functioning of many organ systems (Christina et al., 1999). The most abundant free amino acid in the body, comprising nearly 60% of the intracellular amino acid in the skeletal muscle (Kenari et al., 2009). It serves as an important carrier for the ammonia (nitrogen) from muscle to the splanchnic area and immune system (Deutz et al., 1992). Glutamine also acts as donor of nitrogen in the synthesis of purines and pyrimidines and helps in the proliferation of cells (Limin et al., 2006). Similar values of glutamic acid have also been reported earlier in mackerel (Hou et al., 2011) and Indian Freshwater food fishes (Mohanty et al., 2014). The present study shows the presence of a better amount of essential and non-essential amino acids in *L. bata*, *L. calbasu*, *L. fimbriatus*, *C. reba* and *P. javanicus*.
CONCLUSION

The nutrient profile of five carps viz. *Labeo bata*, *Labeo calbasu*, *Labeo fimbriatus*, *Cirrhinus reba* and *Puntius javanicus* reveal that they are rich in essential nutrients like protein, fat, ash, energy, minerals, vitamins, amino acid and fatty acid content which are required for human health. The important fatty acids eicosapentaenoic acid and docosahexaenoic acid are present in these carps. The nutritional information of these fish species are not documented properly so these findings will help in preparation of database. The data on the nutrient composition of these fish species will help the nutritionists, researchers medical practitioners, dieticians and other related stakeholders to advise consumers to take fish as health food.

ACKNOWLEDGEMENT

This work was supported by Ministry of Agriculture, Government of India under ICAR Outreach Activity on Nutrient Profiling and Evaluation of fish as a Dietary component. The authors greatly acknowledge the help of DDG (Fy, ICAR) and Director, CIFA for providing necessary support and facility to conduct the work. The help extended by Mrs. Puja Singh during analysis of samples is duly acknowledged.

REFERENCES


NUTRITIONAL VALUES OF CARPS


SOCIOECONOMIC ANALYSIS OF HYDROPONIC FODDER PRODUCTION IN SELECTED AREAS OF BANGLADESH: PROSPECTS AND CHALLENGES

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Mymensingh-2202, Bangladesh

ABSTRACT
The study was conducted to assess the prospects and challenges of hydroponic fodder production in Bangladesh. A total of 40 farmers were selected purposively from Kishoregonj and Jashore districts as sample for the study. A combination of descriptive, mathematical and statistical techniques was used to analyze the data. The findings of the study revealed that average household and farm size of the farmers were 5.0 persons and 0.48 hectare, respectively. Average annual income of the farmers was Tk. 92312, of which 56.1% income was from farming activities and 43.9% income was from non-farming activities. Majority of the technology adopting farmers (35.8% farmers) were within the late majority group. Profitability analysis showed that net return and benefit cost ratio of hydroponic fodder production were Tk. 5400 per decimal and 1.82, respectively. Farm size, farming experience, training and extension contact had significant impact on farmers’ adoption of hydroponic fodder production technology. Nutritional quality of fodder, high installation cost, medicinal value for human consumption and sensitivity to temperature were the major strength, weakness, opportunity and threat of hydroponic fodder, respectively. This fodder production technology is sustainable from the perspectives of energy use, environmental safety, economic viability and social/political equity. The study recommended that input support (especially seed), motivation, training programmes and extension services by different government and non-government organizations should be properly organized and implemented in town and water logging areas to raise the farmers’ awareness for the adoption of hydroponic fodder production technology.

Keywords: Adoption, hydroponic fodder, profitability, prospects and challenges, sustainability

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INTRODUCTION

Bangladesh is a densely populated country with limited land resources where livestock gets very small places for grazing. It is important to serve green grass for getting increased productivity from livestock. The scarcity of animal feed and fodder has been identified as major constraint for the development of livestock in Bangladesh. Poor quality roughages having deficiencies in fermentable carbohydrates, protein, minerals and vitamins yield lower milk and meat of the animals (Migwi, 1997). At present, about 83% of the total cultivable land is used for cereal crops, where only 0.10% for cultivation of fodder crops (BBS, 2015). As a result, animal fodder shortage is aggravating day by day and recently it has emerged as an acute problem for rearing livestock in Bangladesh.

