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CORRELATION AND PATH COEFFICIENT ANALYSIS IN ADVANCED WHEAT GENOTYPES

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ABSTRACT

A field experiment was conducted in alpha lattice design in subtropical region of Nepal in the wheat crop to determine the association between yield and yield attributing traits through correlation and path analysis. The result showed highly significant variations among the genotypes for all the traits under study. Simple correlation coefficients revealed that the association of grain yield with biological yield followed by harvest index, plant height, thousand grain weight and Area Under SPAD Retread Curve (AUSRC) at anthesis were positive and highly significant (at 1% level of significance). The positive and significant (at 5% level of significance) association of grains per spike followed by flag leaf area with grain yield was also found. Path analysis showed that biological yield and harvest index had the highest positive direct effect on grain yield. While other traits contribute to the grain yield significantly indirectly via biological yield and harvest index. This suggests that biological yield and harvest index having significant positive correlation and high direct effect on grain yield explained the true relationship and the direct selection of the genotypes through these traits is effective for improving yield potentiality.

Keywords: Correlation, genotypes, path analysis, selection, traits, wheat

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the third most important staple food crop both in terms of area and production after rice and maize in Nepal with yield potential of 2.49 t ha⁻¹ (MoAD, 2014). This yield is far below than the most wheat producing countries of the world and is not sufficient to fulfill the demands of growing population of Nepal. To minimize the prevalent yield gap and to provide food security in developing countries like Nepal, major efforts of wheat breeders have been directed towards improving its grain yield and development of varieties which

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are high yielding and adaptable to wide range of environment. Chhibber and Jain (2014) reported that the traits affecting and influencing yield needs to be identified and selection has to be exerted on those characters which show a close association with grain yield. In agronomic and breeding studies, correlation coefficients are generally employed to determine the relation of grain yield and yield components. Simple correlation coefficients revealed that thousand-grain weight, number of grains per spike, and plant height showed significant positive correlations with grain yield (Reza et al., 2014) days to maturity had significant positive correlation with spike length and biological yield and grain yield (Gelalcha and Hanchinal, 2013), grain yield had positive correlation with peduncle length, spike length, grains per spike and 1000-grain weight, whereas, negative correlation with days to heading, plant height and tillers per plant (Iftikhar et al., 2012). Mohammadi et al. (2012), Tsegaye et al. (2012) and Zafarnaderi et al. (2013) also reported negative relationship between days to flowering and grain yield per plant in their studies in advanced wheat lines.

Ali and Shakor (2012), Anwar et al. (2009) and Bhutta et al. (2005) also reported that estimation of the correlation between yield and its components alone is not sufficient to understand the importance of each one of these components in determining the grain yield. Path coefficient analysis provides more information among variables than do correlation coefficients since this analysis provides the direct effects of specific yield components on yield and indirect effects via other yield components (Arshad et al., 2006, Del Moral et al., 2003). In agriculture, path analyses have been used by plant breeders to assist in identifying traits that are useful as selection criteria to improve crop yield (Dewey and Lu, 1959, Milligan et al., 1990). A study on path analysis, Iftikhar et al. (2012), indicated that 1000-grain weight had the highest positive direct effect on yield followed by spike length and days to heading while, plant height, grains per spike and peduncle length had negative direct effect on yield. So, these results suggested that traits such as spike length and 1000-grain weight having positive correlation and direct effect on grain yield can be used as suitable selection criteria to develop high yielding genotypes. The purpose of this study, therefore, was to estimate correlation between yields and yield attributing traits as well as the direct and indirect effects of these component traits on yield. The information so derived could be exploited in devising further breeding strategies and selection procedures to develop new varieties of wheat with high yield potentiality.

MATERIALS AND METHODS

Field Experiment

Fifty wheat genotypes including Gautam (a released variety) were investigated in the research field of the Institute of Agriculture and Animal Science (IAAS), Rampur Campus, Chitwan, Nepal in the normal growing season of 2014-2015. The soil type was sandy loam. The experiment was laid out in alpha lattice design with two replications. The plot size was 4m. X 1.5m. = 6 m² each and row spacing in each

plot for wheat sowing was 25 cm. The spacing between two plots was 50 cm and inter spacing between two replications was 1m. Other recommended cultural practices for wheat production were applied during the growing season to raise a good crop.

Data Recorded and Analysis

Observations and measurements were recorded from five randomly selected plants per plot (excluding border plants) for each genotype separately for the following sixteen quantitative yield attributing traits: days to flag leaf emergence (DFL), days to booting (DB), days to heading (DH), days to anthesis (DA), flag leaf area (FLA), area under SPAD retreat curve (AUSRC), days to flag leaf senescence (DFLS), days to maturity (DM), plant height (PH), spike length (SL), peduncle length (PL), grains per spike (GS), thousand grain weight (TGW), biological yield (BY), harvest index (HI) and grain yield (GY). DFL, DB, DH, DA, DF and DM were recorded based on the 50% population observation, FLA was recorded in cm², DFLS was recorded as the date in which 50% of the flag leaves had lost their 90% green color and turned yellow, PH, SL, PL were measured in cm., TGW in grams, AUSRC with SPAD value and yield was converted to Kilograms per hectare for the analysis. Statistical analysis was carried out using Microsoft Office Excel 2013, R-studio (v. 0.99) and SPSS (v. 21). Analysis of Variance (ANOVA) for alpha lattice design was estimated by using methods suggested by Donner and Osman (2007).

RESULTS

Correlation Coefficients

Days to flag leaf emergence vs grain yield

Days to flag leaf emergence had non-significant but negative correlation with grain yield. Days to flag leaf emergence had highly significant and positive correlation with days to anthesis followed by days to heading, days to booting, days to maturity, days to flag leaf senescence. It had highly significant negative correlation with 1000 grain weight followed by HI, FLA and peduncle length.

Days to booting vs grain yield

Days to booting had non-significant and negative correlation with grain yield. It had highly significant and positive correlation with days to anthesis followed by days to heading, days to maturity and days to flag leaf senescence. It exhibited highly significant negative correlation with 1000 grain weight followed by HI. It had significant negative correlation with flag leaf area.

Days to heading vs grain yield

It showed non-significant and negative correlation with grain yield. It exhibited highly significant and positive correlation with days to anthesis followed by days to maturity, and days to flag leaf senescence. It had highly significant negative correlation with harvest index. It showed significant but negative correlation with thousand grain weight.

Days to anthesis vs grain yield

It exhibited non-significant but negative correlation with grain yield. It had highly significant positive correlation with days to maturity followed by days to flag leaf senescence. It showed highly significant negative correlation with 1000 grain weight followed by HI and FLA. It showed significant positive correlation with AUSRC at anthesis followed by spike length. It indicated significant negative correlation with peduncle length.

Flag leaf area vs grain yield

FLA exhibited significant positive correlation with grain yield. It had highly significant positive correlation with peduncle length followed by thousand grains weight and BY. It showed significant positive correlation with plant height.

Area under SPAD retread curve (AUSRC) at anthesis vs grain yield

AUSRC showed highly significant positive correlation with grain yield. It also showed highly significant positive association with GS followed by BY. It had significant positive correlation with days to flag leaf senescence followed by plant height and days to maturity.

Days to flag leaf senescence vs grain yield

Days to flag leaf senescence showed non-significant and positive correlation with grain yield. It had highly significant and positive correlation with DM followed spike length, plant height and BY.

Days to maturity vs grain yield

Grain yield had non-significant negative correlation with DM. DM showed highly significant and positive correlation with spike length. It showed highly significant negative correlation with 1000 grain weight. It exhibited significant but negative correlation with HI.

Plant height vs grain yield

Grain yield exhibited highly significant and positive correlation with plant height. PH had highly significant positive correlation with BY followed by peduncle length.

Spike length vs grain yield

Spike length showed positive correlation with grain yield. It had significant negative correlation with 1000 grain weight but non-significant positive correlation with grains per spike followed by peduncle length and BY.

Peduncle length vs grain yield

It indicated positive correlation with grain yield. It showed highly significant positive correlation with 1000 grain weight and significant positive correlation with biological yield.

Grains per spike vs grain yield

It indicated significant positive correlation with grain yield. It showed significant positive correlation with BY. It showed positive correlation with HI and negative correlation with 1000 grain weight.

Thousand grain weight vs grain yield

It indicated highly significant positive correlation with grain yield. It exhibited significant positive correlation with HI and positive correlation with BY.

Biological yield vs grain yield

It exhibited the highest highly significant positive correlation with grain yield. It showed positive association with HI.

Harvest index vs grain yield

It indicated highly significant positive correlation with grain yield.

Path Analysis

In this paper, the results shown are interpreted for the values with low, moderate and high effects only.

Direct effects on grain yield

The highest (0.30-0.99) positive direct effect on grain yield was exhibited by biological yield (0.737) followed by harvest index (0.555). The positive direct effect on grain yield was also exhibited by thousand grain weight (0.072) followed by days to flag leaf emergence (0.063), days to maturity (0.054), days to booting (0.043), days to heading (0.032), flag leaf area (0.018) and grains per spike (0.010). While days to anthesis followed by AUSRC at anthesis, days to flag leaf senescence, plant height, spike length and peduncle length had negative direct effect on grain yield with the values of -0.072, -0.044, -0.037, -0.028, -0.006 and -0.006 respectively.

Indirect effects on grain yield***Days to flag leaf emergence vs grain yield***

Days to flag leaf emergence exhibited positive direct effect on grain yield with value of 0.063. It also showed moderate (0.20-0.29) indirect negative effect on grain yield via harvest index (-0.242).

Days to booting vs grain yield

Days to booting exhibited negligible direct positive effect on grain yield (0.043) while days to booting showed moderate negative indirect effect on grain yield via harvest index (-0.206).

Days to heading vs grain yield

Days to heading had positive direct effect (0.032) on grain yield. It also exhibited moderate negative indirect effect on grain yield via harvest index (-0.209).

Days to anthesis vs grain yield

Days to anthesis exhibited negative direct effect (-0.072) on grain yield. It also showed moderate negative indirect effect on grain yield via harvest index (-0.255).

Flag leaf area vs grain yield

Flag leaf area exhibited positive direct effect (0.018) on grain yield. It also had moderate positive indirect effect on grain yield via biological yield (0.274).

AUSRC at anthesis vs grain yield

AUSRC at anthesis (SPAD chlorophyll) had negative direct effect (-0.044) and had moderate positive indirect effect via biological yield (0.296) while low positive indirect effect via harvest index (0.106) on grain yield.

Days to flag leaf senescence vs grain yield

Days to flag leaf senescence exhibited negative direct effect (-0.037) and moderate positive indirect effect on grain yield via biological yield (0.274).

Days to maturity vs grain yield

Days to maturity showed positive direct effect (0.054) and low (0.10-0.19) negative indirect effect on grain yield via harvest index (-0.163).

Plant height vs grain yield

Plant height unveiled negative direct effect (-0.028) and had high (0.30-0.99) positive indirect effect on grain yield via biomass yield (0.412).

Spike length vs grain yield

Spike length revealed negative direct effect (-0.006) and low positive indirect effect on grain yield via biological yield (0.107).

Peduncle length vs grain yield

Peduncle length demonstrated negative direct effect (-0.006) and moderate positive indirect effect via biological yield (0.227).

Grains per spike vs grain yield

Grains per spike exhibited positive direct effect (0.010) and moderate positive indirect effect on grain yield via biological yield (0.238) and low positive indirect effect via HI (0.133).

Thousand grain weight vs grain yield

Thousand grain weights showed direct positive effect (0.072) and low positive indirect effect on grain yield via biological yield (0.166) and harvest index (0.193).

Biological yield vs grain yield

The highest (0.30-0.99) positive direct effect on grains per plant was exhibited by biological yield (0.737).

Harvest index vs grain yield

The high positive direct effect of harvest index (0.555) on grain yield was exhibited.

DISCUSSION

There were significant differences among the genotypes for all characters reported here due to diverse genetic background of advanced wheat genotypes used in this experiment.

Correlation and Path Analysis

In the present research, for BY and HI, highly significant and positive correlation was observed with grain yield with values of 0.836** and 0.700** respectively and the direct effects were also positive and highest with values of 0.737 and 0.555 respectively (Table 1 & 2). This suggests that there were little or no indirect effects of these traits on grain yield and whatever relationship existed with grain yield was direct. Singh and Chaudhary (1979) suggested that if the correlation coefficient between a causal factor and the effect is almost equal to its direct effect, the correlation explains the true relationship and the direct selection through these traits is effective. Therefore, these traits (BY and HI) could be used as selection criteria for improving wheat grain yield. Fellahi et al. (2013), Gelalcha and Hanchinal (2013), Tsegaye et al. (2012) also obtained similar result. This implies that selection of wheat genotypes on the basis of biomass yield and harvest index would be beneficial for increasing wheat grain yield.

The correlation coefficient of plant height (0.390**), thousand grain weight (0.386**) and AUSRC at anthesis (0.372**) were also observed to be highly significant and positive with grain yield. The direct effects of these traits with values -0.028, 0.072, -0.044 respectively indicated the negligible effect on grain yield (Table 1 & 2). Similar results for 1000 grain weight with grain yield were also found by Suleiman et al. (2014), significant positive correlation of plant height with grain yield by (Reza et al., 2014) and negative direct effect of plant height on grain yield by Iftikhar et al. (2012) and Suleiman et al. (2014). This indicates that indirect effect seems to be the cause of high correlation showing indirect positive effect through BY and HI on grain yield are the possible cause of positive correlation and negative direct effects are because of the negative indirect effects of the other traits, so these traits are to be considered simultaneously for the selection of wheat genotypes. These findings also tell that increase in thousand grain weight and AUSRC at anthesis increases the grain yield by increasing biomass yield and harvest index. So, while selection of the genotypes for higher grain yield through these traits, BY and HI

Table 1. Correlation coefficients of fifteen traits for grain yield in advanced wheat genotypes

	DFL	DB	DH	DA	FLA	AUSRC	DFLS	DM	PH	SL	PL	GS	TGW	BY	HI	GY
DFL	1															
DB	0.824**	1														
DH	0.835**	0.716**	1													
DA	0.969**	0.844**	0.841**	1												
FLA	-0.433**	-0.341*	-0.263	-0.371**	1											
AUSRC	0.248	0.162	0.197	0.295*	0.164	1										
DFLS	0.500**	0.395**	0.473**	0.533**	0.093	0.328*	1									
DM	0.753**	0.551**	0.702**	0.751**	-0.093	0.289*	0.735**	1								
PH	0.065	0.230	0.099	0.150	0.282*	0.301*	0.410**	0.110	1							
SL	0.238	0.240	0.086	0.294*	0.248	0.161	0.437**	0.440**	0.271	1						
PL	-0.380**	-0.161	-0.230	-0.279*	0.644**	0.116	-0.038	-0.218	0.544**	0.145	1					
GS	0.070	0.226	0.084	0.120	0.089	0.409**	0.001	0.030	0.145	0.149	0.137	1				
TGW	-0.514**	-0.405**	-0.349*	-0.533**	0.374**	0.073	-0.236	-0.506**	0.248	-0.299*	0.394**	-0.112	1			
BY	-0.052	0.025	-0.030	-0.024	0.371**	0.401**	0.372**	0.103	0.558**	0.145	0.307*	0.323*	0.226	1		
HI	-0.437**	-0.372**	-0.376**	-0.459**	0.148	0.191	-0.133	-0.293*	0.005	-0.012	0.042	0.239	0.348*	0.213	1	
GY	-0.259	-0.163	-0.195	-0.260	0.348*	0.372**	0.184	-0.075	0.390**	0.077	0.236	0.361*	0.386**	0.836**	0.700**	1

*Means significance at 5% level, ** means significance at 1% level, without asterisk means non – significance at 5% level. DFL=Days to Flag Leaf emergence, DB= Days to booting, DH= Days to heading, DA= Days to anthesis, FLA= Flag leaf area, AUSRC= Area under SPAD retread curve at anthesis, DFLS= Days to flag leaf senescence, DM= Days to maturity, PH= Plant height, SL= Spike length, PL= Peduncle length, GS= Grains per spike, TGW= Thousand grain weight, BY= Biological yield, HI= Harvest index, GY=Grain yield in kilograms per hectare.

Table 2. Path Analysis Matrix of direct and indirect effects of fifteen traits on grain yield of advanced wheat genotypes

	DFL	DB	DH	DA	FLA	AUSRC	DFLS	DM	PH	SL	PL	GS	TGW	BY	HI
DFL	<u>0.063</u>	0.052	0.053	0.061	-0.027	0.016	0.032	0.048	0.004	0.015	-0.024	0.004	-0.032	-0.003	-0.028
DB	0.036	<u>0.043</u>	0.031	0.037	-0.015	0.007	0.017	0.024	0.010	0.010	-0.007	0.010	-0.018	0.001	-0.016
DH	0.027	0.023	<u>0.032</u>	0.027	-0.008	0.006	0.015	0.022	0.003	0.003	-0.007	0.003	-0.011	-0.001	-0.012
DA	-0.070	-0.061	-0.061	-0.072	0.027	-0.021	-0.038	-0.054	-0.011	-0.021	0.020	-0.009	0.038	0.002	0.033
FLA	-0.008	-0.006	-0.005	-0.007	<u>0.018</u>	0.003	0.002	-0.002	0.005	0.004	0.011	0.002	0.007	0.007	0.003
AUSRC	-0.011	-0.007	-0.009	-0.013	-0.007	-0.044	-0.014	-0.013	-0.013	-0.007	-0.005	-0.018	-0.003	-0.017	-0.008
DFLS	-0.019	-0.015	-0.018	-0.020	-0.003	-0.012	-0.037	-0.027	-0.015	-0.016	0.001	0.000	0.009	-0.014	0.005
DM	0.041	0.030	0.038	0.041	-0.005	0.016	0.040	<u>0.054</u>	0.006	0.024	-0.012	0.002	-0.027	0.006	-0.016
PH	-0.002	-0.006	-0.003	-0.004	-0.008	-0.008	-0.012	-0.003	-0.028	-0.008	-0.015	-0.004	-0.007	-0.016	0.000
SL	-0.001	-0.002	-0.001	-0.002	-0.002	-0.001	-0.003	-0.003	-0.002	-0.006	-0.001	-0.001	0.002	-0.001	0.000
PL	0.002	0.001	0.001	0.002	-0.004	-0.001	0.000	0.001	-0.003	-0.001	-0.006	-0.001	-0.002	-0.002	0.000
GS	0.001	0.002	0.001	0.001	0.001	0.004	0.000	0.000	0.001	0.001	0.001	<u>0.010</u>	-0.001	0.003	0.002
TGW	-0.037	-0.029	-0.025	-0.038	0.027	0.005	-0.017	-0.037	0.018	-0.022	0.028	-0.008	<u>0.072</u>	0.016	0.025
BY	-0.039	0.018	-0.022	-0.018	0.274	0.296	0.274	0.076	0.412	0.107	0.227	0.238	0.166	<u>0.737</u>	0.157
HI	-0.242	-0.206	-0.209	-0.255	0.082	0.106	-0.074	-0.163	0.003	-0.007	0.023	0.133	0.193	0.118	<u>0.555</u>
Correlation	-0.259	-0.163	-0.195	-0.260	0.348*	0.372**	0.184	-0.075	0.390**	0.077	0.236	0.361*	0.386**	0.836**	0.700**

Residual effect: 0.0081. Underlined numbers are positive direct effects (bold face), double underlined numbers are high in magnitude. Values in the off diagonal or columns show indirect effects on grain yield. DFL=Days to Flag Leaf emergence, DB= Days to booting, DH= Days to heading, DA= Days to anthesis, FLA= Flag leaf area, AUSRC= Area under SPAD retreat curve at anthesis, DFLS= Days to flag leaf senescence, DM= Days to maturity, PH= Plant height, SL= Spike length, PL= Peduncle length, GS= Grains per spike, TGW= Thousand grain weight, BY= Biological yield, HI= Harvest index, GY=Grain yield in kilogram per hectare. (High = 0.30-0.99, Moderate = 0.20-0.29, Low = 0.10-0.19)

should also be considered simultaneously in selection. Grains per spike (0.361*) exhibited significant positive association with grain yield and also showed positive direct effect on grain yield with value of 0.010 which is negligible. This indicates that the positive and significant correlation of GS is due to the moderate positive indirect effect of the GS on grain yield through BY (0.238) and low positive indirect effect via HI (0.133). GS had positive and significant correlation with grain yield which was also reported by Gelalcha and Hanchinal (2013). Flag leaf area (0.348*) depicted significant and positive correlation and negligible direct effect (0.018) with grain yield but moderate positive indirect effect on grain yield via BY (0.274). This indicates that casual factor BY should be considered in selection if the selection is to be made through flag leaf area. This also indicates that higher the flag leaf area higher will be the grain yield. Suleiman et al. (2014) also revealed that leaf area index had negative direct effect on yield.

DFLS, SL and PL also showed positive correlation and negative direct effect on grain yield. It indicates that DFLS and PL contribute to grain yield indirectly moderately via BY and SL contribute with low indirect effect via BY. This indicates that these three traits also have importance in breeding of wheat and the genotypes with longer reproductive phase and longer spike length and peduncle length should be selected for wheat improvement. Hence, for selection breeding, indirect casual factor BY and other positively contributing factors should be considered if selection is made through DFLS, SL and PL. Similar correlation results for SL and PL were also revealed by Zafarnaderi et al. (2013). Negative direct effect of peduncle length on grain yield was also reported by Iftikhar et al. (2012). DFL, DB, DH and DM exhibited negative correlation with grain yield but negligible positive direct effect on grain yield. The negative correlation of DA and DH with gain yield was also reported by Mohammadi et al. (2012), Tsegaye et al. (2012) and Zafarnaderi et al. (2013). The negative correlation is due to the moderate negative indirect contribution of the DFL, DB and DH on grain yield via HI (-0.242, -0.206, -0.209 respectively) and that of DM via low indirect effect of HI (-0.163). Days to anthesis had negative correlation and negative direct effect on grain yield. The negative correlation is due to the negative indirect effect of DA on grain yield via HI (-0.255) and other negatively indirectly contributing factors indicating that the early maturing genotypes with longer reproductive phase are better for obtaining high grain yield. These traits indicating relatively non-significant correlation and negligible direct or indirect effect on grain yield are of relatively poor importance in selection breeding for increasing grain yield in these advanced wheat genotypes. Biological yield with flag leaf area, AUSRC at anthesis, days to flag leaf senescence and plant height, individually, showed highly significant and positive inter se association. Similarly, harvest index with days to flag leaf emergence, days to booting, days to heading and days to anthesis, individually, showed highly significant negative inter se association.