In natural conditions, soil acts as a mineral nutrient reservoir but the soil itself is not essential to plant growth. When the mineral nutrients in the soil are dissolved in water, plant roots are able to absorb them. When the required mineral nutrients are introduced into a plant's water supply artificially, soil is no longer required for the plant to thrive. Almost any terrestrial plant can grow like this. This method of growing plants using mineral nutrient solutions, in water, without soil is known as hydroponics. It requires just 480 sq. ft area to produce 1000 kg every day against 5-30 acres of land under conventional system saving water by 95% (Jemimah et al., 2015).

It is possible by hydroponic techniques to achieve better than normal farm production, immune to natural weather variations, as well as organic and more nutritive, in just about 5% of the space and 5% of the irrigation water. Hydroponic fodder production requires only about 2-3% of that water used under field conditions to produce the same amount of fodder (Al-Karaki and Al-Hashimi, 2012). There are two chief merits of the soil-less cultivation of plants: 1st, hydroponics may potentially produce much higher crop yields; and 2nd, hydroponics can be used in places where in-ground agriculture or gardening is not possible.

In Bangladesh, the demand for green fodder is increasing on the account of diversified uses of agricultural residues. Adequate attention is not being given to the production of fodder crops due to increasing pressure on land for production of food grains, oil seeds and pulses. In order to meet this increasing demand for green fodder, the next best alternative is to produce hydroponics fodder to supplement the meager pasture resources. Under this system, grass is grown without soil and the technology has been used in the developed countries for a long time. The most common sprouting crop is barley; although alfalfa, clover, corn, cow peas, oats, sorghum and wheat are possible grains to grow fodder. Determining the best forage crop is an important matter in producing highest fodder yield and quality and at the same time considering the economic dimensions in the process of hydroponic green fodder production by saving of seeds costs (Al-Karaki and Al-Hashimi, 2012).
Hydroponic grass is economically more lucrative to many dairy farmers because of its high productivity, and non-susceptibility to diseases and infections. The grasses that grow in open fields sometimes cause diarrhoea and other diseases to the animals. The seed germination rate in this system is approximately 98 percent. The fodder produced through hydroponic system can be stored for up to 10 days. An indoor facility with a hydroponic fodder unit can provide enough green grass to meet the nutrition needs of the animals. Some dairy proprietors in India have started growing grass in indoor facilities. The technology is quite simple and easy (UNB, 2017). Fodders grown in this system are more nutritious, rich in minerals and micronutrients, and also germ-free, and they could increase milk production by up to 15 percent at a dairy farm (Pramanik, 2017).

Importance of such farming technology has been portrayed in a good number of literatures. A reticent effort has been made here to appraise the previous research studies which are: Islam et al. (2016) carried out a study on the effect of seed rate and water level on production and chemical analysis of hydroponic fodder in Bangladesh and found that high production performances and nutritive value can be found by cultivating hydroponic fodder using two seeds (maize and wheat) in the housing condition; Salam et al. (2014) examined the feasibility of tomato production using different substrates in aquaponic system in Bangladesh and concluded that the gravels substrate gave the highest tomato production than the brick lets and gravels mixed with saw dust substrate; Islam et al. (2013) conducted a study on cost-return analysis of fodder production in selected areas of Bangladesh and declared that the BCR was the highest (2.18) in Jashore district and the lowest (2.18) in Kurigram district for fodder producer cum seller; Naik et al. (2013) studied on low cost devices for hydroponics fodder production in Goa, India and revealed that hydroponics fodder can be produced in low cost green houses with locally available or home-grown grains; Saha (2010) focused on soilless cultivation for landless people as an alternative livelihood practice through indigenous hydroponic agriculture in flood-prone Bangladesh and demonstrated that farmers can use their submerged lands for crop production where plants can be grown on the water in a bio-land or floating bed of water-hyacinth, algae and other plant residues.