Therefore, while selection of the wheat genotypes for increasing grain yield, the yield attributing traits which shows significant correlation and exhibit positive

direct and indirect effect with considerable magnitude on grain yield are to be considered in selection and are of importance in breeding strategies.

CONCLUSION

Correlation analysis results showed that the association of grain yield with biological yield followed by harvest index, plant height, thousand grain weight and AUSRC at anthesis were positive and highly significant whereas positive and significant with grains per spike and flag leaf area indicating that these traits were yield determinative traits. Besides, path analysis revealed that the biomass yield followed by harvest index had the highest positive direct effect on grain yield whereas high positive indirect effect of plant height and moderate positive indirect effects of AUSRC at anthesis, flag leaf area, days to flag leaf senescence, peduncle length and grains per spike on grain yield through biological yield. Strong correlation and positive direct effect of thousand grain weight with grain yield on the one hand and negative direct effect and strong correlation of plant height with grain yield revealed that the selection of these genotypes with high thousand grain weight and moderate plant height should be emphasized while selection for improving grain yield. Therefore, selection of wheat genotypes with high biological yield and high harvest index along with simultaneous consideration of moderate plant height and high thousand grain weight is a prerequisite for attaining improvement in wheat grain yield.

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INFLUENCE OF WEATHER FACTORS ON THE ABUNDANCE AND POPULATION DYNAMICS OF *Spodoptera litura* F. AND *Pieris brassicae* L. ON CABBAGE

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ABSTRACT

In order to study the influence of weather factors on the abundance and population dynamics of *Spodoptera litura* F. and *Pieris brassicae* L. on cabbage, a field experiment was conducted. Larval population of *S. litura* ranged from 0.56 to 1.57 larvae/plant during 8 January to 12 February 2014 crop season while the highest peak was on 5 February 2014 (1.57 larvae/plant) at 29.5 °C temperature. In case of *Pieris brassicae*, larval population ranged from 0.58 to 1.98 larvae/plant and the highest peak of *P. brassicae* was also on 5 February. The highest peak was on 5 February 2014 at 96% and 38% relative humidity of both maximum and minimum categories and the highest peak of *P. brassicae* was on 5 February. There was a strong positive correlation ($r = 0.824$ and $r = 0.920$) between population of *Spodoptera litura* and temperature (maximum and minimum). On the other hand, there was a negative correlation ($r = -0.439$) between population of *S. litura* and maximum relative humidity and a strong negative correlation ($r = -0.716$) between population of *S. litura* and minimum relative humidity. The population of *Pieris brassicae* was positively correlated ($r = 0.899$ and $r = 0.956$) with maximum and minimum temperatures. There was a negative correlation ($r = -0.443$) between population of *P. brassicae* and maximum relative humidity and a strong negative correlation ($r = -0.645$) between population of *P. brassicae* and minimum relative humidity.

Keywords: Cabbage, *Spodoptera litura*, *Pieris brassicae*, weather parameters

INTRODUCTION

Cabbage (*Brassica oleracea* L. var. *capitata*) is one of the most popular vegetables in the world as well as in Bangladesh. It is grown in winter and the

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annual production of cabbage is 217 thousand tons (BBS, 2014) in this country. The production and yield of cabbage is greatly hampered by several insect pests viz., cabbage butterfly (*Pieris brassicae*), diamond back moth (*Plutella xylostella* Linnaeus), tobacco caterpillar (*Spodoptera litura* Fabricius) and cabbage aphid (*Brevicoryne brassicae* L.). Out of these, cabbage butterfly, diamond back moth and tobacco caterpillar are the most destructive pests causing severe yield loss to cabbage every year (Rao and Lal, 2005; Mahla et al., 2005; Kumar et al., 2007). Cabbage butterfly (*P. brassicae*) was recorded as serious pest of cabbage, cauliflower, kohlrabi, broccoli, brussels and sprouts in cauliflower growing areas of the world (Hasan and Ansari, 2011).

In Bangladesh, leaf eating caterpillars such as diamondback moth (DBM) and prodenia caterpillar or common cutworm (*Spodoptera litura*) are considered as the major pests of cabbage (Anonymous, 2013). After hatching, the caterpillars start feeding on the under surface of the leaves. Leaves of heavily damaged plants have many feeding holes and sometimes the leaves take a 'sieve-like' appearance. Larvae also bore into the newly formed head through reaching to the newly emerging little leaf and consume it. As a result of feeding, the plants either fail to form compact cabbage heads or produce deformed heads (Uddin et al., 2007). Though the agro-climatic condition of Bangladesh is highly favourable for the successful cultivation of cabbage, this has not yet translated into higher yield mainly due to the attack of insect pests. The severity of the abundance of different insect pests of cabbage is greatly influenced by the prevailing climatic conditions which vary from region to region, even place to place of the country. Therefore, up to date knowledge about the abundance pattern of major insect pests and their population dynamics on a particular crop is a prerequisite for the implementation of an effective and successful insect pest management programme against them. Keeping these views in mind, the present study was conducted to know the abundance and population dynamics of *Spodoptera litura* F. and *Pieris brassicae* L. on cabbage in relation to weather parameters.

MATERIALS AND METHODS

The field experiment was conducted at agricultural farm of Patuakhali Science and Technology University, Dumki, Patuakhali to study the influence of weather factors on the abundance and population dynamics of *Spodoptera litura* F. and *Pieris brassicae* L. on cabbage during *rabi* season of 2013-2014. Two cabbage varieties viz., Atlas 70 (V_1) and Super tropic (V_2) were included in this study. Experiment was laid out in randomized block design (RBD) with three replications. The entire field was divided into three blocks and each block was again divided into ten plots. The distance between both blocks and plots was 1.0 m. The area of each experimental

plot was 9 m². Seeds of the selected cultivars were sown in the month of November and were transplanted in December. Thirty day-old cabbage seedlings were transplanted in the plot of 9 m² area with 45cm x 45cm spacing on 29 December, 2013. The chemical fertilizers viz., urea, TSP and MP were applied at the rate of 285, 145 and 218 kg ha⁻¹. Fertilizer, irrigate ion and all other agronomic practices were carried out in the experimental field as and when needed. All inputs for example fertilizer application, irrigation, hoeing and other agronomic practices remained same for all cultivars. Recommended management practices except plant protection measures were followed for raising the crop. Weekly observations were taken since one week of transplanting till maturity of the crop.

Observation on population dynamics of *Spodoptera litura* and *Pieris brassicae* started as soon as their infestation was noticed. Population density was determined on the basis of number of larvae per plant on randomly selected plants at weekly interval. All the open leaves and heads of the selected plants were observed thoroughly and the larvae found were recorded. Larvae of tobacco caterpillar and cabbage butterfly were counted from randomly selected 10 plants per replication.

Meteorological parameters (temperature and humidity) were collected from meteorological office of Patuakhali district and correlated with the population of tobacco caterpillar (*Spodoptera litura*) and cabbage butterfly (*Pieris brassicae*) through Microsoft Excel program.

RESULTS AND DISCUSSION

Influence of temperature

Trend of incidence of *Spodoptera litura* and *Pieris brassicae* on cabbage in relation to maximum and minimum temperature on different dates of observations is presented in figure 1. Larval population of *S. litura* ranged from 0.56 to 1.57 larvae/plant during 8 January to 12 February 2014 crop season. *S. litura* was first noticed in the field on 8 January, 2014 (0.68 larvae/plant) with decreasing and increasing pattern which results fluctuation of population on remaining dates of observations. However, the highest peak was on 5 February 2014 (1.57 larvae/plant) at 29.5 °C temperature (Figure 1). In case of *Pieris brassicae*, larval population ranged from 0.58 to 1.98 larvae/plant and more or less similar trend of population fluctuation was observed on various dates of observations. The highest peak of *P. brassicae* was also on 5 February (Figure 1). Maximum and minimum temperature had positive influence on population growth of both species.

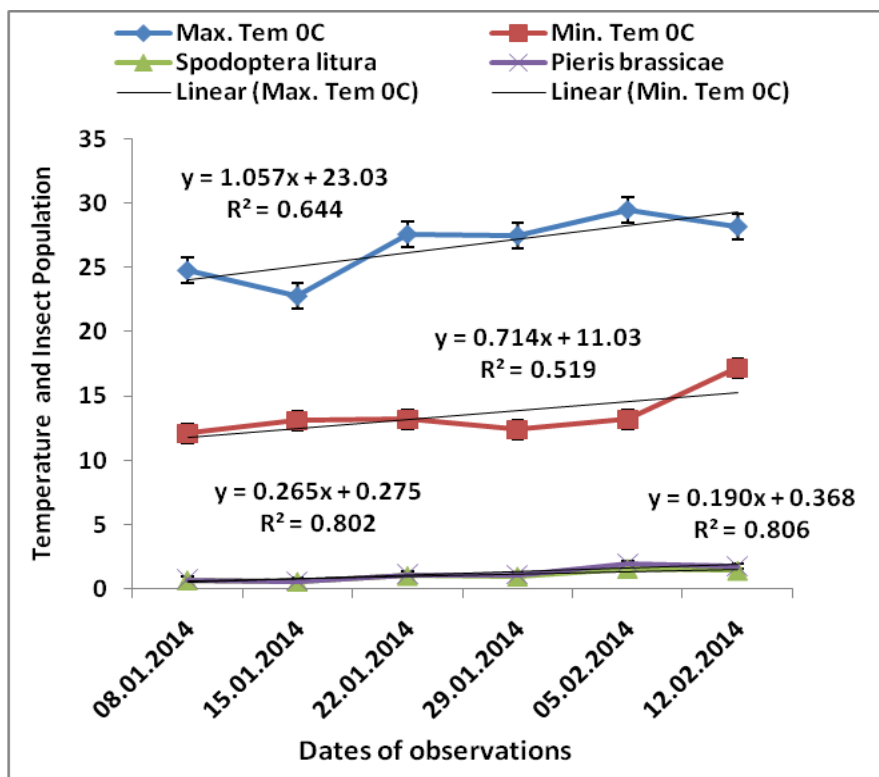


Figure 1. Trend of incidence of *Spodoptera litura* and *Pieris brassicae* on cabbage at different dates of observations as influenced by maximum and minimum temperature

Influence of humidity

Trend of incidence of *Spodoptera litura* and *Pieris brassicae* on cabbage in relation to maximum and minimum humidity on different dates of observations is presented in figure 2. Larval population of *S. litura* ranged from 0.56 to 1.57 larvae/plant during 8 January to 12 February 2014 crop season. The highest peak was on 5 February 2014 (1.57 larvae/plant) (Figure 2) at 96% and 38% relative humidity of both maximum and minimum categories. In case of *Pieris brassicae*, larval population ranged from 0.58 to 1.98 larvae/ plant and more or less similar trend of population fluctuation was observed on various dates of observations. Likewise, the highest peak of *P. brassicae* was on 5 February (Figure 2) at similar conditions of humidity. Maximum and minimum humidity had negative influence on population growth of both species. As incidence of pest depends on host suitability and climatic condition, therefore, incidence and peak infestation of pest vary from variety to variety and due to variation of management practices in cabbage field.

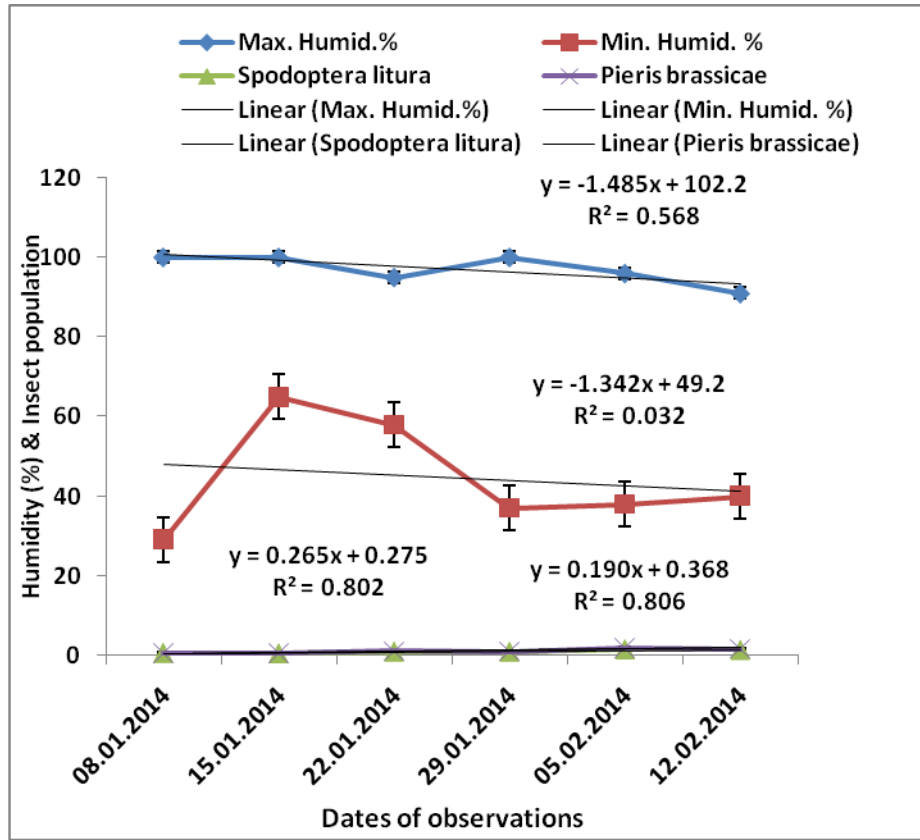


Figure 2. Trend of incidence of *Spodoptera litura* and *Pieris brassicae* on cabbage at different dates of observations as influenced by maximum and minimum humidity

Relationship of *Spodoptera litura* population with weather factors

There was a strong positive correlation ($r = 0.824$ and $r = 0.920$) between population of *Spodoptera litura* and temperature (maximum and minimum) presented in figure 3 (A&B). It indicates that the population of *S. litura* increases with increasing of both maximum and minimum temperatures. The contribution of the regression ($R^2 = 0.679$ and $R^2 = 0.847$) was 68% and 85%, respectively. On the other hand, there was a negative correlation ($r = -0.439$) between population of *S. litura* and maximum relative humidity. However, a strong negative correlation ($r = -0.716$) between population of *S. litura* and minimum relative humidity (Figure 3, C&D). Likewise, the contribution of the regression ($R^2 = 0.193$ and $R^2 = 0.513$) was 19% and 51%, respectively (Figure 3).

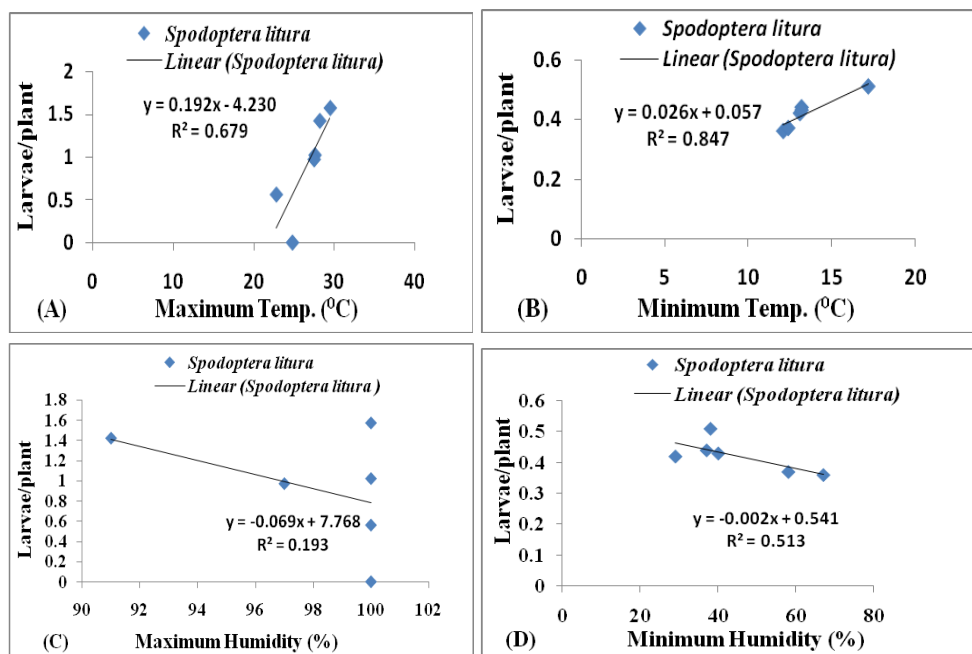


Figure 3. Relationship between *Spodoptera litura* population with (A) maximum temperature ($^{\circ}\text{C}$), (B) minimum temperature ($^{\circ}\text{C}$), (C) maximum humidity (%) and minimum humidity (%) in cabbage

Relationship between *Pieris brassicae* population with weather factors

The population of *Pieris brassicae* was positively correlated ($r = 0.899$ and $r = 0.956$) with maximum and minimum temperatures (Figure 4, A&B). The relationship indicated that the maximum and minimum temperatures had strong influence on the population of *P. brassicae*. The relationship can be expressed as 81% ($R^2 = 0.809$) and 91% ($R^2 = 0.913$), respectively by the contribution of regression. On the other hand, there was a negative correlation ($r = -0.443$) between population of *P. brassicae* and maximum relative humidity and a strong negative correlation ($r = -0.645$) between population of *P. brassicae* and minimum relative humidity (Figure 4, C&D). Likewise, the contribution of the regression ($R^2 = 0.196$ and $R^2 = 0.421$) was 20% and 42%, respectively (Figure 4).

Hemchandra and Singh (2007) reported that higher temperature, lower relative humidity, lower total rainfall, longer duration of sunshine hours and higher wind speed seem to favour the pest population build up. Patait et al. (2008) found that the population of *Crociodolomia binotalis* [*C. pavonana*], *Hellula undalis*, *Plutella xylostella* and *Spodoptera litura* on cabbage varied from 3.8 to 44.0, 1.0 to 6.2, 0.6 to 1.6 and 0.6 to 3.2 and 1.0 to 5.0, 1.0 to 1.6, 1.6 to 20.4 and 0.2 to 1.0 larvae/quadrat

during rainy and winter seasons of 2006-07 in Latur, Maharashtra, India, respectively. The population of *H. undalis* and *S. litura* was affected positively by the action of minimum temperature and rainy days and negatively by forenoon relative humidity and rainfall (Patait et al., 2008). They also reported that the population of *S. litura* was influenced positively by forenoon relative humidity and negatively by minimum temperature and afternoon relative humidity. The findings of Patait et al. (2008) is slightly contradict with the findings of the present study.

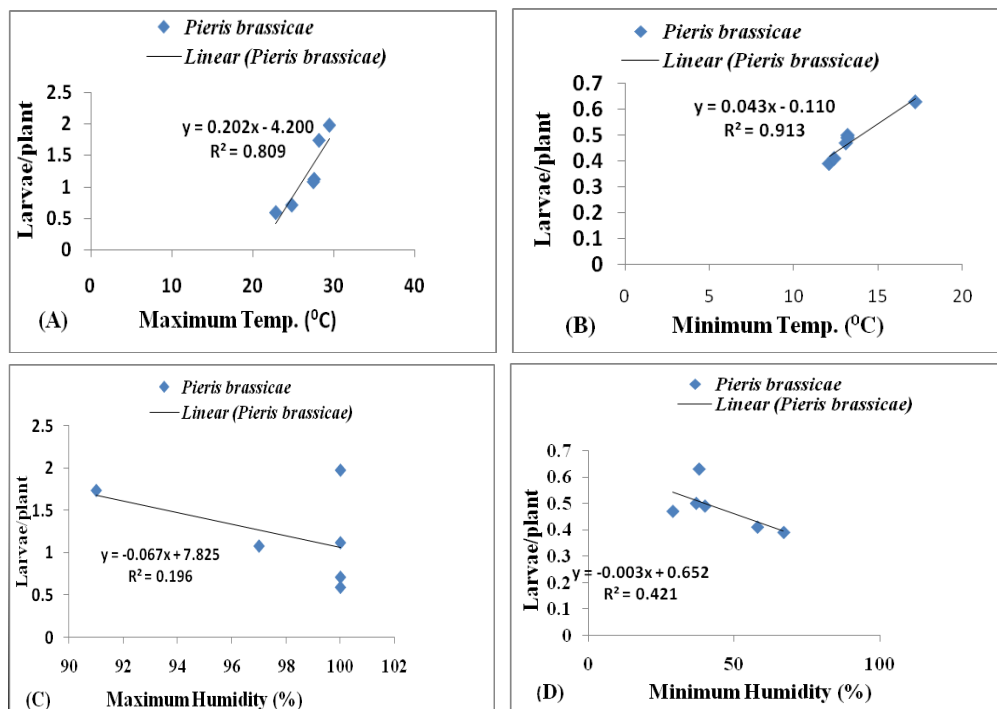


Figure 4. Relationship between *Pieris brassicae* population with (A) maximum temperature ($^{\circ}\text{C}$), (B) minimum temperature ($^{\circ}\text{C}$), (C) maximum humidity (%) and minimum humidity (%) in cabbage

Patra et al. (2012) reported negative influence of temperature; relative humidity and rainfall on population build up of aphid at Meghalaya condition. Both maximum and minimum temperature had major role to build up the population of diamond back moth, *C. plutellae* and coccinellids beetle while aphid population was enhanced only by maximum temperature (Patra et al., 2013). Vanlaldiki et al. (2013) found that the larvae of diamondback moth first appeared at the end of January (0.20 and 0.15/plant), increased gradually and reached its peak by the end of March with a population (12.05 and 11.20/plant) during the two seasons respectively. The larval population declined (2.20 and 0.60/ plant) by the second week of April in both the

years. The correlation studies indicated a significant positive correlation between larval population of diamondback moth and maximum and minimum temperature with an exception to the second year while maximum temperature showed a non-significant interaction. During 2009-10, the relative humidity (R.H), total rainfall and bright sunshine hours (BSSH) had negative correlation, whereas in the second year (2010-2011), R.H and rainfall had positive relation with the larval population of diamondback moth. There was a marked variation of diamondback moth incidence in different dates of planting. The result showed that early planted crop (15 November) harbored least number of the target pest (0.06/plant) with highest yield (20.80 t ha⁻¹) whereas late planting (14 January) resulted in highest population (3.37 /plant) with the lowest yield of 6.15 t ha⁻¹ and devoid of any marketable heads. Bana et al. (2012) reported that the maximum and minimum temperatures showed significant negative correlation with aphid and larval population of diamondback moth, whereas, relative humidity and sunshine hours showed non-significant correlation. Ahmed and Ansari (2010) found that temperature and humidity recorded maximum and minimum i.e., 24.15° to 32.91°C and 68.60 to 91.30 percent, respectively. Population build up is usually observed in II to IV week of September. *Cotesia plutellae* was found to be a dominant larval parasitoid while, *Oomyzus sokolowskii* parasitized relatively few pupae of *P. xylostella*. 34.77°C significantly ($p < 0.01$) enhanced the population of DBM also on 8 September, 8 October, 2004 and 26 January, 2005. Rainfall negatively affected the DBM population in 2004–2005 and 2005–2006. Venkateswarlu et al. (2011) reported that among different abiotic factors, maximum and minimum temperature had significant positive correlation whereas morning and evening relative humidity showed significant negative correlation with DBM population. Sarkar et al. (2007) reported that DBM was the most abundant during January-February when the maximum and minimum temperature varied from 21.1 to 31.40C and 7.9 to 19.90C, respectively on yellow sarson under West Bengal condition.