It is evident from the above discussion that no empirical study has been conducted yet on prospects and challenges of hydroponic fodder production in Bangladesh. Thus, there exists a scope to identify the present adoption scenario and production practice of hydroponic fodder in Bangladesh. Research on socioeconomic aspects of hydroponic fodder production in Bangladesh is scarce and many policy level questions still are remained unanswered. Therefore, the study is highly relevant to the national goal of the government stated in the National Agriculture Policy. The specific objectives of the study are: i) to document the socioeconomic status of hydroponic fodder producers in Bangladesh; ii) to estimate the profitability of hydroponic fodder production in the study area; iii) to examine the factors affecting adoption of hydroponic fodder technology by the farmers; and iv) to address the
prospects and challenges as well as suggest policy recommendations for sustainable hydroponic fodder production in Bangladesh.

MATERIALS AND METHODS

Study area, sample size and data acquisition methods
The study was conducted at different villages of Kishoregonj and Jashore districts. A total of 40 sample farmers were interviewed using pre-tested questionnaire. Purposive random sampling technique was followed to select the sample farmers. Moreover, key informant interviews (KII) were also performed in Dhaka, Gazipur and Narayangonj for collecting the necessary information. Secondary data sources like reports, publications, handouts, etc. relevant with this study were also consulted.

Analytical techniques
For analyzing the data, a combination of descriptive (sum, averages, percentages, etc.), mathematical and statistical techniques were used to achieve the objectives and to get the meaningful result.

Profitability analysis
Profitability of hydroponic fodder production was measured in terms of gross return, gross margin, net return, and benefit cost ratio (undiscounted). The formula needed for the calculation of profitability is discussed as follows:

Gross return (GR)
Gross return was calculated by multiplying the total volume of output by the price in the harvesting period (Dillon and Hardaker, 1993). The equation was as follows:

$$GR = X_{mp}P_{mp} + X_{bp}P_{bp}$$

Where,

- \(X_{mp}\) = Yield of main product (kg decimal\(^{-1}\));
- \(P_{mp}\) = Price of main product (Tk. kg\(^{-1}\));
- \(X_{bp}\) = Yield of by-product (kg decimal\(^{-1}\)); and
- \(P_{bp}\) = Price of by-product (Tk. kg\(^{-1}\)).

Gross margin (GM)
Gross margin was calculated by the difference between gross return and total variable cost. The following equation was used to calculate GM:

$$GM = GR - \Sigma C_v$$

Where,

- \(GR\) = Gross return (Tk. decimal\(^{-1}\)); and
- \(\Sigma C_v\) = Total variable cost (Tk. decimal\(^{-1}\)).
Net return (NR)

Net return was calculated by deducting all costs (variable and fixed) from the gross return. The following algebraic form of NR was used for estimation:

\[ NR = GR - \Sigma C_v - \Sigma C_f \]

Where,

- \( GR \) = Gross return (Tk. decimal\(^{-1}\));
- \( \Sigma C_v \) = Total variable cost (Tk. decimal\(^{-1}\)); and
- \( \Sigma C_f \) = Total fixed cost (Tk. decimal\(^{-1}\)).

Benefit cost ratio (BCR)

Benefit cost ratio (BCR) is a relative measure which is used to compare the return per unit of cost. BCR was estimated as a ratio of gross return to gross cost. The formula used for calculating BCR (undiscounted) was as follows:

\[ BCR = \frac{GR}{GC} \]

Where,

- \( GR \) = Gross return (Tk. decimal\(^{-1}\)); and
- \( GC \) = Gross cost (i.e. \( \Sigma C_v + \Sigma C_f \)) (Tk. decimal\(^{-1}\)).

Logit model

In order to identify the factors influencing adoption of hydroponic fodder technology by the farmers, the following logistic regression analysis (i.e. Logit model) was used (Gujarati, 2003):

\[
K_i = \ln \left[ \frac{P_i}{1 - P_i} \right] = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X_4 + \beta_5X_5 + \beta_6X_6 + \beta_7X_7 + \beta_8X_8 + E_i
\]

Where,

- \( P_i \) is the probability of adoption and non-adoption of hydroponic fodder production technology, \( P_i = 1 \) indicates adoption and \( P_i = 0 \) indicates non-adoption.
- Dependent variable: Adoption of hydroponic fodder production technology
  - (Adopters = 1, otherwise 0)
- \( K_i \) = Probability of adoption of hydroponic fodder technology.