CONCLUSION

The highest peak populations of *Spodoptera litura* and *Pieris brassicae* were found on 5 February 2014 at 29.5 °C temperature and at 96% relative humidity. Temperature had positive influence on populations of both species while humidity had negative influence on them.

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HORSE PERSULANE MANAGEMENT IN COTTON BY ALLELOPATHIC CROP WATER EXTRACTS ALONG WITH REDUCED DOSES OF HERBICIDES

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ABSTRACT

The experiment was conducted at the field of University of Agriculture, Faisalabad, Pakistan to evaluate the performance of lower dose of herbicides in combination with allelopathic crop water leachates for controlling horse persulane (*Trianthemaportulacastrum* L.) weed in cotton field during two consecutive years of 2007 and 2008. Seven treatments viz., T₁= control as weedy, T₂= Pendimethalin @ 1.25 kg active ingredient (a.i.) ha⁻¹ (full dose) as pre-emergence, T₃= Paraquat @ 200 g a.i. ha⁻¹ (full dose) direct shielded at 40 days after sowing (DAS), T₄= Pendimethalin @ 0.417 kg a.i. ha⁻¹ (1/3 dose) as pre-emergence, T₅= Paraquat @ 67g a.i. ha⁻¹ (1/3 dose) direct shielded at 40 DAS, T₆=sorghum + sunflower + brassica + mulberry water extracts each @ 18 l ha⁻¹ + Pendimethalin @ 0.417 kg a.i. ha⁻¹ (1/3 dose) as pre-emergence, and T₇ = sorghum + sunflower + brassica + mulberry water extracts each @ 18 l ha⁻¹ + Paraquat @ 67g a.i. ha⁻¹ (1/3 dose) direct shielded at 40 DAS were compared. Combination of water leachates of allelopathic crops with either 1/3 dose of Paraquat or with 1/3 dose of Pendimethalin were found more effective than other treatments for reducing density and dry weight of horse persulane at both the stages. Higher but identical number of bolls per plant, boll weight per plant and seed cotton yield were recorded in water leachates + 1/3 dose of Pendimethalin as pre-emergence, and water leachates + 1/3 dose of Paraquat direct shielded at 40 DAS. Higher gross return and gross margin were also obtained from the same treatments. The results revealed that sorghum, sunflower, brassica and mulberry water extracts each @ 18 l ha⁻¹ in combination with 1/3 dose of Paraquat direct shielded at 40 DAS or with 1/3 dose of Pendimethalin as pre-emergence is economical, environment friendly and sustainable strategy for controlling horse persulane weed in cotton field in Pakistan.

Keywords: Allelopathic crop water extracts, cotton, horse persulane, paraquat, pendimethalin and water leachates.

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INTRODUCTION

Cotton is the main fiber crop in Pakistan. It plays a significant role in economy of the country. Share of cotton in value addition in agriculture accounts for 8.6% and that in GDP is 1.8%. Based on area of cotton, Pakistan ranks 5th in the world while in production, it ranks 3rd position (Economic Survey of Pakistan, 2006). The average yield of cotton in Pakistan is much lower than potential yield. The yield gap among research farms, progressive farmers and common farmers is primarily due to lack in adoption of advanced management practices specially plant protection. Plant protection and weed management are the most neglected components, which result in yield loss up to 20-40% in different crops (Rehman et al., 2013). Chemical weed control is an efficient and effective method, and widely practiced in all crops especially in cotton. Although herbicides are effective tool for weed control yet certain limitations confront their use. Specific knowledge is needed for their use, which is lacking in Pakistan. It is due to unawareness and limited trained manpower (Rehman et al., 2013). Sometimes, selection of improper herbicide leads to create resistance in weeds, and weeds remain uncontrolled. Moreover, herbicides may pollute soil and water. So, it is a dire need to find out new environment friendly techniques and methods for controlling weeds. One possible option may be the reduction of herbicide usage. Another possibility is to develop natural products, bio-herbicides and utilizing allelopathic crop water leachates as foliar spray or combined application of these leachates with lower doses of the recommended herbicides. They are effective, economical, viable and environment friendly. There are two fundamental approaches to use natural products for weed management. First one is to use them as allelochemicals in crops or cover crops to manage weeds, insects, pests and pathogens (Duke et al., 2002). The other strategy is to use them directly as herbicides in the form of crop water leachates. Use of sorgaab (sorghum water leachate) as natural weed inhibitor in maize has been suggested by Ahmad et al. (1995). Cheema et al. (2003) stated that sorgaab reduces density as well as dry weight of horse persulane weed and thereby increased maize yield by 13-37%.

Similarly, Singh et al. (2003) reported that weeds cause enormous loss of crop yield by interfering agro-ecosystems. Worldwide efforts are being made to reduce the usage of synthetic herbicides for weed control. So, the use of allelochemical is an alternative environment friendly method of weed control in all crops. The crops possessing allelopathic potential should be included in crop rotation, or use them as cover crop, smother crop, green manures, or intercropped for sustainable weed management. However, literature regarding allelopathic weed control in Pakistan is meager. Hence, this experiment was conducted to evaluate the performance of lower dose of herbicides in combination with allelopathic crop water leachates for controlling horse persulane weed in cotton field.

MATERIALS AND METHODS

A field experiment was undertaken at the University of Agriculture, Faisalabad, Pakistan on loamy soil having moderate fertility during two consecutive years of 2007 and 2008 to determine suitable combination of sorghum (*Sorghum bicolor* L.), sunflower (*Helianthus annuus* L.), mulberry (*Morus alba* L.) and Brassica (*Brassica spp.*) water extracts @ 18 l ha⁻¹ with reduced doses of Paraquat (Gramoxon 20 SL), and Pendimethalin (Stomp 330 E) for weed management in cotton. The experiment was laid out in Randomized Complete Block Design with four replications having a net plot size of 7m x 3m. Seven treatments were: T₁= control as weedy, T₂= Pendimethalin @ 1.25 kg active ingredient (a.i.) ha⁻¹(full dose) as pre-emergence, T₃= Paraquat @ 200 g a.i. ha⁻¹ (full dose) direct shielded at 40 days after sowing (DAS), T₄= Pendimethalin @ 0.417 kg a.i. ha⁻¹(1/3 dose) as pre-emergence, T₅= Paraquat @ 67g a.i. ha⁻¹(1/3 dose) direct shielded at 40 DAS, T₆=sorghum + sunflower + brassica + mulberry water extracts each @ 18 l ha⁻¹ + Pendimethalin @ 0.417 kg a.i. ha⁻¹(1/3 dose) as pre-emergence, and T₇ = sorghum + sunflower + brassica + mulberry water extracts each @ 18 l ha⁻¹ + Paraquat @ 67g a.i. ha⁻¹(1/3 dose) direct shielded at 40 DAS were compared.

Crop herbage (sorghum, sunflower, brassica and mulberry) was harvested at maturity, dried under shade and then chopped into 2 cm pieces with the help of fodder cutter. This chopped material was soaked in distilled water with a ratio of 1:10 (w/v) for 24 hours. Water extracts were collected by passing through sieves. The filtrate was boiled at 100°C for reducing the volume by 20 times (Cheema and Khaliq, 2000).

Cotton cultivar MNH-786 was sown on a well prepared seedbed in 75 cm spaced rows with single row hand drill on 5 May 2007 and 12 May 2008, using 15 kg ha⁻¹ delinted seed. Thinning was done to maintain 30 cm plant to plant distance. Fertilizer @ 125-57-23 NPK kg ha⁻¹ was applied during both the years. Half of nitrogen with full doses of phosphorous and potassium was applied at the time of sowing, while the remaining half of nitrogen was applied followed by first irrigation. First irrigation was given at 35 DAS and subsequent irrigations were adjusted according to the climatic conditions and need of the crop. The crop was harvested at maturity stage.

Data on weed density and weed dry weight were taken at 40 and 60 DAS. Germination count was performed after emergence of the crop. Leaf area per plant was taken from randomly collected three plants from each plot at 90 and 120 DAS. Plant height, number of monopodial and sympodial branches per plant, number and weight of bolls per plant were collected at maturity stage. Seed cotton yield was taken from whole plot. Ginning out turn (GOT) was calculated by using the following formula:

$$\text{GOT\%} = \frac{\text{Weight of lint}}{\text{Weight of seed cotton}} \times 100$$

Economic analyses were performed based on the cost occurred for weed control practices in different treatments.

The trend of data collected during both the years was found similar. So, the data were averaged. The average data were analyzed statistically and differences among the treatment means were compared by using the Least Significant Difference (LSD) test at 0.05 probability level with the help of M-Stat C programme.

RESULTS AND DISCUSSION

A. Horse persulane density and dry matter weight

Density of horse persulane was high at 40 DAS and then declined at 60 DAS in all treatments (Table 1). Pendimethalin suppressed the weed by 47.2, 56.8 and 66.4% at 40 DAS and 47.0, 63.6 and 62.1% at 60 DAS when applied at the rate of 1/3 dose, full dose and 1/3 dose with leachates, respectively. But Paraquat suppressed the weeds by 9.6, 13.6 and 19.2% at 40 DAS and 34.9, 39.4 and 40.9% at 60 DAS when applied at the rate of 1/3 dose, full dose and 1/3 dose with leachates, respectively. These results indicated that weed suppression by Pendimethalin with water leachates was more than those of Paraquat with water leachates at both the stages. This was because of pre-emergence application of Pendimethalin which killed most of the weeds at early stages. Weed mortality with Paraquat as post-emergence spray was comparatively lower. These results are partially similar to the findings of Cheema et al. (2000a) who reported that Pendimethalin dose could be reduced to 67% in combination with Sorgaab.

Weed dry weight at 40 DAS was more than at 60 DAS when Paraquat was applied. But at 60 DAS, higher dry weight with less density was observed when Pendimethalin was applied owing to less intra specific competition (Table 1). However, dry weight reduction of weeds in Paraquat treatments at 60 DAS was 58.4, 85.0 and 86.1% as against to 32.1, 49.4 and 63.3% in Pendimethalin when applied at 1/3 dose, full dose and 1/3 dose with water leachates, respectively. These results partially support the findings of Cheema et al. (2003a) who suggested that 67% reduction in Pendimethalin dose in combination with allelopathic crop water leachates is very effective to suppress weeds flora in wheat. While findings regarding Paraquat and allelopathic crop water leachates are encouraging and in line with the work of Cheema et al. (2003b) who suggested that allelopathic crop water leachates in combination with lower herbicides rate could be used for weed control in cotton.

Table 1. Effect of combined application of allelopathic crop water leachates and lower herbicide rates on density and dry weight of horse persulane

Treatment	Weed density (no. m ⁻²)		Weed dry weight (g m ⁻²)	
	40 DAS	60 DAS	40 DAS	60 DAS
T ₁	125	66	320	1190
T ₂	54 (-56.8)	24 (-63.6)	227 (-29.1)	602 (-49.4)
T ₃	108 (-13.6)	40 (-39.4)	283 (-11.6)	178 (-85.0)
T ₄	66 (-47.2)	35 (-47.0)	249 (-22.2)	808 (-32.1)
T ₅	113 (-09.6)	43 (-34.8)	286 (-10.6)	496 (-58.4)
T ₆	42 (-66.4)	25 (-62.1)	210 (-34.4)	437 (-63.3)
T ₇	101 (-19.2)	39 (-40.9)	266 (-16.9)	166 (-86.1)
LSD _(0.05)	9.23	6.86	34.8	20.7

*Figures within parenthesis indicate % decrease (-) or increase (+) over control

T₁= control as weedy, T₂= Pendimethalin @ 1.25 kg active ingredient (a.i.) ha⁻¹(full dose) as pre-emergence, T₃= Paraquat @ 200 g a.i. ha⁻¹(full dose) direct shielded at 40 days after sowing (DAS), T₄= Pendimethalin @ 0.417 kg a.i. ha⁻¹(1/3 dose) as pre-emergence, T₅= Paraquat @ 67 g a.i. ha⁻¹(1/3 dose) direct shielded at 40 DAS, T₆=sorghum + sunflower + brassica + mulberry water extracts each @ 18 l ha⁻¹ + Pendimethalin @ 0.417 kg a.i. ha⁻¹(1/3 dose) as pre-emergence, and T₇ = sorghum + sunflower + brassica + mulberry water extracts each @ 18 l ha⁻¹ + Paraquat @ 67 g a.i. ha⁻¹(1/3 dose) direct shielded at 40 DAS

B. Cotton

Germination remained unaffected among the treatments (Table 2). These results are against the findings of Vasilakoglou et al. (2005) who reported the positive effect of allopathic water extracts on germination of cotton.

Cotton plant height was significantly more in all treatments than control and the tallest plants were observed in T₇ and T₆ treatments which were statistically at par with each other (Table 2). Similar observations were reported by Cheema et al. (2000) who reported that when weeds were managed, plant height of cotton crop was more as compared to weeds infested crop.

Number of monopodial and sympodial branches was similar to those of plant height showing that taller plants had more number of branches (Table 2). More branching in taller plant was observed due to better weed control. These results

confirmed the findings of Cheema et al. (2000b) who stated that both monopodial and sympodial branches increased with weed suppression in cotton.

Table 2. Effect of combined application of allelopathic crop water leachates and lower herbicide rates on cotton germination, plant height and branches of cotton

Treatment	Germination (m ⁻²)	Plant height (cm)	No. of Branches (plant ⁻¹)	
			Monopodial	Sympodial
T ₁	6.25	90.0	3.30	14.8
T ₂	5.75	100.6	3.34	19.2
T ₃	5.68	101.0	4.05	23.6
T ₄	5.63	100.0	3.30	18.9
T ₅	5.50	97.1	3.30	18.2
T ₆	5.37	103.8	4.50	26.8
T ₇	5.38	105.0	4.23	25.8
LSD _(0.05)	NS	4.09	0.330	1.99

*NS = Non-significant

T₁= control as weedy, T₂= Pendimethalin @ 1.25 kg active ingredient (a.i.) ha⁻¹(full dose) as pre-emergence, T₃= Paraquat @ 200 g a.i. ha⁻¹(full dose) direct shielded at 40 days after sowing (DAS), T₄= Pendimethalin @ 0.417 kg a.i. ha⁻¹(1/3 dose) as pre-emergence, T₅= Paraquat @ 67 g a.i. ha⁻¹(1/3 dose) direct shielded at 40 DAS, T₆=sorghum + sunflower + brassica + mulberry water extracts each @ 18 l ha⁻¹ + Pendimethalin @ 0.417 kg a.i. ha⁻¹(1/3 dose) as pre-emergence, and T₇ = sorghum + sunflower + brassica + mulberry water extracts each @ 18 l ha⁻¹ + Paraquat @ 67 g a.i. ha⁻¹(1/3 dose) direct shielded at 40 DAS

Maximum number of bolls per plant were recorded in T₆ (30.6) and T₇ (29.3) treatments (Table 3). These treatments were statistically at par with each other but significantly higher than other treatments. More number of bolls per plant resulted in increase of boll weight per plant in these treatments (Table 3). Increase in number of bolls and boll weight per plant was possibly due to better weed management. These results supported the findings reported by Cheema et al. (2000b) who narrated that sorgaab, sorghum mulch and herbicidal treatments significantly influenced the number of bolls and boll weight per plant due to better weed control.

Maximum leaf area at both stages (515.3 cm² at 90 DAS and 559.3 cm² at 120 DAS) was recorded in T₇ treatment. But in other treatments, leaf area at both stages was similar (Table 3).

Seed cotton yield varied significantly due to different weed management treatments (Table 4). The increase in seed cotton yield over control ranged from

48.6% to 97.5%. Maximum seed cotton yield (2305 kg ha^{-1}) was observed in T_7 treatment and it was statistically identical to T_6 treatment (2276 kg ha^{-1}). The reason for higher seed cotton yield in those treatments was due to more number of bolls and boll weight per plant. Almost similar effects of allelopathic crop water leachates on seed cotton yield were observed by Cheema et al. (2002) who demonstrated that combination of allelopathic crop water leachates and 1/3 dose of Paraquat increased seed cotton yield.

Table 3. Effect of combined application of allelopathic crop water leachates and lower herbicide rates on bolls, boll weight and leaf area of cotton

Treatment	No. of Bolls (plant ⁻¹)	Boll weight (g plant ⁻¹)	Leaf area (cm ²)	
			90 DAS	120 DAS
T ₁	18.4	55.9	444.4	459.5
T ₂	26.8	106.6	446.0	495.4
T ₃	27.8	119.0	467.6	510.0
T ₄	22.9	82.9	443.0	448.4
T ₅	21.5	71.3	459.4	475.6
T ₆	30.6	129.7	469.5	510.1
T ₇	29.3	132.6	514.3	559.3
LSD _(0.05)	2.14	14.24	15.11	19.51

T₁= control as weedy, T₂= Pendimethalin @ 1.25 kg active ingredient (a.i.) ha⁻¹(full dose) as pre-emergence, T₃= Paraquat @ 200 g a.i. ha⁻¹(full dose) direct shielded at 40 days after sowing (DAS), T₄= Pendimethalin @ 0.417 kg a.i. ha⁻¹(1/3 dose) as pre-emergence, T₅= Paraquat @ 67 g a.i. ha⁻¹(1/3 dose) direct shielded at 40 DAS, T₆=sorghum + sunflower + brassica + mulberry water extracts each @ 18 l ha⁻¹ + Pendimethalin @ 0.417 kg a.i. ha⁻¹(1/3 dose) as pre-emergence, and T₇ = sorghum + sunflower + brassica + mulberry water extracts each @ 18 l ha⁻¹ + Paraquat @ 67 g a.i. ha⁻¹(1/3 dose) direct shielded at 40 DAS

Ginning out turn (GOT) was not affected by any of the treatments (Table 3). Similar findings were reported by Cheema et al. (2000b) who found no effect on GOT% by different weed control practices. This behavior is due to the genetic potential of variety.

C. Economic analysis

Gross return increased with increasing seed cotton yield and the highest gross return (US\$ 864.375 ha⁻¹) was recorded in T₇ treatment which was close to T₆ treatment (US\$ 853.500 ha⁻¹). Weed control cost includes herbicide cost, extract cost, sprayer rent and labour cost. Weed control cost was maximum (US\$ 5.15 ha⁻¹) in T₇ and it was very close to T₆ (US\$ 5.12 ha⁻¹). Gross margin ranged from US \$ 437.625

to 859.225 in different treatments. The highest gross margin was obtained from T₇ treatment and it was close to T₆ treatment (Table 4). The results indicated that T₇ and T₆ treatments were more profitable than other weed management treatments.

Table 4. Effect of combined application of allelopathic crop water leachates and lower herbicide rates on seed cotton yield, ginning out turn (GOT) and economics

Treatment	Seed cotton yield (kg ha ⁻¹)	GOT (%)	Gross return** (US\$ ha ⁻¹)	Weed control cost*** (US\$ ha ⁻¹)	Gross margin (US\$ ha ⁻¹)
T ₁	1167	40.1	437.625	0	437.625
T ₂	1831 (56.9)	42.3	686.625	3.04	683.585
T ₃	1915 (64.1)	41.4	718.125	3.15	714.975
T ₄	1742 (49.4)	40.2	653.250	2.32	650.930
T ₅	1734 (48.6)	40.2	650.250	2.35	647.900
T ₆	2276 (95.1)	41.1	853.500	5.12	848.380
T ₇	2305 (97.5)	41.3	864.375	5.15	859.225
LSD _(0.05)	48.4	NS	-	-	-

*NS = Non-significant, **Market price of seed cotton: US\$ 0.375 kg⁻¹

*** Weed control cost includes: herbicide cost, extract cost, sprayer rent and labour cost

T₁= control as weedy, T₂= Pendimethalin @ 1.25 kg active ingredient (a.i.) ha⁻¹(full dose) as pre-emergence, T₃= Paraquat @ 200 g a.i. ha⁻¹(full dose) direct shielded at 40 days after sowing (DAS), T₄= Pendimethalin @ 0.417 kg a.i. ha⁻¹(1/3 dose) as pre-emergence, T₅= Paraquat @ 67 g a.i. ha⁻¹(1/3 dose) direct shielded at 40 DAS, T₆=sorghum + sunflower + brassica + mulberry water extracts each @ 18 l ha⁻¹ + Pendimethalin @ 0.417 kg a.i. ha⁻¹(1/3 dose) as pre-emergence, and T₇ = sorghum + sunflower + brassica + mulberry water extracts each @ 18 l ha⁻¹ + Paraquat @ 67 g a.i. ha⁻¹(1/3 dose) direct shielded at 40 DAS

CONCLUSION

From the study, it might be concluded that combination of sorghum, sunflower, brassica and mulberry water leachates at the rate of 18 l ha⁻¹ with 1/3 dose of Paraquat at 40 DAS direct shielded or 1/3 dose of Pendimethalin as pre-emergence is highly effective, economical, environment friendly and sustainable strategy for controlling horse persulane weed in cotton field for increasing cotton yield in Pakistan.