Independent variables:

- \( X_1 \) = Household size (no.);
- \( X_2 \) = Educational level of household head (years of schooling);
- \( X_3 \) = Age of household head (years);
- \( X_4 \) = Farm size (ha);
- \( X_5 \) = Annual income (Tk.);
X\_6 = Farming experience (years of farming);
X\_7 = Training (P_i = 1 indicates having training on hydroponic fodder production and P_i = 0 indicates having no training on hydroponic fodder production);
X\_8 = Extension contact (P_i = 1 indicates having extension contact and P_i = 0 indicates having no extension contact);
β\_0 = Intercept;
β\_1 to β\_8 = Regression coefficients of the independent variables; and
E_i = Error term.

The marginal probabilities of the key determinants of adopting hydroponic fodder technology were estimated based on expressions derived from the marginal effect of the Logit model which was as follows:

\[ \frac{dK}{dX} = \beta_i \{P_i(1 - P_i)\} \]

Where,

\[ \beta_i = \text{Estimated Logit regression coefficient with respect to the } i^{th} \text{ factor; and} \]

\[ P_i = \text{Estimated probability of farmers’ adoption status.} \]

**SWOT analysis**

SWOT analysis was done to identify the problems and potentials of hydroponic fodder technology. The SWOT analysis guided to identify the positives and negatives inside of the organization (S-W) and outside of it in the external environment (O-T) (Gürel and Tat, 2017).

**Sustainability perspective of hydroponic fodder production technology**

Sustainability describes a condition in which natural systems and social systems survive and thrive together indefinitely. A sustainable condition allows people to meet the needs of the present without compromising the ability of future generations to meet their own needs (IISD, 2015). 4E sustainability framework analysis uses perspectives from energy, environment, economics and equity in social/political aspects to explain how hydroponic fodder production technology contributes to maintain sustainability (Braun, 2017).

**RESULTS AND DISCUSSION**

**Socioeconomic profile of the farmers**

Table 1 represents the basic information of the farmers in the study areas. It is found that average household size of the farmers was 5.0 which was higher than the national average of 4.1 (HIES, 2016); and farm size was 0.48 ha. Average dependency ratio of the farmers (1.8) indicated that on average, about 2 household members were dependent on the economically working and earning person of that household. The percentages of male and female respondents were 64.2 and 35.8,
respectively in the study areas. Average age of the farmers was found to be 39 years. Majority of the farmers completed at least primary and above educational level (47.5% farmers).

Table 1. Basic information about the selected farmers

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Respective information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average household size (no.)</td>
<td>5.0 (male: 70.0%; female: 30.0%)</td>
</tr>
<tr>
<td>Average farm size (ha)</td>
<td>0.48</td>
</tr>
<tr>
<td>Average dependency ratio (no.)</td>
<td>1.8</td>
</tr>
<tr>
<td>Average sex distribution (% of farmers)</td>
<td>Male 64.2</td>
</tr>
<tr>
<td>Average age (years)</td>
<td>Female 35.8</td>
</tr>
<tr>
<td>Average dependency ratio (no.)</td>
<td>1.8</td>
</tr>
<tr>
<td>Average sex distribution (% of farmers)</td>
<td>Male 64.2</td>
</tr>
<tr>
<td>Average age (years)</td>
<td>Female 35.8</td>
</tr>
<tr>
<td>Average age (years)</td>
<td>39</td>
</tr>
<tr>
<td>Literacy rate (% of farmers)</td>
<td>Illiterate 18.3</td>
</tr>
<tr>
<td>Primary and above</td>
<td>Sign only 34.2</td>
</tr>
<tr>
<td>Agricultural status (% of farmers)</td>
<td>Agriculture only 31.7</td>
</tr>
<tr>
<td>Occupational status (% of farmers)</td>
<td>Agriculture and others 68.3</td>
</tr>
<tr>
<td>Farm income</td>
<td>51793 (56.1% of total income)</td>
</tr>
<tr>
<td>Non-farm income</td>
<td>40519 (43.9% of total income)</td>
</tr>
<tr>
<td>Total income</td>
<td>92312</td>
</tr>
</tbody>
</table>


Most of the farmers (68.3% farmers) were engaged in agriculture as well as other income generating activities like labour selling, service, small business, etc. It is also shown that average annual income of the farmers was Tk. 92312, of which 56.1% income was from farming activities (i.e. income from crop, livestock, poultry, homestead and agro-forestry) and 43.9% income was from non-farming activities (i.e. income from small business, wage labour, shop keeping, van/rickshaw pulling and other sources) (Table 1).