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DISTRIBUTION OF FOOT AND MOUTH DISEASE VIRUS SEROTYPES IN CATTLE OF BANGLADESH

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ABSTRACT

Foot and mouth disease (FMD) is a highly contagious viral infection in cloven hoofed domestic and wild animals and endemic in many countries of the world including Bangladesh. Clinical investigation was carried out to identify natural cases of FMD and characteristics signs of FMD like salivation, ulceration in oral and pedal tissues and lameness was seen. The specific serotypes of FMD viruses involved in infected cattle were, therefore, identified using reverse transcriptase polymerase chain reaction (RT-PCR). Samples (N=97) from oral lesions was collected from infected cattle from seven divisions of Bangladesh during May to December, 2013. Viral RNA was extracted from the infected oral tissues and FMD virus specific uniplex RT-PCR was designed to detect FMD viruses. Multiplex RT-PCR was adapted to detect serotype specific amplicons. Out of 97 samples tested in uniplex and multiplex RT-PCR, 92 and 90 samples showed amplification reaction for FMD virus and viral serotypes respectively. Among the 90 FMD virus specific positive identification, single infectivity due to FMD viral Serotypes O, A and Asia 1 were seen in 56 (62.2%), 13 (14.4%) and 16 (17.8%) cases respectively. Three cattle (3.3%) were co-infected with FMD viral Serotypes O and Asia 1 and two (2.2%) with FMD viral Serotypes O and A. FMD viral serotype O was dominating all over the country followed by Asia1 and A. Cattle of Bangladesh were infected with FMD viral serotype O, A and Asia 1 alone or in combination. The RT-PCR protocols designed and adapted successfully detected FMD viruses and viral serotypes in a fraction of the time required for virus isolation and serological detection. These RT-PCR protocols can be used for rapid serotyping of FMD viruses from field infectivity and selection of vaccine viruses.

Keywords: Amplicon, FMD, FMDV, RNA, RT-PCR, serotype, vaccine

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INTRODUCTION

Foot and mouth disease (FMD) is an acute, highly contagious viral disease affecting all cloven-hoofed animals including cattle, swine, sheep, goats, wild pigs, wild ruminants and buffaloes. Clinically, FMD is characterized by vesicles formation in the mouth and on the muzzle, feet and sometimes on udders. Severe myocarditis causes high rate of mortality in infected newborns. The infection in adult animals is rarely lethal, it results in significant losses due to a decrease in production through reduced milk yield, loss of weight, abortions, delayed conception, and the restriction on international trade of animals and animal products (James and Rushton, 2002; Grubman and Baxt, 2004). Foot and Mouth disease virus (FMDV) is a non-enveloped, single stranded and positive sense RNA virus of the genus *Aphthovirus* belonging to the family *Picornaviridae* (Mumford, 2007). There are seven FMD viral serotypes namely O, A, C, SAT1, SAT2, SAT3, Asia1 and about 65 subtypes; vaccination with one serotype or subtype does not confer solid protection against others (Brown, 2003; OIE 2006; Saiz et al., 2002). Serotypes O and A are widely distributed, where as serotypes SAT1, SAT2 and SAT3 are restricted to Africa and serotype Asia1 to Asia (Knowles, and Samuel, 2003), rare incursions into other regions have been recorded. The last outbreak due to serotype C FMDV was reported in Bangladesh during 1996 (Kitching, 1998) and in Ethiopia during 2005 (WAHID 2009) and serotype C viruses may no longer exist outside the laboratories.

FMD is endemic in Bangladesh (Islam et al., 2001) and sequence analysis of the capsid protein encoding genome region of the isolates collected in 1999 and 2000 showed that serotype O and serotype A were circulated in Bangladesh (Loth et al., 2011). FMD viruses isolated from outbreak areas in Bangladesh in late 2009 were also belonged to the viral serotype O (Nandi et al., 2013). Recently, the country has faced severe outbreaks of FMD due to serotypes O and A, which is closely related to the viruses circulating in India and Nepal (Sarker et al., 2011). Diagnosis of FMD is commonly made by observing clinical signs. The viral serotypes in the laboratory can be done by virus isolation, demonstration of the FMD viral antigens or nucleic acid in a sample tissue or fluid by RT-PCR (OIE 2009). Detection of specific anti viral antibodies can also be made by using ELISA. This study was aimed to identify the naturally infected cattle by clinical examination and detect FMD viruses and viral serotypes from infected cases by means of RT-PCR, a sensitive, reliable and specific laboratory technology. Detection of FMD viral serotypes is required to understand distribution pattern of FMD, prepare vaccine seed using specific serotypes and design future preventive strategies.

MATERIALS AND METHODS

Investigation of infected cattle and collection of samples

The infected cattle were investigated for the presence of ulcerative lesions in oral and pedal tissues and salivation. The salivation was visible by distance

observation and oral and pedal lesions were investigated before collecting samples. A total of 97 clinically ill cattle were examined and samples (epithelial tissues from oral lesions) were collected in transport media (MEM with antibiotic and antimycotic agents). Samples were collected (Figure 1) randomly from selected areas of Barisal (n=9), Chittagong (n=15), Dhaka (n=25), Khulna (n=10), Rajshahi (n=16), Rangpur (n=11) and Sylhet (n=11) divisions of Bangladesh during May to December, 2013. The samples were shifted to the laboratory, Department of Pathology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh in chilled condition and preserved at -80°C until used in RNA extraction and RT-PCR detection of the viruses.

RNA extraction and R-T PCR detection of viruses

Total RNA was extracted from oral tissue homogenate using RNeasy Mini Kit (Qiagen #74106) following manufacturer's instruction. The concentration and purity of extracted RNA were measured by analyzing the samples in a spectrophotometer (Islam et al., 2016). FMD virus specific uniplex RT-PCR (uRT-PCR) was carried out with 50-60ng RNA/ reaction to detect fragment of Lpro gene of FMD viruses with designed primer pairs FMDF (5'-ctctctgttacacgctctcag-3') and FMDF (5'-cgaacacagcgtgttcttggc-3'). The SuperScript III one step RT-PCR kit with Platinum Taq (Invitrogen, USA) was used to amplify Lpro gene. Extracted RNA of the uRT-PCR positive samples was then subjected to multiplex RT-PCR (mRT-PCR) for the detection of viral serotypes using serotype specific published primers (Table 1). The RT-PCR was carried out with 25µl reaction volume (Table 2) in a thermocycler using the thermal profile stated in table 3. Finally, the cDNAs were gel electrophoresed in a transilluminator (Alphaimager HP, California, USA) and the serotypes of the viruses were identified by observing selecting bands in agarose gel.

Table 1. Primer sequences used for the detection of FMD viral serotypes by using multiplex RT-PCR (Callens and De Clercq, 1997)

Primer Name	Sequence (5'-3')	Orientation	Amplicon size(bp)	Serotypes
P33	AGCTTGTACCAGGGTTTGGC	Reverse		
P38	GCTGCCTACCTCCTTCAA	Forward	402	O
P40	GTTTCTGCACTTGACAACACA	Forward	596	C
P74	GACACCACTCAGGACCGCCG	Forward		
P75	GACACCACCCAGGACCGCCG	Forward	292	Asia1
P76	GACACCACACAAGACCGCCG	Forward		
P77	GACACGACTCAGAACCGCCG	Forward		
P110	GT(G:A:T:C)ATTGACCT(G:A:T:C)	Forward	732	A

Distribution analysis of viral serotypes

Results obtained from the RT-PCR with the samples collected from different divisions of Bangladesh was analyzed by using Microsoft Office excel 2007. The percentage analysis was carried out using Office excel and fraction point 0.45 and above was considered as single point. Distribution of FMD viral serotypes O, Asia 1 and A throughout the country was plotted.

Table 2. Composition of reaction mixture for mRT-PCR. The amplification reaction was carried out in 25µl volume

SL. No.	Components	Volume
1	2X reaction mixture	12.5 µl
2	Forward primer (20pmol/ µl) -P38	0.5 µl
3	Forward primer (20pmol/ µl) -P40	0.5 µl
4	Forward primer (20pmol/ µl) -P74	0.5 µl
5	Forward primer (20pmol/ µl) -P75	0.5 µl
6	Forward primer (20pmol/ µl) -P76	0.5 µl
7	Forward primer (20pmol/ µl) -P77	0.5 µl
8	Forward primer (20pmol/ µl) -P110	0.5 µl
9	Reverse primer(20pmol/ µl) -P33	0.5 µl
10	Taq polymerase enzyme	0.5 µl
11	RNAse out	1 µl
12	Nuclease free water	2 µl
13	RNA template	5 µl
Total volume		25 µl

RESULTS AND DISCUSSION

Foot and Mouth Disease (FMD), popularly known as Aphthous fever, khura, jhora appeared as an acute illness in cattle. FMD is a highly contagious viral disease of almost all the cloven-hoofed domestic animals including cattle, buffalo, sheep, goats and swine (Ullah et al., 2015). Infected animals showed high fever, vesicular lesions and ulceration in oral and pedal skin (Figure 1). The ulcerative lesions in oral mucosa and interdigital skin was used to establish a diagnosis of FMD (OIE, 2009). In this study the vesicular lesion were not seen in any case and the epithelial tissues from the infected oral lesions were, therefore, collected to extract viral RNA and detection of Lpro and VP1 genes of FMD viruses in RT-PCR.

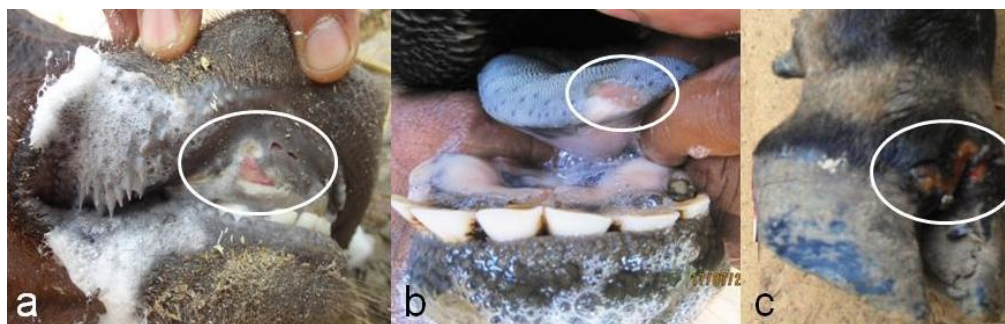


Figure 1. Naturally infected cattle due to FMD. Profuse frothy salivation (a and b), ulceration and erosion in oral and pedal tissues were commonly seen.

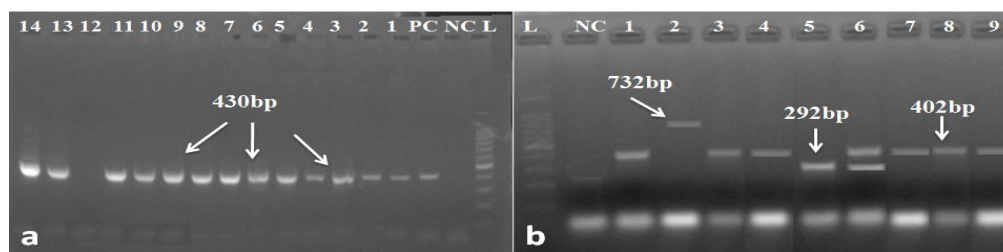


Figure 2. Viral RNA (L-pro gene) from the field samples (a) were tested in uRT-PCR detection of FMD viruses and in positive cases 430bp amplicon was generated. The lane L is for 100bp Ladder, NC is for negative control, PC is for positive control and lane 1 to 14 and 1 to 9 are for test samples. Viral RNAs (b) was also used in mRT-PCR detection of FMD viral serotypes and generation of 292bp, 402bp and 732bp amplicons were specific for FMD viral Serotype Asia1, O and A respectively. Mixed infection was detected in RT-PCR (b, lane 6).

FMD in cattle from naturally infected cattle was diagnosed by observing characteristics clinical signs. But most difficulty is lying with the detection of viral serotypes. FMD viruses and viral serotypes were identified from field samples using uniplex and multiplex RT-PCR. Results of uRT-PCR (Figure 2a) showed amplification of 430bp amplicons in 92 cases as documented in agarose gel; the amplification was specific for FMD viruses (Islam et al., 2016). A mRT-PCR was standardized using published primer sequences (Callens and De Clercq, 1997). The reverse primers of this RT-PCR was designed from a sequence of VP1 gene common to all serotypes and forward primers was designed from selective region of VP1 gene; that determine the specific serotype of FMD. The extracted RNA from the field samples (N=92) while tested in mRT-PCR, 292bp, 402bp and 763bp amplicons (Figure 2b) were generated in 90 cases; there were specific for FMD viral serotypes Asia1, O and A respectively. FMD viral Serotype O, A, Asia1 were detected alone in 56 (62%), 13 (14%) and 16 (18%) cases respectively (Table 3). Three cattle (3%) were co-infected with FMD viral Serotypes O and Asia 1 and two (2 %) with FMD

viral Serotypes O and A (Table 3). There was lacking of non-specific amplification in RT-PCR. Specificity of the RT-PCR was found higher and detected FMD viral serotypes O, A and Asia 1 in a reaction (Reid et al., 2000). The multiplex RT-PCR did not generate 596bp amplicon and FMD viral serotype C was, therefore, remain undetected in this study.

Geographic distribution of FMD viral serotypes in Bangladesh

Results of RT-PCR showed that there was about 62%, 13% and 18% infectivity in cattle due to FMD viral serotypes O, A and Asia1. About 3% and 2% co-infectivity in cattle was seen due to FMD viral serotype O and Asia1 and O and A respectively (Table 4). FMD viral serotype O found dominating in cattle followed by serotype Asia1 and A (Figure 3). FMD viral serotype O was dominating and common in all division. FMD viral serotype O and Asia 1 was prevailing at an equal level at Rajshahi division of Bangladesh. Serotype A was not detected in Chittagong, Khulna and Sylhet divisions where as serotype Asia1 was absent in Barisal division (Figure 4 & Table 3).

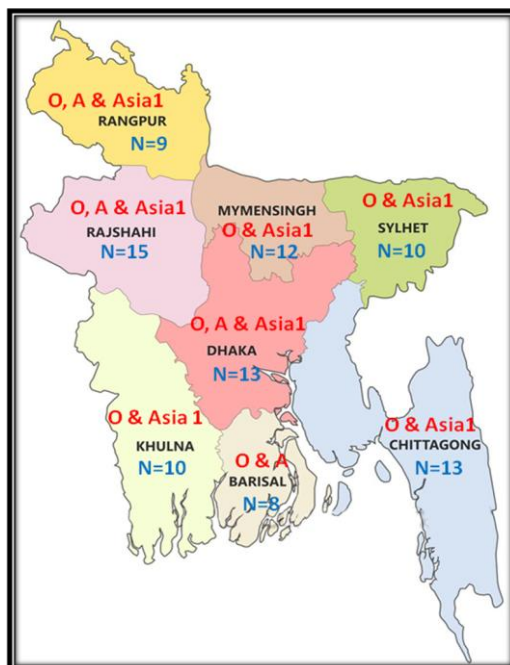


Figure 3. Map of Bangladesh showing different divisions and distribution FMD viral serotypes. N represent the number of cases examined in this study.

Previously the dominance of FMD viral serotype O in cattle was also reported in Bangladesh during the year 2011 (Sarker et al., 2011) and identified 80%, 12% and 8% infectivity due to FMD viral serotype O, Asia1 and A in cattle respectively. The

dominancy of FMD viral serotype O in 2011 was much higher than the present infectivity. Recently a different observation was noted in Bangladesh and found dominancy of FMD viral serotype A over Asia1 (Hossen et al., 2014); this might be due to differences in the coverage of sample collection areas. Infectivity of cattle due to FMD viral serotypes O (80%), A (8%) and Asia1 (12%) in India was also reported during April 2006 to March 2011 (Subramaniam et al., 2013), where FMD viral serotype O was dominating. FMD viral serotypes O, A and Asia1 are consistently circulating in Bangladesh, Bhutan, India, Nepal, and Sri Lanka (Pool 2) with a dominancy of serotype O (FAO, 2015). There is unlimited and uncontrolled animal movements throughout the SAARC countries, the scenario is worsening during religious festival. Now a day cross border cattle movements through Bangladesh, India and Myanmar although reduced but there are still some gate/point that allows unlawfully movement of cattle. Cattle, buffaloes, sheep and goats movement throughout the cross border and in country make ubiquitous distribution of FMD viral serotypes O, Asia1 and A in the SAARC countries (Siddique et al., 2014; Subramaniam et al., 2015). The FMD viral serotype O has been dominating in the SAARC countries. From the very beginning the FMD viral serotypes O was dominated over all other serotypes (Chowdhury et al., 1993; Pervin et al., 2011; Ullah et al., 2015) and this situation still exists (Islam et al., 2015). In country movement of animals may have contributed spreading of FMD and viral serotype.

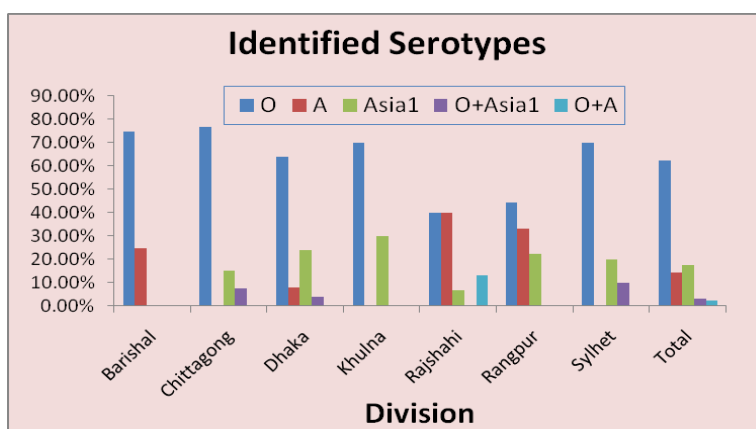


Figure 4. Division wise distribution of FMD viral serotypes in Bangladesh. Mixed infection due to FMD viral serotypes O plus Asia1 and O plus A was also cases

Table 3. Results of uniplex and multiplex RT-PCR to detect FMD viruses and viral serotypes From the natural outbreaks in cattle

Division	Number of samples	Positive in uRT-PCR (%)	Positive in mRT-PCR (%)	FMDV Serotypes identified by mRT-PCR (%)				
				O	A	Asia1	O+Asia1	O+ A
Barishal	9	8 (88.89)	8 (100)	6 (75)	2 (25)	-	-	-
Chittagong	15	14 (93.33)	13 (92.85)	10 (76.92)	-	2 (15.38)	1 (7.69)	-
Dhaka	25	25 (100)	25 (100)	16 (64)	2 (8)	6 (24)	1 (4)	-
Khulna	10	10 (100)	10 (100)	7 (70)	-	3 (30)	-	-
Rajshahi	16	15 (93.75)	15 (100)	6 (40)	6 (40)	1 (6.67)	-	2 (13.33)
Rangpur	11	10 (90.91)	9 (90)	4 (44.44)	3 (33.33)	2 (22.22)	-	-
Sylhet	11	10 (90.91)	10 (100)	7 (70)	-	2 (20)	1 (10)	-
Total	97	92 (94.85)	90 (97.83)	56 (62.22)	13 (14.44)	16 (17.78)	3 (3.33)	2 (2.22)

CONCLUSIONS

This study designed and adapted uniplex and multiplex RT-PCR protocols for rapid detection of FMD viruses and serotyping of the viruses using RNA extracted from the infected oral tissues. Out of 97 bovine samples tested in uniplex RT-PCR, 430bp amplicon was generated in 92 cases (94.85% detection level), suggestive for infectivity due to FMD viruses. While viral RNA from the uRT-PCR test positive samples (N=92) were used in mRT-PCR protocol, specific FMD viral serotypes was detected in 90 cases (98% test positivity). The RT-PCR protocols appeared highly sensitive (more than 90%) in terms of detecting FMD viruses and viral serotypes and were specific. FMD viral serotypes O, Asia1 and A were circulated in cattle during 2013 outbreaks in Bangladesh. FMD viral serotype O found dominating over serotype Asia 1 and A. Mixed infection with O & Asia1 and O and A was also reported. Infectivity in cattle due to FMD viral serotype C is still absent in Bangladesh. Overlapping of infectivity due to FMD viral serotypes as seen in this study recommend the use of polyvalent vaccine consisting of FMD viral serotypes O, A and Asia 1. The FMD viral vaccines used in Bangladesh lack information about their antigenic and genetic properties and protective efficacy; these may contribute vaccination failure and regular outbreak of FMD in the field.

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EFFECT OF INTEGRATED NUTRIENT MANAGEMENT ON NUTRIENT UPTAKE AND SUSTAINABLE GRAIN YIELD IN TRANSPLANTED AMAN RICE

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ABSTRACT

A field experiment was carried out at Sher-e-Bangla Agricultural University (SAU) Farm during July to November 2011 to evaluate the effect of integrated nutrient management (INM) on T. Aman rice (cv. BRRI dhan40). The field belongs to Red Brown Terrace soil under Tejgoan series having silt loam texture and 6.0 pH. Application of 70 % NPKS fertilizers + 4 t ha⁻¹ dhaincha (*Sesbania rostrata*) (T₁) produced the highest grain yield (5.90 t ha⁻¹), the second highest yield (5.85 t ha⁻¹) was obtained from 80% NPKS + 2 t ha⁻¹ dhaincha (T₂) treatment. The grain yield increased by 31.2 to 86.3% over control depending on the treatments. Treatments T₁ and T₂ resulted in higher N, P, K and S uptake by rice. The N, P, K and S concentrations of rice grain was higher for the treatments where *Sesbania* and cow dung were together applied in combination with NPKS fertilizers. The combined application of chemical fertilizers and organic manure increased organic carbon (OC), total N, available P, K and S contents in post-harvest soil. The overall results indicate that the integrated use of chemical fertilizer and organic manure can help increase grain yield of rice without deteriorating soil fertility.

Keywords: Integrated nutrient management, rice yield, soil fertility

INTRODUCTION

Agriculture in Bangladesh is predominately rice based and Bangladesh is the fourth rice (*Oryza sativa*) producing country in the world (BRRI, 2006). It is grown in 28.06 million hectares of land in which T. aman (rainfed wet season rice) covers about 13.99 million hectares and produces about 12.21 million metric tons of rice (BBS, 2010) by utilizing varied soil fertility levels and fertilizer management. Soil fertility deterioration is a major constraint for higher rice yield in Bangladesh. The increasing land use intensity without adequate and balanced use of chemical fertilizers and with little or no use of organic manures have caused severe fertility

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deterioration of our soils resulting in stagnating or even declining of crop productivity. The farmers of this country use, on an average, 172 kg nutrients/ha (follow similar style) annually (132 kg N + 27 kg P + 17 kg K + 4 kg S, and 2 kg Zn), while the crop removal is about 250 kg ha⁻¹ (Islam, 1995). Since fertile soil is the fundamental resource for higher crop production, its maintenance is a prerequisite for long-term sustainable crop productivity. Soil organic matter is a key factor for sustainable soil fertility and crop productivity. A good soil in Bangladesh should have an organic matter content of at least 2.5% (BARC, 2012). According to an appraisal report of Bangladesh soil resources, soils of about 6.10 m ha contain very low (less than 1%) organic matter, 2.15 m ha contain low (1-2%) organic matter and the remaining 0.90 m ha contain more than 2 % organic matter (Mondal, 2000). Green manure prepared from *Sesbania spp.* is a cheaper and feasible alternative to other organic sources. Green manuring species such as *Sesbania rostrata* can fix sufficient amounts of N for optimum rice yield without any inorganic N input (Meelu et al., 1992). Soil fertility declining day by day is a major reason for lower crop yield in Bangladesh. Intensive cropping, use of modern varieties, use of higher doses of nitrogenous fertilizers with little or no addition of organic manure are the principal factors for soil fertility depletion in the country. A crop production system with high yield targets cannot be sustainable unless balanced nutrient inputs are supplied to soil against nutrient removal by crops (Bhuiyan et al., 1991). Available data indicate that the soil fertility in Bangladesh is in declining trend (Karim et al., 1994; Ali et al., 1997) which is responsible for declining crop yields (Cassman et al., 1995). Neither organic manure nor chemical fertilizer alone can increase satisfactory yield under intensive farming.

Organic fertilizers has positive effect on root growth by improving the root rizosphere conditions (structure, humidity, etc.) and also plant growth is encouraged by increasing the population of microorganisms (Shaheen et al., 2007). Organic fertilizers contain plant nutrients. Organic acids which occur in decomposition increases the benefits of nutrients (Anonymous, 2010). Organic sources of nutrients applied to preceding crop can benefit the succeeding crop to a great extent (Hedge, 1998) through integrated use of organic and inorganic sources of nutrients (Singh et al., 2001). Nambiar (1997) viewed that integrated use of organic manure and chemical fertilizers would be quite promising not only in providing greater stability in production, but also in maintaining better soil fertility. Based on the soil fertility problem as discussed above, the present study was undertaken to investigate the effect of combined use of chemical fertilizers and organic manures in T. aman (BRRI dhan40) production.

MATERIALS AND METHODS

The experiment was conducted in the experimental field of Sher-e-Bangla Agricultural University, Dhaka, Bangladesh during July to November 2011. The experiment was laid out in a randomized complete block design (RCBD), with three

replications. Each block was divided into eight unit plots as treatments with raised bunds around. The unit plot size was 3 m x 2 m (6 m²). The treatment combinations used were T₀ (Control, no fertilizer), T₁ (N₁₂₀ P₁₅ K₄₅ S₂₀ kg ha⁻¹, recommended dose), T₂ (80% NPKS + 2 t ha⁻¹ DH), T₃ (80% NPKS + 4 t ha⁻¹ CD), T₄ (80% NPKS + 1 t ha⁻¹ DH + 2 t ha⁻¹ CD), T₅ (70% NPKS + 4 t ha⁻¹ DH), T₆ (70% NPKS + 8 t ha⁻¹ CD), T₇ (70% NPKS + 2 t ha⁻¹ DH + 4 t ha⁻¹ CD), T₈ (50% NPKS + 6 t ha⁻¹ DH), T₉ (50% NPKS + 12 t ha⁻¹ CD), T₁₀ (50% NPKS + 3 t ha⁻¹ DH + 6 t ha⁻¹ CD) and assigned in a randomized complete block design with three replications. The unit plot size was 3-x2-m x 2.0m. Full amount of P as TSP, K as MP and S as gypsum were applied during final land preparation. Urea was applied in 3 equal splits: one third at basal, one third at 30 days after transplanting (DAT) and the remaining one third was applied at 55 DAT. Two different types of organic manure viz., dhaincha (*Sesbania rostrata*) and cow dung (CD) were used. Dhaincha was applied before 2 weeks and CD was applied before one week of transplanting. Forty days old seedlings of BRRI dhan40 were uprooted from seedbed and transplanted on 14 August 2011 at a spacing of 20 cm x 20 cm using 3 seedlings hill⁻¹. The crop was harvested when 80-90% of the grains were turned into straw colored on 26 November 2011. The plant height was measured from the ground level to the top of the panicle. From each plot, plants of 10 hills were measured and averaged. The measurement of panicle length was taken from basal node of the rachis to the apex of panicle. Each observation was an average of 10 hills. Ten hills were taken randomly from each plot and total numbers of effective tillers hill⁻¹ were recorded. Filled and unfilled grains per panicle were counted and averaged from the panicles of ten hills. 1000 grains were taken from the samples and after sun drying the weight was recorded by using electrical balance. The harvested crops was threshed, cleaned, dried and weighed. Straw and grain yields were adjusted to 14% moisture content. Before land preparation, initial composite soil sample were collected at 0-15 cm depth from different spots and prepared for physical and chemical analysis. The experimental site is fairly level, well drained and belongs to Deep Red Brown Terrace Soil under Madhupur Tract (AEZ-28). The physical and chemical characteristics of initial soils are given in the table 1.

Table 1. Soil properties of the experimental site

Location	pH	OM	Ca	Mg	K	Total N	P	S	B	Mn	Zn
		%	µg/g		cmol kg ⁻¹	(%)	µg/g soil				
SAU, Dhaka	6.0	1.19	09	0.23	0.12	0.045	16	14.4	0.1	3.0	0.4
Critical level	-	-	2.0	0.5	0.12	0.12	10	10	0.2	1.0	0.6

Table 2. Nutrient status of organic manure used in the experiment (oven dry basis)

Sources	Nutrient content					
	C (%)	N (%)	P (%)	K (%)	S (%)	C:N
Cow dung	36	1.20	1.00	0.75	0.21	30
Green manure	46	2.00	0.26	1.56	0.24	23

Plant nutrient analysis: Grain and straw samples were collected from each plot and were dried for 48 hrs in oven at $65 \pm 5^\circ\text{C}$. These dried samples were partitioned into grain and husk. Finally ground samples were passed through 0.5 mm mesh sieve and were used for chemical determination of N, P and K concentration as described by Jackson (1973). The significance of the differences among pairs of treatment means was estimated by the least significant difference (LSD) test at 5% level of probability (Gomez and Gomez, 1984). The mean comparisons of the treatment were evaluated by DMRT (Duncan's Multiple Range Test).

RESULTS AND DISCUSSION

Yield: The highest grain (5.9 t ha^{-1}) and straw (8.59 t ha^{-1}) yields were obtained from treatment T_5 (70% NPKS + 4 t ha^{-1} DH) and T_3 (80% NPKS + 4 t ha^{-1} CD), respectively and the lowest grain yield (3.63 t ha^{-1}) and straw yields (4.66 t ha^{-1}) were observed in control plots (Table 3). Grain yield was positively correlated with the number of effective tiller, panicle length and filled grains panicle⁻¹ and 1000 grain weight. The grain and straw yields due to different treatments ranked in order of $T_5 > T_2 > T_3 > T_4 > T_1 > T_7 > T_6 > T_8 > T_{10} > T_9 > T_0$ and $T_3 > T_1 > T_2 > T_4 > T_5 > T_7 > T_6 > T_8 > T_{10} > T_9 > T_0$ respectively. Hoque et al. (2001) reported significantly increased grain and straw yields due to application of organic manure and chemical fertilizers. This is also in agreement with the findings of Laxminarayan (2000), Dwivedi and Thakur (2000) and Rajni Rani et al. (2001).

Nitrogen content in grain and straw: Nitrogen contents in rice grain and straw ranged from 1.14 to 1.28% and 0.710 to 0.862%, respectively. The highest N contents (1.28%) in rice grain and (0.862%) in rice straw was observed in the treatment T_1 ($N_{120} P_{15} K_{45} S_{20} \text{ kg ha}^{-1}$) due to application of recommended doses of chemical fertilizers and the lowest N content in grain (1.14%) and in straw (0.710%) was noted in T_0 (control) treatment (Table 4). The effect of T_5 treatment was more pronounced in increasing the N content in rice grain and straw of BRRI dhan40 as compared to cow dung. The results revealed that N content in rice grain was higher than straw. Significant increase in N contents in rice grain and straw due to the application of organic manure and fertilizers have been reported by many investigators (Azim, 1999; Hoque, 1999).

Table 3. Effects of INM on the grain and straw yields of T. Aman rice (BRRI dhan40)

Treatments	Grain		Straw	
	Yield (t ha ⁻¹)	Increase over control (%)	Yield (t ha ⁻¹)	Increase over control (%)
T ₀ =Control	3.63h	-	4.66e	-
T ₁ =N ₁₂₀ P ₁₅ K ₄₅ S ₂₀ kg ha ⁻¹ (RDF)	5.62d	75.7	8.08ab	65.5
T ₂ =80% NPKS + 2 t ha ⁻¹ DH	5.85ab	84.4	8.00abc	75.6
T ₃ = 80% NPKS + 4 t ha ⁻¹ CD	5.82b	83.3	8.59a	87.2
T ₄ =80% NPKS + 1 t ha ⁻¹ DH + 2 t ha ⁻¹ CD	5.75c	80.6	7.82bc	86.5
T ₅ =70% NPKS + 4 t ha ⁻¹ DH	5.90a	86.3	7.47bc	72.5
T ₆ =70% NPKS + 8 t ha ⁻¹ CD	5.18e	58.9	7.34c	65.9
T ₇ =70% NPKS + 2 t ha ⁻¹ DH + 4 t ha ⁻¹ CD	5.20e	59.7	7.37bc	63.7
T ₈ = 50% NPKS + 6 t ha ⁻¹ DH	4.53f	34.2	6.24d	30.2
T ₉ =50% NPKS +12 t ha ⁻¹ CD	4.45g	31.2	6.06d	35.2
T ₁₀ =50% NPKS + 3 t ha ⁻¹ DH + 6 t ha ⁻¹ CD	4.52f	33.84	6.16d	37.84
\pm SE	0.11	-	0.13	-
LSD (P=0.05)	0.42	-	0.45	-

Means having same letter(s) do not differ significantly at 5% level of probability

RDF= Recommended Dose of Fertilizer, N= Nitrogen, P= Phosphorus, K= Potassium, S= Sulphur, CD= Cow dung, DH = Dhaincha, \pm SE = Standard Error of Means, CD₁ = Critical Difference

Total N uptake: The highest total N uptake (141.9 kg ha⁻¹) was recorded in the treatment T₁ which was statistically identical to treatment T₅ (Table 5). This result showed that total N uptake was high in recommended doses of chemical fertilizer T₁ (N₁₂₀ P₁₅ K₄₅ S₂₀ kg ha⁻¹) followed by combined application of GM with chemical fertilizers (treatment T₅). This might be due to (i) increased supply of all essential nutrients directly through organic and inorganic source to crop, (ii) indirectly through checking the losses of nutrient from soil solution and (iii) by increasing in the nutrient use efficiency. Sengar et al. (2000) reported that the N uptake by rice grain and straw increased significantly with the combined application of organic manure and chemical fertilizers. Duhan et al. (2002); Rahman (2001); Hoque (1999) and Azim (1999) also reported similar results.

Table 4. Effect of different treatments on N, P, K and S concentration in grain and straw of T. Aman rice (cv. BRRI dhan 40)

Treatments	Concentration (%)							
	Grain				Straw			
	N	P	K	S	N	P	K	S
T ₀ =Control	1.14d	0.200b	0.258b	0.090c	0.710c	0.051d	1.573de	0.055c
T ₁ =N ₁₂₀ P ₁₅ K ₄₅ S ₂₀ (RDF)	1.280a	0.280a	0.324a	0.114ab	0.862a	0.065bc	2.120ab	0.073ab
T ₂ =80% NPKS + 2 t ha ⁻¹ DH	1.209bc	0.292a	0.336a	0.114ab	0.770bc	0.081a	2.152ab	0.083a
T ₃ = 80% NPKS + 4 t ha ⁻¹ CD	1.150cd	0.273a	0.317ab	0.094c	0.787b	0.076a	1.960abc	0.070b
T ₄ =80% NPKS + 1 t ha ⁻¹ DH + 2 t ha ⁻¹ CD	1.146d	0.280a	0.330a	0.102b	0.768bc	0.058c	2.052ab	0.078a
T ₅ =70% NPKS + 4 t ha ⁻¹ DH	1.260ab	0.289a	0.341a	0.116a	0.778b	0.056c	2.250a	0.086a
T ₆ =70% NPKS + 8 t ha ⁻¹ CD	1.164cd	0.285a	0.315ab	0.096bc	0.750bc	0.079ab	1.910bc	0.071b
T ₇ =70% NPKS + 2 t ha ⁻¹ DH + 4 t ha ⁻¹ CD	1.182cd	0.282a	0.323a	0.099bc	0.761bc	0.063bc	1.385e	0.077ab
T ₈ = 50% NPKS + 6 t ha ⁻¹ DH	1.157cd	0.279a	0.312ab	0.113ab	0.779b	0.077b	2.150ab	0.079ab
T ₉ =50% NPKS +12 t ha ⁻¹ CD	1.160cd	0.255ab	0.298ab	0.106b	0.754bc	0.079ab	1.722cd	0.070b
T ₁₀ =50% NPKS + 3 t ha ⁻¹ DH + 6 t ha ⁻¹ CD	1.150cd	0.251ab	0.290ab	0.096bc	0.752bc	0.055c	1.690cde	0.073ab
\pm SE	1.15	0.28	0.23	0.001	0.002	0.056	1.18	0.03
CD ₁ (P=0.05)	0.33	0.81	0.45	0.004	0.004	0.045	0.37	0.08

Means having same letter(s) do not differ significantly at 5% level of probability

RDF= Recommended Dose of Fertilizer, N= Nitrogen, P= Phosphorus, K= Potassium, S= Sulphur, CD= Cowdung, DH = Dhaincha, \pm SE = Standard Error of Means, CD₁= Critical Difference

Phosphorus content in grain and straw: Phosphorus content in rice grain and straw ranged from 0.200 to 0.292% and 0.051 to 0.081%, respectively. The highest P content (0.292%) in grain and (0.081 %) in rice straw was observed in the treatment T₂ (0% NPKS + 2 t ha⁻¹ DH) and the lowest P concentration was noted in the treatment T₀ (Table 4). The next highest P content in rice grain and straw was found in the treatment T₅ (70% NPKS + 4t ha⁻¹DH). Lower grain P content was observed in those treatment received NPKS fertilizers with CD compared to 100% NPKS and NPKS fertilizers with GM. Verma (1992) reported that incorporation of organic manure significantly increased the concentration of P in rice grain and straw yields of rice.

Table 5. Nitrogen and Phosphorus uptake by grain and straw of T. Aman rice (cv. BRRI dhan 40)

Treatments	N uptake (kg ha ⁻¹)		Total N uptake (kg ha ⁻¹)	P uptake (kg ha ⁻¹)		Total P uptake (kg ha ⁻¹)
	Grain	Straw		Grain	Straw	
T ₀ =Control	41.38 e	33.08 e	74.46f	7.26 f	2.38 g	9.64 i
T ₁ =N ₁₂₀ P ₁₅ K ₄₅ S ₂₀ (RDF)	71.94 a	69.65 a	141.59a	15.74 b	5.25 c	20.99cd
T ₂ =80% NPKS + 2 t ha ⁻¹ DH	70.73 a	61.40 b	132.13bc	17.05 a	6.32 a	23.40 a
T ₃ = 80% NPKS + 4 t ha ⁻¹ CD	70.30 b	67.60 a	138.9ab	15.89 b	6.53 a	22.42 b
T ₄ =80% NPKS + 1 t ha ⁻¹ DH + 2 t ha ⁻¹ CD	65.90 b	60.06 bc	125.96c	16.10 b	4.53 d	20.63 d
T ₅ =70% NPKS + 4 t ha ⁻¹ DH	69.85 a	58.10 bc	132.44bc	17.08 a	4.16 e	21.15 c
T ₆ =70% NPKS + 8 t ha ⁻¹ CD	60.30 c	55.05 c	115.35d	14.76 c	5.95 b	20.71 cd
T ₇ =70% NPKS + 2 t ha ⁻¹ DH + 4 t ha ⁻¹ CD	61.46 c	56.09 c	117.55d	14.67 c	4.64 d	19.31 e
T ₈ = 50% NPKS + 6 t ha ⁻¹ DH	52.41 d	48.61 d	101.02e	12.64d	4.80 d	17.44 f
T ₉ =50% NPKS +12 t ha ⁻¹ CD	51.62 d	45.69 d	97.31e	11.35 e	4.79 d	16.14 g
T ₁₀ =50% NPKS + 3 t ha ⁻¹ DH + 6 t ha ⁻¹ CD	51.98 d	46.32 d	98.3e	11.35 e	3.39 f	14.74 h
\pm SE	4.18	2.3	1.23	0.001	0.002	0.456
CD ₁ (P=0.05)	3.37	2.8	3.45	0.004	0.004	1.45

Means having same letter(s) do not differ significantly at 5% level of probability

RDF= Recommended Dose of Fertilizer, N= Nitrogen, P= Phosphorus, K= Potassium, S= Sulphur, CD= Cow dung, DH = Dhaincha, \pm SE = Standard Error of Means, CD₁= Critical Difference

Total P uptake: The total P uptake ranged from 9.64 to 23.40 kg ha⁻¹. The highest total P uptake (23.40 kg ha⁻¹) was recorded in the treatment T₂ (80% NPKS + 2 t ha⁻¹ DH), and the lowest value (9.64 kg ha⁻¹) was observed in the treatment T₀ (Table 5). This results shows that total P uptake by grain and straw were significantly due to combined application of GM with chemical fertilizers. Sengar et al. (2000) reported that the P uptake by rice grain and straw increased significantly with the combined application of organic manure and chemical fertilizers.

Potassium content in grain and straw: The highest K content (0.341%) in grain and (2.25%) straw was observed in the treatment T₅ (70% NPKS + 4 t ha⁻¹ DH) that was significantly higher than the rest of the treatments and the lowest K concentration was noted in the treatment T₀ (Table 4). From the results it was observed that the K content in rice straw was higher than grain in all the treatments K contents both in grain and straw of rice increased due to combined application of organic manure and chemical fertilizers (Islam, 1997; Khan et al., 1998).

Table 6. Potassium and S uptake by grain and straw of T. Aman rice (BRRI dhan40)

Treatments	K uptake (kg ha ⁻¹)		Total K uptake (kg ha ⁻¹)	S uptake (kg ha ⁻¹)		Total S uptake (kg ha ⁻¹)
	Grain	Straw		Grain	Straw	
T ₀ =Control	9.37 f	73.32 e	82.69 d	3.27 h	2.57 h	5.84g
T ₁ =N ₁₂₀ P ₁₅ K ₄₅ S ₂₀ (RDF)	18.21c	171.29 a	189.5 a	6.41 b	5.90 bc	12.31 b
T ₂ =80% NPKS + 2 t ha ⁻¹ DH	19.66 ab	172.15 a	191.81 a	6.67 ab	6.64 a	13.31 a
T ₃ = 80% NPKS + 4 t ha ⁻¹ CD	18.45 bc	168.38 a	186.83 a	5.47 d	6.01 bc	11.48 c
T ₄ =80% NPKS + 1 t ha ⁻¹ DH + 2 t ha ⁻¹ CD	18.98abc	160.46 ab	179.44ab	5.87 c	6.10 abc	11.97 bc
T ₅ =70% NPKS + 4 t ha ⁻¹ DH	20.12 a	168.01 a	188.13 a	6.84 a	6.42 ab	13.26 a
T ₆ =70% NPKS + 8 t ha ⁻¹ CD	16.32 d	148.51 abc	164.83 ab	4.97 e	5.21 de	10.18e
T ₇ =70% NPKS + 2 t ha ⁻¹ DH + 4 t ha ⁻¹ CD	16.80 d	123.55 cd	140.3 bc	5.15 e	5.67 cd	10.82d
T ₈ = 50% NPKS + 6 t ha ⁻¹ DH	14.13 e	132.13 bcd	146.27 b	5.12 e	4.93 ef	10.05 e
T ₉ =50% NPKS +12 t ha ⁻¹ CD	13.26 e	104.35 d	117.61 c	4.72 f	4.24 g	8.96 f
T ₁₀ =50% NPKS + 3 t ha ⁻¹ DH + 6 t ha ⁻¹ CD	13.11 e	104.11 d	117.22 c	4.34 g	4.50 fg	8.84 f
\pm SE	0.002	0.003	2.15	0.44	2.6	0.002
CD ₁ (P=0.05)	0.003	0.005	4.54	0.35	6.67	0.003

Means having same letter(s) do not differ significantly at 5% level of probability

RDF= Recommended Dose of Fertilizer, N= Nitrogen, P= Phosphorus, K= Potassium, S= Sulphur, CD= Cow dung, DH = Dhaincha, \pm SE = Standard Error of Means, CD₁= Critical Difference

Total K uptake: The highest total K uptake (191.81 kg ha⁻¹) was recorded in the treatment T₂ (80% NPKS + 2 t ha⁻¹ DH), and the lowest value (82.69 kg ha⁻¹) was observed in the treatment T₀ (Table 6). This results shows that total K uptake were influenced more due to combined application of GM with chemical fertilizers. Sengar et al. (2000) reported that the K uptake by rice grain and straw increased significantly with the combined application of organic manure and chemical fertilizers.