**Adopter categories**

It is evident from table 2 that in the case of adopting an innovation like hydroponic fodder technology, the percentages of innovators were 5.0%, early adopters were 15.8%, early majority were 29.2%, late majority were 35.8% and laggards were 14.2%. Though majority of the farmers were pessimistic about this fodder farming technology at the beginning, the adoption of this technology was ultimately successful. The result is similar with Uddin et al. (2017) where the authors revealed that in the case of adopting an innovation like conservation agriculture, majority of the farmers were within the late majority category.
Table 2. Trend of adopting hydroponic fodder technology

<table>
<thead>
<tr>
<th>Adopter categories</th>
<th>Characteristics</th>
<th>% of adopting farmers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Innovators</td>
<td>- Were willing to take risks</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>- Had the highest social status and financial liquidity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Had the closest contact to scientific sources and interaction with other farmers</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Had the highest degree of opinion leadership</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Had a higher social status, financial liquidity and advanced education</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Used judicious choice of adoption to maintain a central communication position</td>
<td></td>
</tr>
<tr>
<td>Early adopters</td>
<td>- Adopted the innovation after a varying degree of time</td>
<td>15.8</td>
</tr>
<tr>
<td></td>
<td>- Had above average social status and seldom hold positions of opinion leadership in a system</td>
<td></td>
</tr>
<tr>
<td>Early majority</td>
<td>- Approached the innovation after the majority of society had adopted the innovation</td>
<td>29.2</td>
</tr>
<tr>
<td>Late majority</td>
<td>- Were typically incredulous about the innovation</td>
<td>35.8</td>
</tr>
<tr>
<td></td>
<td>- Had below average social status and little financial liquidity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Had little to no opinion leadership</td>
<td></td>
</tr>
<tr>
<td>Laggards</td>
<td>-Were oldest among adopters having lowest social status and lowest financial liquidity</td>
<td>14.2</td>
</tr>
<tr>
<td></td>
<td>- Had an aversion to change-agents typically and a tendency to be focused on traditions</td>
<td></td>
</tr>
</tbody>
</table>


**Profitability of hydroponic fodder production**

Profitability of hydroponic fodder production was measured in terms of gross return, gross margin, net return and benefit cost ratio. Variable and fixed costs were taken into deliberation to estimate the total cost of production. Variable costs included human labour, seed, tray and other equipments, and watering; and fixed cost included land use cost and depreciation cost of equipments and shed. It is seen from table 3 that total variable cost was Tk.3900 and total fixed cost was Tk.2700 per decimal, respectively. Total cost of hydroponic fodder production was estimated at Tk. 6600 per decimal, of which 36.4 and 34.8 percent of total cost were incurred as for seed purchasing and depreciation cost, respectively. Table 3 also represents that gross return from hydroponic fodder production was Tk.12000 per decimal. Gross margin
and net return were estimated at Tk.8100 and Tk.5400 per decimal, respectively. BCR of hydroponic fodder production was found to be 1.82 which implied that by investing Tk.100 per decimal in hydroponic fodder production, farmers received Tk.182 in return. The study was slightly similar with Islam et al. (2013) where the authors revealed that the BCRs for fodder producers cum sellers were higher than the producers in all of the study areas of Bangladesh.