Sulphur content in grain and straw: The highest S content (0.116%) in grain and (0.086 %) in rice straw was observed in the treatment T₅ (70% NPKS + 4 t ha⁻¹ DH) that was significantly higher than the rest of the treatments and the lowest S concentration was noted in the treatment T₀ (Table 4). Azim (1999) and Hoque (1999) reported that application of S from manure and fertilizers increased S content both in grain and straw. Ali et al. (2009) also reported the similar results.

Total S uptake: The highest total S uptake (13.31 kg ha⁻¹) was recorded in the treatment T₂ (80% NPKS + 2 t ha⁻¹ DH) and the lowest value (5.84s kg ha⁻¹) was observed in the treatment T₀ (Table 6). Azim (1999) and Hoque (1999) recorded the higher uptake of S with the application of manure and fertilizers in combinations.

Post harvest soil properties: Application of organic manure and chemical fertilizers resulted in considerable influence on the properties of the post-harvest soils (Table

7). In some treatments the pH value slightly decreased as compared to the initial value (6.0). The highest organic matter content (1.42%) was found in T₅ (70% NPKS + 4 t ha⁻¹DH) treatment which was also increased compared to the initial value. Organic manuring increased the organic matter content, total N, available P, exchangeable K and available S in the post harvest soils. Hoque et al. (2001), Mathew and Nair (1997), and Azim (1999) reported that, combined application of organic manure and chemical fertilizer can increased the organic matter content, total N, available P, exchangeable K and available S in the post-harvest soils.

Table 7. Effect of INM on post-harvest soils properties

Treatments	Soil pH	Organic matter (%)	Total N (%)	Available P (ppm)	Exchangeable K (me %)	Available S (ppm)
T ₀ =Control	5.92ab	1.28 c	0.065a	17.48j	0.099e	11.02c
T ₁ =N ₁₂₀ P ₁₅ K ₄₅ S ₂₀ kg ha ⁻¹ (RDF)	5.98a	1.39 ab	0.095a	19.45d	0.135c	18.168a
T ₂ =80% NPKS + 2 t ha ⁻¹ DH	5.80bc	1.34 b	0.089a	19.09f	0.147ab	19.665a
T ₃ = 80% NPKS + 4 t ha ⁻¹ CD	6.00a	1.36ab	0.090a	19.31e	0.134c	16.450ab
T ₄ =80% NPKS + 1 t ha ⁻¹ DH + 2 t ha ⁻¹ CD	5.78c	1.36ab	0.092a	18.56i	0.148ab	17.752a
T ₅ =70% NPKS + 4 t ha ⁻¹ DH	5.86bc	1.42a	0.097a	19.95c	0.156a	18.280a
T ₆ =70% NPKS + 8 t ha ⁻¹ CD	5.82bc	1.38ab	0.088a	23.98a	0.150a	16.225ab
T ₇ =70% NPKS + 2 t ha ⁻¹ DH + 4 t ha ⁻¹ CD	5.88abc	1.36ab	0.089a	20.12b	0.140a	17.558a
T ₈ = 50% NPKS + 6 t ha ⁻¹ DH	5.90abc	1.38ab	0.090a	18.96g	0.142bc	17.770a
T ₉ =50% NPKS +12 t ha ⁻¹ CD	5.84bc	1.38ab	0.082a	18.80h	0.112d	12.520bc
T ₁₀ =50% NPKS + 3 t ha ⁻¹ DH + 6 t ha ⁻¹ CD	6.00a	1.35b	0.079a	18.66i	0.141bc	16.665ab
\pm SE	0.001	0.001	0.001	0.44	0.58	0.55
CD ₁ (P=0.05)	0.002	0.003	0.003	1.77	2.37	1.44

Means having same letter(s) do not differ significantly at 5% level of probability

RDF= Recommended Dose of Fertilizer, N= Nitrogen, P= Phosphorus, K= Potassium, S= Sulphur, CD= Cowdung, DH = Dhaincha, \pm SE = Standard Error of Means, CD₁= Critical Difference

CONCLUSION

From the present study it may be concluded that rice variety BRR1 dhan40 responded better to the nutrient supplied from the organic manure in producing grain and straw yields. The study clearly demonstrates that the benefit of using dhaincha as GM can reduce the N, P, K and S fertilizers for T. Aman rice, giving good economic yield and also slightly increased the soil organic matter content particularly when the fertilizers were applied on soil test basis (STB). This is the one year result. Regular practice of INM may be able to increase different soil qualities including soil organic matter content. BRR1 dhan40 can be cultivated profitably in the Tejgoan silt loam

soil by using combined application of 70% NPKS fertilizers with 4 t ha⁻¹ GM. The overall findings of this study indicate that the combined use of fertilizer and manure in T. Aman rice should be encouraged for maintaining rice yield, quality and soil fertility.

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BIOEFFICACY OF DIFFERENT INSECTICIDES AGAINST BLISTER BEETLE *Mylabris phalerata* (Pallas) ON PIGEONPEA

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ABSTRACT

The experiment was accomplished to evaluate the bioefficacy of different insecticides against *Mylabris phalerata* (Pallas) on pigeonpea. The experiment consisted of seven treatments viz., bifenthrin 62 g a.i. ha⁻¹, lambda-cyhalothrin 24 g a.i. ha⁻¹ (encapsulated with polymers; Matadore), chlorpyrifos 310 g a.i. ha⁻¹+ cypermethrin 31 g a.i. ha⁻¹, cypermethrin 62 g a.i. ha⁻¹, neem oil 1500 ppm, permethrin 154 g a.i. ha⁻¹, chlorpyrifos 173 g a.i. ha⁻¹ compared with untreated (control). The sixth day after each spray, minimum population abundance of *M. phalerata* and maximum percent efficacy in reducing their population was obtained from bifenthrin which was at par with lambda-cyhalothrin, cypermethrin, chlorpyrifos+ cypermethrin and permethrin and all these were significantly effective over chlorpyrifos, neem oil and control plots. On the tenth day after each spray, minimum population abundance and highest per cent efficacy were obtained from bifenthrin which was at par with lambda-cyhalothrin and both these treatments were significantly superior over rest of the treatments. The maximum productivity was observed with bifenthrin, while highest net return per rupee investment was obtained from lambda-cyhalothrin treatment. The results explicitly show that out of all treatments, Lambda-cyhalothrin was effective and imposing lucrative against *M. phalerata* and thereby minimized the yield loss. Three sprays of lambda-cyhalothrin and bifenthrin with an interchange at ten days interval will be effectual and lucrative against *M. phalerata* in short duration pigeonpea crop.

Keywords: Blister beetle, *Mylabris phalerata*, pigeonpea, bioefficacy, insecticides

INTRODUCTION

Pigeonpea (*Cajanus cajan* L.) is one of the most important pulse crop cultivated in more than 25 countries of the world on 6.67 million ha with 4.86 million ton of production, whereas in Asia, it was grown on 5.69 million ha and producing

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3.88 million ton in 2014 (FAO, 2016). The economic loss due to biotic stress factors has been estimated to be US\$ 8.48 billion (Sarika et al., 2013) in the world. The big difference of pigeonpea productivity shows great promises to enhance the crop productivity by management of biotic constraints. Among biotic constraints for productivity in subsistence crop protection pattern, *Mylabris phalerata* is one of the most detrimental insect pests. In pigeonpea, owing to the introduction of short duration, photo-insensitive and determinate varieties with compact floral clusters, damage by blister beetles tends to be manifold.

Blister beetle is a voracious flower feeder and thereby, directly affects the grain yield through flower damage. Pigeonpea is the most preferred host for blister beetle at reproductive stage (Mann and Dhooria, 1993; Balikai, 2000; Durairaj, 2000; Dhakla et al., 2010; Dasbak et al., 2012). Its species damaged pigeonpea flowers with the maximum beetle density 19.4 plant⁻¹ (Durairaj and Ganapathy, 1996). It has significant pest characteristics viz., polyphagous, voracious feeder, high mobility, robustness, high fecundity and the immature stage buried subterranean can safely surpass transitional life cycle in soil. Adult blister beetles are migratory in nature and therefore, all the insecticides may not effectively control those (Blodgett et al., 2010). As per their biology and behaviour, its management is hard headed. Several management options were given to suppress blister beetles population but none of them could overcome the menace (McBride, 2012). Unfortunately, various insecticides do not effectively suppress their population due to its behavioural ability. However, chemical management strategy shows great promises with various potentials viz., practical management, cost effectiveness, farmers' reliance and quick response to overcome their damage. Hence, the present investigation was envisaged to unequivocally substantiate and determine the bio-efficacy of contact, knockdown and deterrent features based insecticides against *M. phalerata* on pigeonpea.

MATERIALS AND METHODS

The experimental materials consisted of pigeonpea, *M. phalerata* and different insecticides. The experiment was accomplished under field conditions at Agronomy Research Farm of School of Agricultural Sciences and Rural Development, Nagaland University, Medziphema, during 2014 and 2015 on the early variety of pigeonpea, UPAS 120. The pigeonpea crop was raised as per recommended package of practices for north east India. The experiment was designed in randomized block design with 8 treatments including control (untreated) and replicated thrice.

The treatments were assigned bifenthrin 62 g a.i. ha⁻¹, lambda-cyhalothrin (encapsulated with polymers; Matadore) 24 g a.i. ha⁻¹, cypermethrin 31 g a.i. ha⁻¹ + chlorpyrifos 310 g a.i. ha⁻¹, cypermethrin 62 g a.i. ha⁻¹, neem oil 1500 ppm (1235 ml ha⁻¹), permethrin 154 g a.i. ha⁻¹, chlorpyrifos 173 g a.i. ha⁻¹ and untreated (control) which were evaluated for their efficacy against blister beetle on pigeonpea. Insecticides were applied thrice during infestation (blooming stage) of crop and

sprays were applied as per requirement basis with high pressure knapsack power sprayer.

The population abundance of blister beetle and grain yield of pigeonpea data were collected from the replicated plots of each treatment. Ten plants were selected randomly from each plot, and population of adults was counted from 0600 to 1000 h. The blister beetle incidence was recorded one day before spray as pre-treatment and on the sixth day and tenth day after sprays as post-treatment. The percent reduction of population over untreated which is expressed as percent efficacy of insecticide was calculated by Henderson and Tilton's formula as given below,

$$\text{Per cent efficacy} = \left[1 - \frac{T_a}{T_b} \times \frac{C_b}{C_a} \right] \times 100$$

Where,

T_a- Population of the treated plot after spray

T_b- Population of the treated plot before spray

C_a- Population of the control plot after spray

C_b- Population of the control plot before spray

The efficacy data were transformed in square root transformation as per transformation rule one (Gomez and Gomez, 1983). The productivity of seed yield was recorded plot⁻¹ and the obtained grain yields were converted in kg ha⁻¹. The avoidable yield loss due to application of different treatments was derived by deducting the yield of untreated from respective treatments. The incremental benefit cost ratio was calculated as per increase in the yield over untreated with experiment year MSP of pigeonpea. The data of each character were subjected to statistical test by applying analysis of variance technique (Gomez and Gomez, 1983).

RESULTS AND DISCUSSION

Blister beetle population abundance and bioefficacy of insecticides

The recorded population abundance of *M. phalerata* and per cent efficacy of different insecticides have been depicted in table 1 and 2. Before the first spray, mean population of *M. phalerata* varied from 1.73 to 1.93 plant⁻¹ and 1.80 to 2.03 plant⁻¹ during 2014 and 2015 respectively from various assigned treatments and statistically identical with each other. On the sixth day after the first spray, the lowest population plant⁻¹ (0.47) and highest efficacy (73.93%) were recorded with bifenthrin followed by lambda-cyhalothrin, cypermethrin, chlorpyrifos+ cypermethrin and permethrin which were at par to each other and significantly effective in reducing the population over chlorpyrifos, neem oil and control (untreated) during 2014 and the same trend was observed in 2015. On the tenth day after first spray, the lowest population plant⁻¹ (1.40) and highest efficacy (36.36%) were recorded with bifenthrin followed by lambda-cyhalothrin which were at par to each other and significantly superior in

efficacy and reducing the population over rest of the treatments including untreated plots. By the tenth day after first spray, the efficacy of chlorpyrifos+ cypermethrin, cypermethrin, neem oil, permethrin and chloropyrifos treatments were statistically at par with control (untreated) during 2014 in reducing the population and efficacy and the similar trend was observed in 2015.

Before the second spray, the population plant⁻¹ varied from 2.23 to 2.60 and 2.03 to 2.33 during 2014 and 2015 respectively, which were statistically at par with each other. On the sixth day after the second spray, the lowest population plant⁻¹ (0.43) and highest efficacy (78.42%) were recorded with bifenthrin followed by lambda-cyhalothrin, cypermethrin, permethrin and chlorpyrifos+ cypermethrin which were statistically at par to each other and significantly superior in efficacy and in reducing the population over chloropyrifos, neem oil and control (untreated) during 2014 and the same trend was observed in 2015. Again, on the tenth day after second spray, the lowest population plant⁻¹ (1.27) and highest efficacy (35.72%) was recorded with bifenthrin followed by lambda-cyhalothrin which were at par to each other and significantly superior in efficacy and reducing the population over rest of the treatments including untreated plots. On the tenth day after second spray, the efficacy of chlorpyrifos+ cypermethrin, cypermethrin, neem oil, permethrin and chloropyrifos treatments were statistically at par with control (untreated) in efficacy and reducing the population during 2014 and the similar trend was observed in 2015.

Before the third spray, population plant⁻¹ varied from 1.73 to 2.43 and 2.13 to 2.33 during 2014 and 2015 respectively, which were statistically at par with each other. On the sixth day after the third spray, the lowest population plant⁻¹ (0.37) and highest efficacy (78.44%) were recorded with bifenthrin followed by lambda-cyhalothrin, chlorpyrifos+ cypermethrin, cypermethrin and permethrin which were statistically at par to each other and significantly superior in efficacy and reducing the population over chloropyrifos, neem oil and control (untreated) during 2014 and the same trend was observed in 2015. Again, on tenth day after third spray, the lowest population plant⁻¹ (0.90) and highest efficacy (22.17%) were recorded with bifenthrin followed by lambda-cyhalothrin which were at par to each other and significantly superior in reducing the population and efficacy over rest of the treatments including untreated plots. By the tenth day after third spray, the efficacy of chlorpyrifos+ cypermethrin, cypermethrin, neem oil, permethrin and chloropyrifos treatments were statistically at par with control (untreated) in efficacy and reducing the population of *M. phalerata* during 2014 and the similar trend was observed in 2015.

Effects of various treatments on the productivity and incremental benefit cost ratio

The pigeonpea crop productivity (kg ha⁻¹) of various treatments has been depicted in table 3. The efficacy of all the treatments to reduce the *M. phalerata* population was reflected on the pigeonpea productivity. The data explicitly showed that the highest yield (1402.91 kg ha⁻¹) was recorded with bifenthrin followed by

lambda-cyhalothrin, cypermethrin, chlorpyrifos+ cypermethrin and permethrin which were statistically at par to each other and significantly superior over chlorpyrifos, neem oil treatments and untreated plots during 2014 and the similar trend was observed in 2015.

The cost of control measures of *M. phalerata* and their effects on productivity was reflected on incremental benefit cost ratio of the various treatments which has been depicted in table 3. The incremental benefit cost ratios revealed that the bifenthrin, lambda-cyhalothrin, chlorpyrifos+ cypermethrin, cypermethrin and permethrin treatments were lucrative in comparison with untreated plots while chlorpyrifos and neem oil were economically not viable during both experimental years. The highest incremental benefit cost ratio (1:9.45) was obtained from lambda-cyhalothrin (matador) followed by bifenthrin (1:8.62), cypermethrin (1:6.87), chlorpyrifos+ cypermethrin (1:6.08), permethrin (1:5.90), chlorpyrifos (1:0.99) and neem oil (1:0.73) during 2014 and an almost similar trend was obtained during 2015. The highest net return per rupee of investment (8.45) was obtained from lambda-cyhalothrin (matador) followed by bifenthrin (7.62), cypermethrin (5.87), chlorpyrifos+ cypermethrin (5.08), permethrin (4.90), chlorpyrifos (-0.01) and neem oil (-0.27) during 2014 and an almost similar trend was obtained during 2015. The incremental benefit cost ratio variation may be due to yield difference and cost of the insecticides, which has been explicated in above results.

These present findings are in confirmation with Ali et al. (2005) reported that the lowest number of insects spotted bollworms was obtained in bifenthrin and lambda-cyhalothrin treated plots, however, cypermethrin was not as effective as the above mentioned insecticides. Pyrethroids; cypermethrin and lambda-cyhalothrin were most effective than the other groups of insecticides against blister beetles (Sharma et al., 2010; Shende et al., 2013). The cypermethrin and chlorpyrifos+ cypermethrin were promising with consistently lower blister beetle population (Pawar et al., 2013). There was significant difference in per cent efficacy on sixth day after sprays of all pyrethroids based pesticides in comparison to chlorpyrifos and neem oil. Pyrethroids treatments effectively controlled blister beetles in comparison to neem-Azal (Dikshit et al., 2001).

The significant difference in per cent efficacy on the tenth day after sprays of bifenthrin in comparison to other insecticides might be attributed due to its long persistence. The bifenthrin is more stable than chlorpyrifos (Baskaran et al., 1999). At high dose, residues of bifenthrin persisted up to 15 days in leguminous crop (Mukherjee et al., 2010). The significant difference in per cent efficacy on the tenth day after sprays of lambda-cyhalothrin (matador) in comparison to other pesticides might be attributed due to lambda-cyhalothrin encapsulated with polymers. The encapsulation process provides the long-lasting characteristic due to its ability curtail to its degradation. Micro-encapsulation technology is reduced evaporative losses and

Table 1. Bioefficacy of different insecticides against *M. phalerata* on pigeonpea during 2014

Treatment	<i>M. phalerata</i> population plant ⁻¹ (1 st insecticidal spray)			Population reduction (%) after 1 st spray		<i>M. phalerata</i> population plant ⁻¹ (2 nd insecticidal spray)			Population reduction (%) after 2 nd spray		<i>M. phalerata</i> population plant ⁻¹ (3 rd insecticidal spray)			Population reduction (%) after 3 rd spray	
	Before spray	6 th day after spray	10 th day after spray	6 th day after spray	10 th day after spray	Before spray	6 th day after spray	10 th day after spray	6 th day after spray	10 th day after spray	Before spray	6 th day after spray	10 th day after spray	6 th day after spray	10 th day after spray
Bifenthrin 62 g a.i. ha ⁻¹	1.80	0.47	1.40	73.93 (8.62)	36.36 (6.07)	2.23	0.43	1.27	78.42 (8.88)	35.72 (6.02)	1.73	0.37	0.90	78.44 (8.88)	22.17 (4.75)
Lambda-cyhalothrin 24 g a.i. ha ⁻¹	1.90	0.50	1.47	73.74 (8.61)	36.98 (6.11)	2.37	0.47	1.37	77.94 (8.85)	34.29 (5.89)	1.83	0.40	0.97	77.19 (8.80)	21.10 (4.65)
Chlorpyrifos 310 gai. + Cypermethrin 31 g a.i.	1.87	0.53	2.27	71.42 (8.48)	0.75 (1.05)	2.27	0.57	2.03	72.60 (8.54)	0.57 (0.97)	1.93	0.50	1.33	72.79 (8.56)	0.00 (0.71)
Cypermethrin 62 g a.i.	1.93	0.53	2.33	71.57 (8.48)	0.97 (1.13)	2.33	0.53	2.03	75.29 (8.71)	3.33 (1.55)	1.97	0.50	1.33	72.84 (8.55)	1.13 (1.13)
Neem oil 1500 ppm	1.87	1.83	2.30	1.51 (1.29)	0.00 (0.71)	2.37	2.17	2.30	1.36 (1.23)	0.26 (0.85)	2.13	2.03	1.47	0.55 (0.99)	1.13 (1.13)
Permethrin 154 g a.i. ha ⁻¹	1.77	0.53	2.17	69.52 (8.37)	0.68 (1.00)	2.27	0.53	2.03	74.54 (8.66)	0.26 (0.85)	1.90	0.50	1.33	73.01 (8.56)	0.00 (0.71)
Chlorpyrifos 173 g a.i.	1.73	1.73	2.17	1.63 (1.37)	0.00 (0.71)	2.60	2.37	2.33	1.34 (1.20)	1.28 (1.17)	2.17	2.07	1.60	0.19 (0.82)	3.87 (1.63)
Untreated	1.83	1.83	2.23	0.00 (0.71)	0.00 (0.71)	2.60	2.40	2.30	0.00 (0.71)	0.00 (0.71)	2.43	2.33	1.63	0.00 (0.71)	0.00 (0.71)
SEM±	0.19	0.13	0.23	0.27	0.20	0.12	0.10	0.09	0.25	0.39	0.15	0.10	0.11	0.25	0.33
CD (P=0.05)	0.56	0.39	0.69	0.82	0.59	0.38	0.29	0.28	0.75	1.18	0.45	0.30	0.33	0.75	0.99
CV	17.53	22.19	19.37	8.14	15.47	9.09	14.39	8.12	7.35	29.99	13.12	15.53	14.12	7.47	29.42

Square root transformed value in parentheses

Table 2. Bioefficacy of different insecticides against *M. phalerata* on pigeonpea during 2015

Treatment	<i>M. phalerata</i> population plant ⁻¹ (1 st pesticides spray)					Population reduction (%) after 1 st spray		<i>M. phalerata</i> population plant ⁻¹ (2 nd insecticidal spray)			Population reduction (%) after 2 nd spray		<i>M. phalerata</i> population plant ⁻¹ (3 rd insecticidal spray)			Population reduction (%) after 3 rd spray	
	Before spray	6 th day after spray	10 th day after spray	6 th day after spray	10 th day after spray	Before spray	6 th day after spray	10 th day after spray	6 th day after spray	10 th day after spray	Before spray	6 th day after spray	10 th day after spray	6 th day after spray	10 th day after spray		
	Bifenthrin 62 g a.i. ha	2.00	0.50	1.43	77.02 (8.80)	34.38 (5.89)	2.03	0.40	1.27	80.60 (9.00)	38.50 (6.24)	2.13	0.47	0.93	78.10 (8.86)	31.77 (5.68)	
Lambda-cyhalothrin 2 g a.i. ha ⁻¹	2.03	0.53	1.47	75.63 (8.72)	34.29 (5.90)	2.07	0.43	1.33	79.35 (8.93)	36.35 (6.06)	2.13	0.50	1.00	76.34 (8.76)	26.71 (5.19)		
Chlorpyrifos 310 gai. + Cypermethrin 31 g a.i.	1.83	0.57	2.03	71.09 (8.46)	0.00 (0.81)	2.07	0.53	2.13	74.65 (8.66)	1.39 (1.19)	2.10	0.47	1.40	77.70 (8.84)	0.00 (0.71)		
Cypermethrin 62 g a.i.	1.83	0.53	1.97	72.35 (8.53)	406 (1.77)	2.03	0.50	2.10	75.90 (8.74)	0.35 (0.89)	2.07	0.43	1.37	78.88 (8.91)	1.33 (1.18)		
Neem oil 1500 ppm	1.80	1.90	1.93	1.52 (1.22)	3.17 (1.53)	2.17	2.17	2.17	1.74 (1.37)	1.80 (1.38)	2.13	2.13	1.40	0.14 (0.79)	0.98 (1.16)		
Permethrin 154 g a.i.	1.90	0.57	2.07	71.28 (8.47)	3.50 (1.90)	2.07	0.53	2.13	73.94 (8.62)	1.51 (1.27)	2.10	0.47	1.40	77.76 (8.84)	0.66 (1.00)		
Chlorpyrifos 173 g ha ⁻¹	1.90	2.00	2.10	1.78 (1.33)	0.00 (0.71)	2.23	2.23	2.23	1.57 (1.28)	1.57 (1.31)	2.17	2.13	1.43	1.66 (1.28)	0.98 (1.16)		
Untreated	1.97	2.10	2.17	0.00 (0.71)	0.00 (0.71)	2.33	2.37	2.37	0.00 (0.71)	0.00 (0.71)	2.33	2.33	1.50	0.00 (0.71)	0.00 (0.71)		
SEm±	0.17	0.1	0.15	0.28	0.42	0.10	0.07	0.10	0.26	0.33	0.08	0.07	0.05	0.24	0.25		
CD (P=0.05)	0.53	0.30	0.46	0.85	1.29	0.30	0.21	0.29	0.79	1.01	0.23	0.21	0.15	0.74	0.77		
CV	15.70	15.86	13.82	8.42	30.64	8.21	11.29	8.52	7.67	24.19	6.06	10.79	6.77	7.22	21.03		

Square root transformed value in parentheses

Table 3. Effects of various treatments against *M. phalerata* on the productivity of pigeonpea and incremental benefit cost ratio

Treatment	2014					2015				
	Average grain yield (kg ha ⁻¹)	Total Return (Rs ha ⁻¹)	Realization over control (Rs ha ⁻¹)	Cost of treatment (Rs ha ⁻¹)	B : C ratio	Average grain yield (kg ha ⁻¹)	Total Return (Rs ha ⁻¹)	Realization over control (Rs ha ⁻¹)	Cost of treatment (Rs ha ⁻¹)	B : C ratio
Bifenthrin 62 g a.i. ha ⁻¹	1402.91	61026.44	25854.08	3000	8.62	1357.33	62776.67	27040.83	3000	9.01
Lambda-cyhalothrin 24 g a.i. ha ⁻¹	1395.27	60694.10	25521.74	2700	9.45	1353.33	62591.67	26855.83	2700	9.95
Chlorpyrifos 310 gai. + Cypermethrin 31 g a.i. ha ⁻¹	1185.71	51578.24	16405.88	2700	6.08	1207.00	55823.75	20087.92	2700	7.44
Cypermethrin 62 g a.i. ha ⁻¹	1229.17	53469.04	18296.68	2662.5	6.87	1211.00	56008.75	20272.92	2662.5	7.61
Neem oil 1500 ppm	856.43	37254.56	2082.20	2850	0.73	822.00	38017.50	2281.67	2850	0.80
Permethrin 154 g a.i. ha ⁻¹	1179.95	51327.68	16155.32	2737.5	5.90	1199.67	55484.58	19748.75	2737.5	7.21
Chloropyrifos 173 g a.i. ha ⁻¹	865.56	37651.86	2479.50	2512.5	0.99	823.00	38063.75	2327.92	2512.5	0.93
Untreated	808.56	35172.36	-	0	-	772.67	35735.83	-	0	-
SEm±	81.64					75.69				
CD (P=0.05)	247.67					229.61				
CV	13.22					12.02				
MSP of Pigeonpea 2014-15 was 43500 ton ⁻¹										
MSP of Pigeonpea 2015-16 was 46250 ton ⁻¹										

therefore, reduces environmental degradation (Shirley et al., 2001; Knowles, 2008). The polymer wall retains the lambda cyhalothrin for extended periods on inert or chemically active surfaces, reducing losses from adsorption and chemical reaction (Wege et al., 1999).

The maximum yield was observed in bifenthrin and lambda-cyhalothrin and minimum in control plots. The yield up to some extent, might be varied due to damage of reproductive parts as flowers by blister beetle and similarly, (Kemal and Kocak, 2008) reported that blister beetle reduces crop yield through direct feeding of its flowers. The highest grain yield of pigeonpea was recorded from bifenthrin, which is significantly identical with lambda-cyhalothrin, although cost of bifenthrin treatment was much higher than the lambda-cyhalothrin. Therefore, the incremental benefit cost ratio analysis revealed superiority of lambda-cyhalothrin and impose lucrative net return per rupee of investment followed by bifenthrin, cypermethrin, permethrin and chlorpyrifos+ cypermethrin. This result was in conformity with the findings of (Singh et al., 2009; Pawar et al., 2013).

CONCLUSION

In conclusion, bifenthrin, lambda-cyhalothrin (encapsulated with polymers), cypermethrin, chlorpyrifos+ cypermethrin and permethrin proved effectual to suppress the *M. phalerata* population up to five days. However, bifenthrin and lambda-cyhalothrin were superior and most effectual to suppress the population with high efficacy up to ten days. The lambda-cyhalothrin was found highest incremental benefit cost ratio. The results inferred that the both bifenthrin and lambda-cyhalothrin confers excellent protection up to ten days, but, blooming period of pigeonpea variety UPAS 120 is about 30 days in normal weather conditions. Hence, three sprays of lambda-cyhalothrin and bifenthrin with an interchange at ten days interval will be effectual and lucrative against *M. phalerata* in short duration pigeonpea crop.

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IN VITRO PRODUCTION OF BANANA PLANTLETS AND THEIR VALIDATION AT HILL VALLEYS

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ABSTRACT

Performances of tissue cultured (TC) plantlets of BARI Kola-3 and BARI Kola-4 were evaluated against transplanting of conventional (CON) sucker at hill valleys during July 2011 to December 2014. The plantlets were produced at Biotech Lab, BARI Gazipur and validation trial was conducted at Ramgarh, Khagrachari hill district during March-April and September-October planting seasons (2011-14). Murashige and Skoog (MS) basal medium supplemented @ 3 mg l⁻¹ and 5 mg l⁻¹ BAP showed the best performance for multiple shoot production in BARI Kola-3 and BARI Kola-4, respectively. Both the varieties produced well developed roots in ½MS medium supplemented @ 0.5 mg l⁻¹ IBA. Among the plantlets 98.5% plantlets were survived when hardened 7 days under polyethylene tunnel after 5 days hardening at room temperature but 100% plantlets died when transplanted plantlets were kept in open conditions at the nursery. 45 day - old healthy plantlets were planted at hill valleys in September-October and April-May planting seasons. BARI Kola-3 produced the highest 45.67 and 44.05 t ha⁻¹ bunch yield at April-May and September-October planting, respectively. The highest 55.89 t ha⁻¹ bunch yield was recorded from TC BARI Kola-4 in March-April planting followed by 49.67 t ha⁻¹ in September-October Planting. TC BARI Kola-3 and BARI Kola-4 produced 3.68 and 12.52% higher yields in March-April planting, respectively than September-October planting. In case of planting materials, TC BARI Kola-3 and TC BARI Kola-4 produced 23.43 and 20.33% higher yield over CON suckered plant in March-April planting. On the other hand, TC BARI Kola-3 and TC BARI Kola-4 also produced 12.34 and 9.35% higher yield than CON Suckered plant in September-October planting. Similarly, TC BARI Kola-4 produced 28.33% higher yield than CON suckered BARI Kola-4 in March-April planting. In every case, TC plants showed better performances than the CON suckered plant.

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Keywords: Banana, tissue culture, phytohormones, planting season, yield

INTRODUCTION

Banana (*Musa* sp.) is the most important fruit crop in Bangladesh. It grows all over the country but production is mainly concentrated in the Chittagong hill tract regions. Recently, commercial cultivation has been started at North-Western part of the country. In Bangladesh, area under banana cultivation is 47 thousand hectares of land and production is 0.77 million metric tons in a year (BBS, 2013-14). The average yield of banana is 16.62 t ha⁻¹ which is much lower than that of other banana growing countries. Bananas are propagated vegetatively by means of sucker which is very slow process. In this way, each plant produced small number sword suckers in a year which is a great limitation for commercial cultivation and this material carries the most devastating disease (Rahman et al., 2002). Such problems further aggravates by common practice of propagation using infected suckers. BARI Kola-3 and BARI Kola-4 are popular high yielding varieties and widely cultivated all over the country. These two varieties are also suitable for hilly regions (Saha, 2010). Although hill valleys are important for banana cultivation but unfortunately, commercial cultivation of BARI Kola-3 and BARI Kola-4 is absent at hilly areas. Hilly peoples grows banana along with zoom, homestead and at the foot of the hills scattered (Uddin et al., 1998). Due to the lack of quality sucker, post harvest processing and transportation system, farmers cannot cultivate banana commercially at hilly areas (Annon, 2010). Tissue culture method can produce a huge amount of disease free banana plantlets in limited time and space (Molla et al., 2004). These plantlets are genetically homogeneous; more than 95% of total products are at a time harvestable with 10-15% higher yield (Akhond and Bhuiyan, 2001). Moreover, this planting material resists bunchy top disease for several ratoon crops with higher yield (Khanam et al., 2014). Biotechnology division, BARI has been developed a protocol for multiplication of Amrithsagar and BARI Kola-1 (Khatun et al., 2003). Usually this protocol varies variety to variety. So far it was reviewed; the protocol for BARI Kola-3 and BARI Kola-4 was not optimized. So, the existing protocol was needed to optimize. Research report on tissue culture banana plantlet cultivation at hilly areas of Bangladesh is not published yet. This is so far the first research report on tissue culture banana plantlet cultivation at hilly areas in Bangladesh. Therefore, the study was conducted to optimize the existing protocol for *in vitro* production of BARI Kola-3 and BARI Kola-4 and validate the performance of tissue cultured banana plantlets at hill valleys.

MATERIALS AND METHODS

Sword sucker of BARI Kola-3 and BARI Kola-4 from disease-free plant were collected from Agricultural Research Station (ARS), Raikhali, Rangamati from mother orchard. Roots and outer layer of tissues of the suckers were removed and the remaining portions were washed with tap water with detergent. The explants were then surface sterilized in 60% Clorox with a few drops of Tween-20 for 30 min. After washing 4 times with sterilized distilled water, the shoot tips were further cut to a size of approximately 1.0 cm portion containing an intact apex under clean bench. The explants were placed on MS medium (Murashige and Skoog, 1962) supplemented with different concentrations of cytokinins (BAP and 2-ip) for *in vitro* multiple shoot production. All cultures were incubated at $25 \pm 1^\circ\text{C}$ with a 16 hrs photoperiod provided by cool white florescent tubes. The pH of the medium was adjusted to 5.8 prior to autoclaving. The materials were sub-cultured at 30 day's interval in the same medium to produce multiple shoots. Well developed shoots were transferred to rooting medium containing $\frac{1}{2}$ MS medium supplemented with different concentrations of IBA for root induction. At the age of 28 days in rooting medium, well developed rooted plantlets were kept at room temperature for 5 days and then transferred to poly bags containing soil, sand and decomposed cow dung (1:1:1). Transplanted plantlets were hardened under polyethylene tunnel for 7 days and then shifted at nursery conditions for growth and development. After 45 days, well established plantlets were transplanted for validation. Plantlets were planted 2.0m \times 2.0m spacing with factorial RCB design having 3 replications during March-April and September-October planting season along with traditional sucker. Before planting, randomly 100 sample plantlets were tested for bunchy top virus using Agdia Bunchy top virus testing kit (USA). Data on plant height (cm), base girth (cm), number of leaves at flowering, days to 50% flowering, days to harvest, no. of finger per bunch, no. of hands per bunch, wt. of individual finger (g.), individual bunch wt. (kg) and yield (t ha⁻¹) etc. were recorded and data were analyzed using Mstat-C statistical package. Differences among the means were compared by the Duncan's Multiple Range Test at 1% level of significance (Gomez and Gomez, 1984).

RESULTS AND DISCUSSIONS

Standardization of protocol for *in vitro* production of BARI Kola-3 and BARI Kola-4

The study was conducted to find out an efficient dose of cytokinin (BAP and 2-ip) for *in vitro* production of BARI Kola-3 and BARI Kola-4. Shoot tip of BARI Kola-3 and BARI Kola-4 were cultured in MS medium supplemented with six different concentrations of BAP (viz., 1, 2, 3, 4, 5 and 6 mg l⁻¹) and three different concentrations of 2-ip (viz., 1.0, 1.5, 2.0, 2.5 mg l⁻¹). BARI Kola-4 produced the highest number of shoots per plant (7.0) in MS medium supplemented with BAP 5.0 mg l⁻¹ followed by BARI Kola-3 (6.1) in BAP 3.0 mg l⁻¹ (Figure 1 & Plate 1) within 40.0 and 42.55 days, respectively.

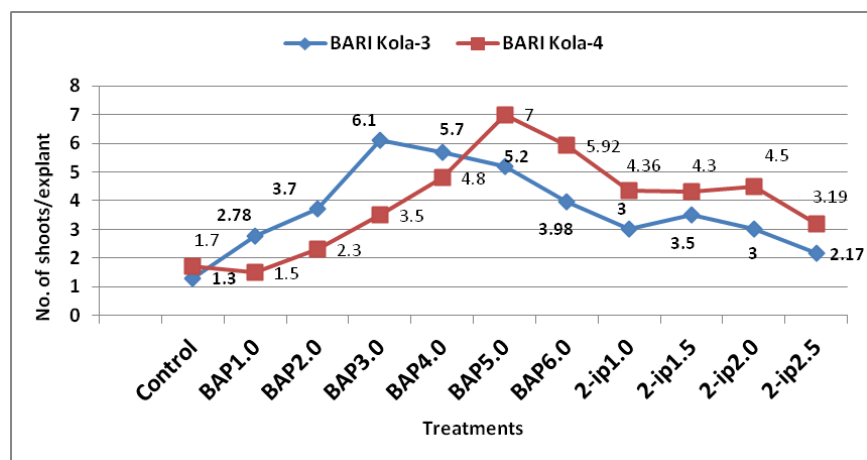


Figure 1. Shoot proliferation and multiple shooting of BARI Kola-3 and BARI kola-4

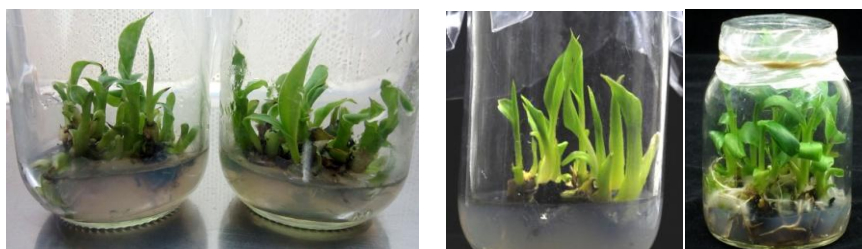


Plate 1.a. Multiple shoots (BARI Kola-3)

Plate 1.b. Multiple shoots (BARI Kola-4)

Control treatment did not produce any lateral shoots. Cytokinin is generally considered necessary for the acquisition of the meristematic competence of the responsive cells. Once this competence has been established, excessive concentration is often found to be inhibitory for further embryonic or adventitious bud development (Khanam et al., 2007, Molla et al., 2004). However, higher concentration of BAP produced maximum number of shoots up to a certain level where lower concentration induced minimum number of shoots. Cytokinin reduced the callus phase and accelerated shoot regeneration. The findings are accordance with Khatun et al., 2006; Khanam et al., 2007.

Effect of IBA on *in vitro* rooting at BARI kola-3 and BARI Kola-4

The study was conducted to find out an efficient dose of IBA for *in vitro* root production of BARI Kola-3 and BARI Kola-4. After five sub-cultures, well developed shoots were transferred to rooting medium. Half MS medium supplemented with different concentration of IBA (0.1, 0.3, 0.5, 0.7 and 0.9 mg l⁻¹) were tested for rooting of BARI Kola-3 and BARI Kola-4. Thirty six explants were

cultured on each treatment where survival percentage was 100%. The maximum 15.6 days required for root induction in BARI Kola-3 only in $\frac{1}{2}$ MS medium whereas only 4 days was needed to root initiation in BARI Kola-4 in $\frac{1}{2}$ MS medium supplemented 0.5 mg l^{-1} IBA.

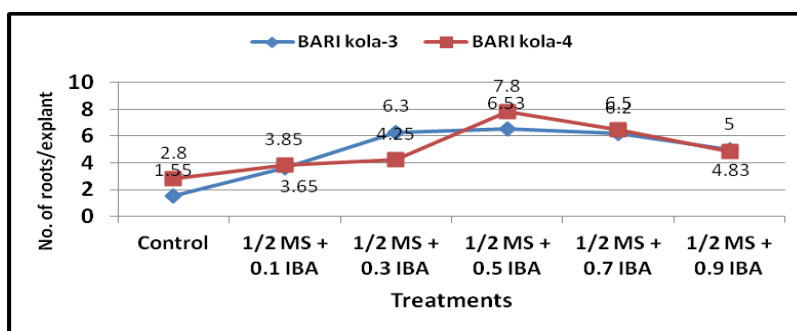


Figure 2. Root induction as influenced by different concentrations of IBA

The maximum number of roots (7.80) was recorded in BARI Kola-4 in $\frac{1}{2}$ MS medium supplemented with 0.5 mg l^{-1} IBA followed by BARI Kola-3 (6.53) with same level of IBA (Figure 2, Plate 2).



Plate 2.a: Plantlets (BARI Kola-3)

Plate 2.b: Plantlets (BARI Kola-4)

Number of root per plant decreased after a certain levels of IBA in $\frac{1}{2}$ MS medium. This might be due to excessive dose of hormone. Control treatment produced minimum number of roots which were very lean and thin. Moreover, number of root/plant varied due to growth and development of shoots cultured in rooting medium. Higher dose of IBA in the medium also reduced the number of root/plant. These findings are in accordance with Molla et al., 2004. Auxin is generally necessary for the acquisition of the meristematic competence of the responsive rooting cells. Once this competence has been established, excessive concentration is often found to be inhibitory for further embryonic or adventitious bud development (Khanam et al., 2007). Lower concentration of auxin and cytokinin influenced the formation of both root and shoot but at higher concentrations plantlets showed reversed trends (Sanavy and Moeini, 2003)

***Ex vitro* establishment**

The rooted plantlets with 5 leaves stage were transferred from culture room and kept at room temperature for 5 days. The plantlets were then removed from the culture tubes and all the adhering media were carefully washed out so that, the root damage was the least. Washed plantlets were planted into small polyethylene bags (24cm×16cm) containing soil, sand and decomposed cow dung at the ratio of 1: 1: 1. More than 98.5% *in vitro* plantlets survived when these plantlets were kept at room temperature for 5 days and hardened inside the polythene tunnel house for 7 days at nursery on moist condition but 100% plantlets were died where the plantlets were transferred to net house at normal temperature under open condition.

Field performance of TC plantlets against CON sucker at March -April and September-October Planting:

Tissue culture (TC) plantlets of BARI Kola-3 and BARI Kola-4 along with CON sucker were planted at hill valleys during March-April and September-October planting seasons. Details results have been presented in table 1.