Table 3. Profitability of hydroponic fodder production

<table>
<thead>
<tr>
<th>Cost of hydroponic fodder production</th>
<th>Tk. decimal&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Percentage (%) of total cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable costs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human labour</td>
<td>300</td>
<td>4.5</td>
</tr>
<tr>
<td>Seed</td>
<td>2400</td>
<td>36.4</td>
</tr>
<tr>
<td>Tray and other equipments</td>
<td>900</td>
<td>13.6</td>
</tr>
<tr>
<td>Watering</td>
<td>300</td>
<td>4.5</td>
</tr>
<tr>
<td>i. Total variable cost</td>
<td>3900</td>
<td>59.0</td>
</tr>
<tr>
<td>Fixed costs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Land use cost</td>
<td>200</td>
<td>3.1</td>
</tr>
<tr>
<td>Depreciation cost</td>
<td>2300</td>
<td>34.8</td>
</tr>
<tr>
<td>Interest on operating capital</td>
<td>200</td>
<td>3.1</td>
</tr>
<tr>
<td>ii. Total fixed cost</td>
<td>2700</td>
<td>41.0</td>
</tr>
<tr>
<td>iii. Total cost</td>
<td>6600</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Return from hydroponic fodder production

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Quantity (kg decimal&lt;sup&gt;1&lt;/sup&gt;)</th>
<th>Price (Tk. kg&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Amount of return (Tk. decimal&lt;sup&gt;1&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>iv. Gross return</td>
<td>600</td>
<td>20</td>
<td>12000</td>
</tr>
<tr>
<td>v. Gross margin (iv - i)</td>
<td></td>
<td></td>
<td>8100</td>
</tr>
<tr>
<td>vi. Net return (iv - iii)</td>
<td></td>
<td></td>
<td>5400</td>
</tr>
<tr>
<td>vii. Benefit cost ratio (BCR) (iv ÷ iii)</td>
<td></td>
<td></td>
<td>1.82</td>
</tr>
</tbody>
</table>

Source: Authors’ estimation, 2018.

Factors influencing adoption of hydroponic fodder technology by the farmers

A Logit model was used to identify the factors influencing adoption of hydroponic fodder production technology by the farmers. In this study, eight independent variables were identified as major determinants of adopting this technology by the farmers. Four out of eight independent variables included in the model were found
significant in explaining the variation in adopting hydroponic fodder production technology by the farmers. These variables were farm size, farming experience, training and extension contact (Table 4).

Table 4. Estimated values of coefficients and marginal effects of logit model

<table>
<thead>
<tr>
<th>Explanatory variables</th>
<th>Coefficients</th>
<th>Standard errors</th>
<th>p-value</th>
<th>dK/dX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>3.810</td>
<td>1.356</td>
<td>0.325</td>
<td>-</td>
</tr>
<tr>
<td>Household size (X_1)</td>
<td>-0.211</td>
<td>0.132</td>
<td>0.214</td>
<td>-0.003</td>
</tr>
<tr>
<td>Educational level of household head (X_2)</td>
<td>0.060</td>
<td>0.195</td>
<td>0.110</td>
<td>0.009</td>
</tr>
<tr>
<td>Age of household head (X_3)</td>
<td>-0.195</td>
<td>0.075</td>
<td>0.164</td>
<td>-0.008</td>
</tr>
<tr>
<td>Farm size (X_4)</td>
<td>0.006*</td>
<td>0.096</td>
<td>0.063</td>
<td>0.013</td>
</tr>
<tr>
<td>Annual income (X_5)</td>
<td>0.690</td>
<td>0.183</td>
<td>0.337</td>
<td>0.013</td>
</tr>
<tr>
<td>Farming experience (X_6)</td>
<td>0.039**</td>
<td>0.121</td>
<td>0.029</td>
<td>0.006</td>
</tr>
<tr>
<td>Training (X_7)</td>
<td>0.123***</td>
<td>0.044</td>
<td>0.000</td>
<td>0.010</td>
</tr>
<tr>
<td>Extension contact (X_8)</td>
<td>0.179*</td>
<td>0.127</td>
<td>0.092</td>
<td>0.008</td>
</tr>
</tbody>
</table>

Source: Authors’ estimation, 2018.
Note: ***, ** and * indicate significant at 1%, 5% and 10% probability level, respectively.

The coefficient estimates of Logit model revealed that educational level of household head, farm size, annual income, farming experience, training and extension contact had positive impact; and household size and age of household head had negative impact on farmers’ hydroponic fodder technology adoption. The significant variables found from the model were farm size, farming experience, training and extension contact (significant at 10, 5, 1 and 10% probability level, respectively).

The marginal effect estimates indicated that if educational level of household head, farm size, farmers’ annual income, experience of farming, training on this technology, and extension contact with government and non-government extension agents are increased by 1 unit, farmers’ probability of adopting hydroponic fodder production technology will be increased by 0.009, 0.013, 0.013, 0.006, 0.010 and 0.008 percent, respectively, holding other factors constant. On the contrary, if household size of the farmers and age of household head are increased by 1 unit, farmers’ probability of adopting hydroponic fodder production technology will be decreased, keeping other factors the same (Table 4). Njima (2016) supported the findings slightly where the author showed that farmers’ number of dependants and access to information through seminars and internet were the factors influencing hydroponics fodder production among the smallholder dairy farmers in Kenya.
SWOT analysis on hydroponic fodder production technology

SWOT analysis on hydroponic fodder production technology is shown in Table 5. In terms of strengths, 80.0% farmers stated that fodder produced by hydroponic technology was more nutritive compared to field grass for adequacy of fermentable carbohydrates, protein, minerals and vitamins. Unlike field grass production system that use run-to-waste irrigation practices, water spraying and recirculation system could be used in hydroponic fodder technology for reducing the amount of waste water.

Table 5. SWOT analysis matrix

<table>
<thead>
<tr>
<th>Statements</th>
<th>% of farmers</th>
<th>Statements</th>
<th>% of farmers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strengths</td>
<td></td>
<td>Weakness</td>
<td></td>
</tr>
<tr>
<td>i) More nutritive than field grass</td>
<td>80.0</td>
<td>i) High installation cost for production</td>
<td>87.5</td>
</tr>
<tr>
<td>ii) Less requirement of water for fodder production</td>
<td>78.3</td>
<td>ii) Unavailability and higher price of seed</td>
<td>72.5</td>
</tr>
<tr>
<td>iii) Less labour and maintenance cost</td>
<td>54.2</td>
<td>iii) Applicable in low temperature</td>
<td>59.7</td>
</tr>
<tr>
<td>Opportunities</td>
<td></td>
<td>Threats</td>
<td></td>
</tr>
<tr>
<td>i) Can be used in places where in-ground fodder production is not possible</td>
<td>69.2</td>
<td>i) Sensitive to temperature and humidity</td>
<td>91.8</td>
</tr>
<tr>
<td>ii) High consumer demand for being organic</td>
<td>82.5</td>
<td>ii) Fungus affected fodder is less digestive</td>
<td>41.7</td>
</tr>
<tr>
<td>iii) Can be used as medicine for human high blood pressure and cardiac diseases</td>
<td>95.8</td>
<td>iii) Cannot be stored for a longer time</td>
<td>61.7</td>
</tr>
</tbody>
</table>


According to 78.3% farmers, hydroponic fodder technology requires nearly 4% of that water used under field conditions to produce the same amount of fodder. The technology was also appreciated by 54.2% farmers for its less labour requirement and lower maintenance cost. The major weakness that farmers faced was the high initial capital investment (stated by 87.5% farmers). They had to invest a big amount of money to acquire production trays, seeds, equipments and construct a production house. Another major weakness was availability and price of seed (according to 72.5% farmers). It was found that availability of seed was sparse in the market and so, respective price of seed was very high (Table 5).

The biggest opportunity of hydroponic fodder was its use for dual purpose, i.e., for livestock feeding as well as human consumption. Majority of the farmers confirmed that this fodder was highly consumed by the people because of its medicinal value to cure blood pressure and cardiac diseases, and had high demand for livestock feeding as no fertilizer, herbicide or pesticide was used to produce the grass. This technique
was useful to produce fodder in the areas where there was shortage of grazing land or
the land was not suitable for grass production (opined by 69.2% farmers). More than
91% farmers opined that hydroponic fodder was highly sensitive to room temperature
and humidity, which was identified as the major threat for this fodder production
technology. Failure to control temperature and humidity could cause to grow mold,
fungi and bacteria to develop. Farmers (41.7%) reported that if fungus affected
fodder would fed to the livestock, it could create digestion problem to livestock and
in case of extreme level, the livestock could result in a death. About two third farmers
stated that hydroponic fodder was highly perishable in nature (Table 5). Mehta and
Sharma (2016) also found some advantages and disadvantages of hydroponic fodder
production technology which are partly supportive with this SWOT analysis.

**Sustainability of hydroponic fodder production technology**

Sustainability of hydroponic fodder production technology is confirmed when the
physical development and institutional operating practices of this technology meet
the needs of present users and consumers without compromising the ability of future
generations to meet their own needs. In this regard, the authors had developed a 4E
sustainability framework (Table 6) and explained the sustainability of this technology
from the perspectives of energy, environment, economics and equity. The framework
is represented and explained as follows:

Table 6. 4E sustainability framework on hydroponic fodder production technology

<table>
<thead>
<tr>
<th>Sustainability perspectives</th>
<th>Sustainability determinants</th>
<th>Determining responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy</td>
<td>Which sources of energy does hydroponic fodder production technology use?</td>
<td>Generally no external energy is used, but sometimes temperature controller is incorporated in the production house</td>
</tr>
<tr>
<td></td>
<td>Are the energy sources polluting?</td>
<td>No, the energy source is not polluting</td>
</tr>
<tr>
<td></td>
<td>What is the impact of energy use?</td>
<td>The temperature controller is used in some production houses for determining the optimum seed germination temperature</td>
</tr>
<tr>
<td>Environment</td>
<td>Does hydroponic fodder production technology harm the environment?</td>
<td>No, the technology is friendly to the environment and ecology</td>
</tr>
<tr>
<td></td>
<td>Does the technology or its applications impact negative to the consumers?</td>
<td>Hydroponic fodder production technology prohibits the use of all chemical fertilizers, pesticides and medicines. So, the consumption of fodder is safe to the consumers</td>
</tr>
<tr>
<td>Economics</td>
<td>What are the inputs used in</td>
<td>The major variable inputs are seed, tray</td>
</tr>
</tbody>
</table>
### Sustainability perspectives

<table>
<thead>
<tr>
<th>Sustainability determinants</th>
<th>Determining responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>hydroponic fodder production? Are they available?</td>
<td>and watering equipments, and fixed input is production house. The major challenge in this case is the availability of seed in the market</td>
</tr>
<tr>
<td>Is the technology profitable?</td>
<td>Yes, the technology is profitable likely field grass production</td>
</tr>
<tr>
<td>How much profit does the technology gain?</td>
<td>The return is almost double in terms of investment</td>
</tr>
<tr>
<td>Who are the beneficiaries?</td>
<td>The producers as well as the consumers</td>
</tr>
<tr>
<td>How hydroponic fodder production technology can contribute to social or political inequalities?</td>
<td>Now-a-days, a number of women entrepreneurs are getting involved in this technology of fodder production. Thus, gender inequality is lessened</td>
</tr>
<tr>
<td>What is the impact of this technology on stakeholders’ livelihood?</td>
<td>Involvement in hydroponic fodder production creates scope for employment, income generation and poverty reduction in some extent</td>
</tr>
</tbody>
</table>

Source: Authors’ observation, 2018.

Sayara et al. (2016) supported the findings where the authors stated that hydroponic and aquaponic systems are essential for sustainable agriculture and environment in Palestine.

### CONCLUSIONS AND POLICY RECOMMENDATIONS

The study comes to an end with a conclusion that hydroponic fodder production technology as a new fodder production system was cherished highly and adopted successfully by the farmers. The production of hydroponic fodder was highly profitable in the study areas. Farm size, farming experience, training and extension contact had significant impact on adoption of hydroponic fodder production technology by the farmers. The nutritional quality of fodder, high start up cost, medicinal value for human consumption and less digestive capability as the major strength, weakness, opportunity and threat of hydroponic fodder. Hydroponic fodder production technology is sustainable from the perspectives of energy, environment, economics and equity. Considering the findings of the study, some policy recommendations have been arisen which are: input support (especially seed), motivation and extension services of government as well as non-government organizations should be properly implemented to raise farmers’ awareness about adopting hydroponic fodder production technology. This technology of fodder production is considerably appropriate in town areas and in water logging areas. So, initiative for scientific and technical training programmes should be arranged so that
farmers’ can get inspiration for moving into this new dimension of fodder production technology.

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