There was a significant variation on plant height due to the effect of planting season and propagating materials. The maximum plant height 356.33cm was recorded in TC BARI Kola-3 in March-April planting followed by CON suckered BARI Kola-3 (347cm). The lowest plant height (319.67cm) was recorded in CON suckered BARI Kola-4. The results revealed that TC BARI Kola-3 gave the highest plant height in both seasons compare to TC BARI Kola-4. This might be due to genetic and seasonal effect. Effect of season and propagating materials had significant effect on base girth which ranged from 65.11 to 85.04cm. The highest base girth 85.04cm was recorded in TC BARI Kola-3 in March-April planting followed by TC BARI Kola-3 in September-October. Lowest base girth (65.11cm) was recorded in CON suckered BARI Kola-4 in September-October planting (Table 1). This might be due to available soil moisture during March-April planting and residual hormonal effect in TC planting material. Distinct variation was found in respect of number of leaves per plant at harvest due to the season and propagating materials. The maximum number of leaves (23.40) was counted in TC BARI Kola-3 at September-October planting while 22.89 was counted in CON BARI Kola-3. On the other hand, TC BARI Kola-4 produced maximum 22.66 leaves per plant followed by CON suckered (22.21) at the same season (Table 1). In case of March-April planting, maximum number of leaves per plant (22.85) was counted in TC BARI Kola-3 followed by CON suckered plant (22.61) which was statistically similar. There was no significant difference between the number of leaves in TC BARI Kola-4 and CON suckered plants (Table 1). Distinct variation was found in respect of days to 50% flowering due to the season and planting materials (Table 1). Seventeen days early flowering was found at TC BARI Kola-3 than CON suckered plant which was statistically significant. It was needed minimum 375 days for 50% flowering. However, TC BARI Kola-4 needed 359.33 days for 50% flowering followed by

370.33 days in CON suckered plant. In case of March-April planting, 19 days early flowering was found in TC BARI Kola-3 than CON suckered plant. Also, TC BARI Kola-4 produced 50% flowering within 343.33 days which was 22 days earlier than CON suckered plant. Both the varieties produced 15 days early flowering in March-April planting. Plants derived from CON sucker produced flower in different times where TC plants produced within 7-10 days from starting. Remarkable variation was found in days to harvest due to the combined effect of season and planting materials (Table 1). Maximum 482.33 days was needed for harvesting CON suckered BARI Kola-3 while 471.67 days was needed in TC plants at September-October planting. But in March-April planting, 449 days needed for TC plants where 474.33 days needed in CON suckered plants of BARI Kola-3 (Table 1). At least 22 days early harvesting was found in March-April planting than September-October planting in BARI Kola-3. Days required for harvesting were ranged from 434.33 to 450. Sixteen days early harvest was done in TC BARI Kola-4 than CON suckered BARI Kola-4 in September-October planting. In case of March-April planting, TC BARI Kola-4 was harvested 20 days earlier than CON suckered plants. Early harvesting was found in March-April planting in both the varieties than September-October planting. These results are in accordance with the findings of Faisal et al., 1998. The combined effects of season and planting materials had significant influence on yield and yield contributing characters (Table 1 & Figure 3). TC plants had significant effect on number of fingers per hand in BARI Kola-3 than CON suckered plants. Maximum number of fingers per hand 15.45 was recorded in TC plant followed by CON suckered plant (14.63). Similar trends were also found in case of individual finger weight (g), finger size (cm²), weight of individual bunch (g) and yield of bunch (kg) at September-October plantation (Table 1). TC BARI Kola-4 also produced higher number of fingers per hand (15.45), number of finger per bunch (164.85), number of hands per bunch (10.67), followed by CON suckered plants 14.63, 150.73 and 10.30, respectively at September-October planting. Similar trend was also found in individual finger weight, finger size, weight of individual hand, weight of individual bunch and bunch yield at both of September-October and March-April planting (Table 1). Wide variation was found in respect of yield due to the effect of season and planting materials. The highest 55.89 t ha⁻¹ bunch yield was found from TC BARI Kola-4 followed by 45.67 t ha⁻¹ in TC BARI Kola-3 in March-April planting (Figure 3). In case of September-October planting, TC BARI Kola-3 and BARI Kola-4 produced 49.67 and 44.05 t ha⁻¹, respectively which was 12.34% and 9.35% higher over CON suckered plant. Hilly peoples usually planted banana during the month of March-April. During this period raining starts at hilly areas and soil contains moisture which helps to better growth and development of plants.

During the period of September-December, hilly areas suffer from drought and water scarcity. Due to the absent of rain water plant cannot get abundant moisture. As a result, growth and development of plants hampered and production reduced. However, TC BARI Kola-3 produced 49.67 t ha⁻¹ followed by TC BARI Kola-4

Table 1. Effect of planting season and propagating materials on the growth and yield of BARI Kola-3 and BARI Kola-4 at hilly areas

Treatments	Plant height (cm)	Base girth (cm)	Total no. of leaves at harvest per plant	Days to 50% flowering	Days to harvest	No. of hands per bunch	No. of fingers per bunch	No. of fingers per hand	Individual finger weight (g)	Finger size (cm ²)		Wt. of indi. hand (kg)	Indi. bunch weight (kg)
										Length	Breadth		
S ₁ M ₁	344.33 bc	82.11 b	23.40 a	375.00 bc	471.67 ab	10.67 a-d	164.85 ab	15.45 a	105.00 b	13.33 c	3.88 b	1.54 c	17.62 c
S ₁ M ₂	337.67 bc	74.89 d	22.89 ab	392.00 a	482.33 ab	10.30 cd	150.73 b	14.63 b	88.00 c	12.11 e	3.50 c	1.36 e	15.68 d
S ₁ M ₃	335.00 c	70.11 e	22.66 b	359.33 e	434.33 b	11.43 a	161.28 ab	14.11 bc	112.33 b	15.07 a	3.96 ab	1.53 cd	18.27 c
S ₁ M ₄	318.00 d	65.11 f	22.21 bc	370.33 cd	450.00 b	10.97 a-c	149.54 b	13.63 c	85.33 c	11.82 f	3.32 c	1.20 f	15.07 d
S ₂ M ₁	356.33 a	85.04 a	22.85 ab	361.00 e	449.33 b	11.17 ab	177.71 a	16.06 a	109.71 b	13.86 b	3.96 ab	1.77 b	19.87 b
S ₂ M ₂	347.00 ab	77.83 c	22.61 b	380.00 b	474.33 a	10.08 d	162.73 ab	16.17 a	93.14 c	12.35 d	3.49 c	1.49 cd	18.17 c
S ₂ M ₃	338.67 bc	73.58 d	21.77 c	343.33 e	417.67 b	11.13 ab	171.62 a	16.15 a	124.44 a	15.11 a	4.10 a	1.99 a	22.36 a
S ₂ M ₄	319.67 d	70.45 e	21.87 c	365.33 de	437.67 b	10.57 b-d	163.17 ab	16.00 a	91.47 c	12.01 ef	3.40 c	1.42 de	17.42 c
Level of significance	**	**	*	**	**	**	**	*	**	**	**	**	**
CV%	6.65	7.19	6.85	6.31	8.98	4.25	5.96	7.93	4.81	7.01	6.11	4.14	7.63

S₁= September-October planting, S₂= March-April planting, M₁= TC BARI Kola-3, M₂ = CON sucker of BARI Kola-3, M₃ = TC BARI Kola-4, M₄ = CON sucker of BARI Kola-4.

Means bearing same letters are not significantly different at 1% level.

(44.05 t ha⁻¹) in September-October planting which was 9.36% and 12.34% over yield on CON suckered plants of BARI Kola-3 and BARI Kola-4, respectively (Figure 3). Rahman et al. (2002) found 9.34% higher yield in TC Amrithsagar banana over plants raised from CON sucker. Faisal et al. (1998) also found higher yield, better growth and development in TC plants against CON suckered plant of banana cv. Champa.

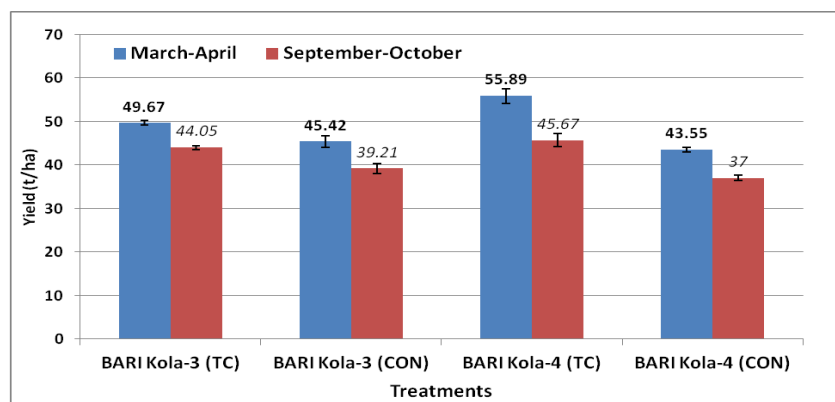


Figure 3. Yield at September-October and March-April plantation

CONCLUSION

From the above results, MS medium supplemented with 3 mg l⁻¹ and 5 mg l⁻¹ BAP was found suitable for *in vitro* multiple shoot production from the shoot tip of BARI Kola-3 and BARI Kola-4, respectively. ½MS medium supplemented with 0.5 mg l⁻¹ IBA has been found suitable for *in vitro* root production for both the varieties. BARI Kola-4 showed the better performance for commercial cultivation at hill valleys than BARI Kola-3. March-April planting season is better than the September-October planting at hilly areas due to available rain water. In every case, TC plants showed the better performance than the plants derived from CON sucker.

ACKNOWLEDGEMENT

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INFLUENCE OF SOWING TIME BASED TEMPERATURE ON FLOWERING AND SEED YIELD OF FRENCH BEAN (*Phaseolus vulgaris* L.)

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ABSTRACT

A field experiment was conducted at the research field of Agronomy Division, BARI, Joydebpur, Gazipur during rabi season of 2015-16 to evaluate the flowering behavior and seed yield of French bean. Three varieties viz., BARI Jharsheem-1, BARI Jharsheem-2 and BARI Jharsheem-3 were sown at 15 November, 30 November, 15 December and 30 December for getting temperature variation. Sowing dates based temperature variation, significantly affect the flowering behaviour and seed yield of French bean varieties. Flowering duration of 15 November and 30 November sowings were longer due to prevailing low temperatures (Min.11.72-13.24⁰C and Max. 25.02-25.82⁰C). On the contrary, minimum flowering duration was recorded in 30 December sown crop (11-17 days). Minimum duration might be due to prevailing high temperature (Min. 16.05-18.61⁰C and Max. 28.89-31.31⁰C) that shorten the flowering duration of all French bean varieties. November sowing performed better in relation to yield components and seed yield than other sowing. BARI Jharsheem-1 produced the maximum seed yield (1734 kg ha⁻¹) in 15 November which was statistically similar to 30 November sowing (1620 kg ha⁻¹). The lowest seed yield (421 kg ha⁻¹) was produced in BARI Jharsheem -2 from 30 December sowing. The highest seed yield was obtained from November sowing might be due to favourable optimum temperature for better flowering and pod setting that resulted in maximum seed.

Keywords: Phenology, temperature, flowering, yield, french bean, varieties

INTRODUCTION

French bean (*Phaseolus vulgaris* L.) is cultivated in Bangladesh as green bean as well as dry seeds. The mature seeds could be used for preparing dal. The crop has gained popularity for its short durability and high nutritive value. Green pods are rich in vitamins, protein and minerals which is also consumed as soup. French bean can

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play an important role to overcome the national protein deficit. Late sowing is one of the major causes of low yield of French bean. In Bangladesh, it is usually cultivated after T. Aman harvest. If delay of T. Aman harvest than the crop faces unfavorable weather conditions at its reproductive phase and gave low yield. Among the weather factors, mainly air temperature and rainfall greatly affect the growth and development of bean plants (Gross and Kigel, 1994; Mouhouche et al., 1998; Ibarra - Perez et al., 1999). Sowing at proper time allows sufficient growth and development of a crop to obtain a satisfactory yield because high temperature is one of the major environmental stresses that affect plant growth and development (Boyer, 1982). The optimum temperature range for optimum bean growth is 16-30°C (Nonneck, 1989). High temperature stress causes substantial loss in crop yield due to damage of reproductive organs (Savin and Nicolas, 1996) and reduced length of reproductive period. So, it is essential to study the crop growth behaviours in changing climatic condition. Therefore, the present experiment was conducted to evaluate the flowering pattern and seed yield under different temperature variation from different sowing time.

MATERIALS AND METHODS

The experiment was conducted at the Agronomy research field of Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur during rabi season of 2015-2016. The experiment site was located at Chhiata Series under Agro-Ecological Zone-28 (AEZ-28), latitude 23°59' N and longitude 90°24' E. Before opening the land, the soil samples were taken from the spots of the experimental area and analyzed from the Soil Science Division, BARI. The soil analysis showed that the soil of the experimental field was loam in texture and low in organic matter (1.27 %). The soil was acidic in nature with pH (6.1) and contained very low amount of total nitrogen (0.067%), phosphorus (9.6 %), sulphur (12 %), zinc (2 meq/100 g) and medium amount of potassium (0.18 meq/100 g). The treatments consisted of four sowing dates at 15 days interval starting from 15 November and end on 30 December in main plot while sub-plot comprises of three varieties (var. BARI Jharsheem-1, BARI Jharsheem-2 and BARI Jharsheem-3) with replicated thrice. The experiment was laid out in a split-plot design. The unit plot size was 3.0 m x 3.0 m. The crop was fertilized with 120-40-60-12-3 kg N-P-K-S-Zn /ha, respectively (FRG, 2012). Half of N and full doses of other fertilizers were applied at the time of final land preparation and the rest urea was top dressed at 35 days after sowing (DAS). Seeds was treated with vitavax and sown continuously in 30cm apart rows. Plant to plant distance was maintained by 15 cm. Hand weeding was done at 25 and 40 days after sowing (DAS). Pre sowing irrigation was given to the crop for uniform emergence. The crops were attacked by cutworm (*Agrotis ipsilon*) and hairy caterpillar (*Spilarctia obliqua*) at early growth (vegetative) stage. The cutworm was controlled through hand picking. Perfecthion 40 EC @ 2.0 ml l⁻¹ of water was sprayed at an interval of 7-10 days for 3 times to control hairy caterpillar. Matured pods were

counted at harvest and pod abscission percentage was calculated as the formula given by Saha (2003).

Pod abscission (%) = [Total open flowers- total matured pods]/ (total open flowers) ×100.

At each harvesting time, five plants were harvested randomly from each plot to record the data on yield components. Seed yield was recorded from an area of 2 m x 2 m avoiding border effect. Data on different parameters were subjected to analysis of variance and the treatment means were compared by Least Significant Difference (LSD) test.

RESULTS AND DISCUSSION

Crop developmental events and growth duration were influenced by prevailing temperature variations (Table 1). Crop growth duration was recorded maximum in BARI Jharsheem-2 sown on 15 November (107 days) followed by 30 November sowing (104 days). The minimum duration (76 days) was recorded in BARI Jharsheem-3 at 30 December sowing. The reasons for variation in growth duration might be due to increased day and night temperature. The late sown crops flowered earlier with reduced vegetative durations than those of early sown ones. The highest duration from emergence to first flowering was recorded in 30 November (39-43 days) sowing. The lowest duration for flowering was recorded (32-38 days) in 30 December sowing.

Table 1. Crop development events and crop duration of French bean varieties as affected by sowing date

Sowing date × variety	Emergence	Days to 1st flowering	Flowering duration (Day)	Crop growth duration (Day)
D ₁ × V ₁	6	32	25	100
D ₁ × V ₂	6	35	29	107
D ₁ × V ₃	6	29	20	100
D ₂ × V ₁	6	41	25	101
D ₂ × V ₂	6	43	30	104
D ₂ × V ₃	6	39	21	96
D ₃ × V ₁	7	39	21	86
D ₃ × V ₂	7	40	22	92
D ₃ × V ₃	7	37	15	88
D ₄ × V ₁	8	36	17	84
D ₄ × V ₂	8	38	15	86
D ₄ × V ₃	8	32	11	76

V₁= BARI Jharsheem -1, V₂= BARI Jharsheem -2, V₃= BARI Jharsheem -3,

D₁ = 15 November, D₂ = 30 November, D₃ = 15 December, D₄ = 30 December.

Temperature is an important factor of flowering in French bean (Table 2). Flowering duration in all varieties was also observed maximum in November sowing (20-30 days). Flowering duration of 15 November and 30 November sowings were longer due to prevailing low temperatures (Min.11.72-13.24^oC and Max 25.02-25.82^oC). On the contrary, minimum flowering duration was recorded in 30 December sown crop (11-17 days). Minimum duration might be due to prevailing high temperature (Min. 16.05-18.61^oC and Max 28.89-31.31^oC) that shorten the flowering duration of all varieties. Similar results were also observed by Helena Labuda and Anna Brodaczewska (2007).

Table 2. Prevailing average temperature during flowering duration of French bean varieties grown at different dates

Sowing Date	Flowering duration					
	BARI Jharsheem -1		BARI Jharsheem -2		BARI Jharsheem -3	
	Max. Tem. ^o C	Min. Tem. ^o C	Max. Tem. ^o C	Min. Tem. ^o C	Max. Tem. ^o C	Min. Tem. ^o C
15 November	25.56	11.74	25.02	11.72	25.52	11.78
30 November	25.18	12.10	25.82	13.24	24.26	11.41
15 December	27.82	15.07	28.75	16.11	26.38	12.74
30 December	30.87	18.41	31.31	18.61	28.89	16.05

Table 3. Total dry matter production of French bean as affected by variety and sowing date

Sowing date × variety	Total dry matter (g/m ²)				
	20 DAS	35 DAS	50 DAS	65 DAS	80 DAS
D ₁ × V ₁	31.81	77.52	150.07	254.83	282.17
D ₁ × V ₂	28.43	72.63	152.53	240.37	270.53
D ₁ × V ₃	29.52	68.67	142.50	224.24	240.40
D ₂ × V ₁	29.17	74.50	146.13	248.17	274.87
D ₂ × V ₂	27.33	71.57	136.50	227.27	257.13
D ₂ × V ₃	26.00	64.67	116.83	211.07	231.77
D ₃ × V ₁	21.71	61.33	112.82	200.53	254.23
D ₃ × V ₂	18.00	60.67	88.80	182.80	216.13
D ₃ × V ₃	15.33	48.04	78.03	152.50	178.57
D ₄ × V ₁	15.66	45.99	86.33	161.87	187.33
D ₄ × V ₂	13.33	38.31	68.33	126.25	146.13
D ₄ × V ₃	12.00	27.56	79.70	152.83	158.53
LSD (0.05)	-	-	12.70	4.18	9.78
CV (%)	11.40	5.08	6.48	4.22	5.51

Total dry matter production

The interaction of sowing date and variety had significant effect on total dry matter (TDM) production at 50, 65 and 80 DAS, except 20 and 30 DAS (Table 3). At the growth stages of 50, 65 and 80 DAS, the highest TDM were recorded 150.07, 254.57 and 282.17 g m⁻², respectively in BARI Jharsheem-1 sown on 15 November (D₂) which was identical with the dry matter produced by the same variety sown on 30 November. At 80 DAS, the lowest dry matter of 146.13 g m⁻² was produced by BARI Jharsheem-2 sown on 30 December (D₄). Among the varieties, BARI Jharsheem-1 sown at different dates accumulated more dry matter at all stages. This might be due to genetic potentiality of the variety (Ali and Tripathi, 1988).

YIELD AND YIELD COMPONENTS

Yield and yield attributes of French bean varieties were significantly affected by different sowing dates (Table 4). BARI Jharsheem-1 produced the tallest plant (52.33cm) in 15 November sowing. The shortest plant (22.33 cm) was obtained in BARI Jharsheem-2 sown on 30 December. The number of flowers plant⁻¹ was significantly affected by the different sowing dates and varieties. BARI Jharsheem -2 produced maximum and identical number of flowers plant⁻¹ (42.56 and 40.72) when sowing done on 15 November and 30 November and the lowest (15.03) also recorded in BARI Jharsheem-2 sown on 30 December. The result was in full agreement with that Graham (1979) who reported that French bean plants did not flower at 35-25 °C but flowered at day-night temperature of 25 - 15°C. The variation of flowering might be due to variations in temperature due to sowing dates. Significantly the highest number of pods plant⁻¹ (15.83) was recorded in BARI Jharsheem-1 sown on 15 November. The lowest (3.47) number of pods plant⁻¹ was obtained in BARI Jharsheem-2 sown on 30 December followed by BARI Jharseem-3 of same sowing date due to high temperature (Figure1). The lowest % pod abscission (59.29) was observed in BARI Jharsheem-1 from 15 November sowing. Both BARI Jharsheem-2 and BARI Jharsheem-3 produced the highest pod abscission in 30 December sowing. Heitholt et al. (1986) also reported that late flowers aborted more frequently than did early flowers in soybean. Pod abscission was increased by delay sowing which was accordance with Fisher (1980). The number of seeds pod⁻¹ also significantly differed by sowing date and varieties (Table 4). The var. BARI Jharsheem-1 produced maximum number of seeds pod⁻¹ (5) irrespective of sowing dates while the lowest (3) was produced in BARI Jharsheem-2 in 30 December sowing. The 100-seed weight of French bean was also significantly affected by different sowing dates and varieties. The 100-seed weight decreased with delay sowing. Weight of 100-seed was maximum (35.26g) in BARI Jharsheem -3 sown on 15 November followed by same variety sown on 30 November. The lowest seed weight (16.45 g) was produced in BARI Jharsheem-2 in 30 December sowing. November sowing received lower day and night temperature that causes longer crop growth duration and ultimately more TDM production and translocation to pods. On the other hand, December sowing

received higher day and night temperature that hasten forced maturity and reduced TDM production and translocation to the yield components.

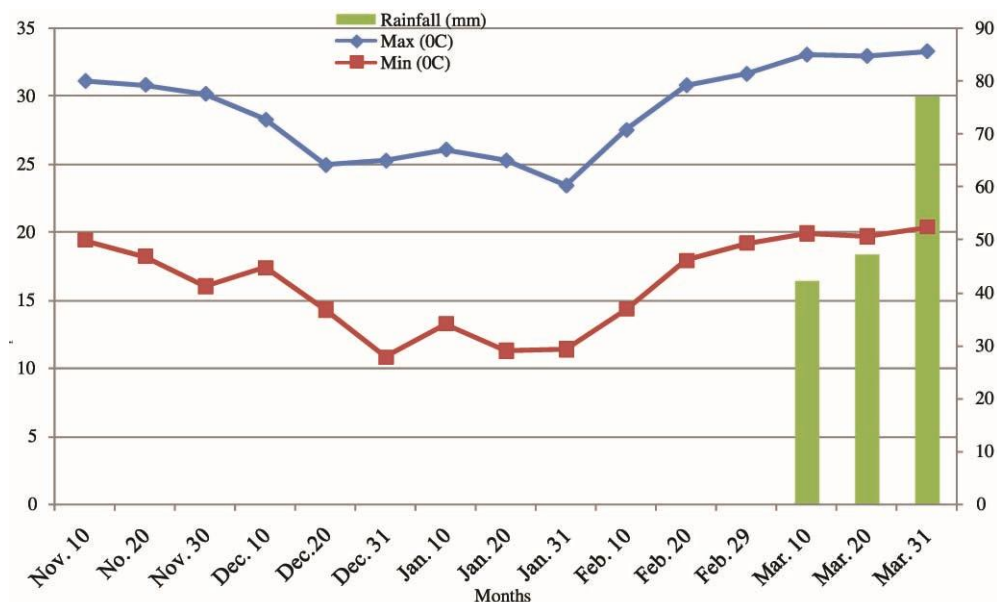


Figure 1. Decadal mean temperatures (min & max) and monthly total rainfall during crop growing periods

Seed yield is the function of number of pods plant^{-1} , seeds pod^{-1} and 100-seed weight. Seed yield decreased with delay sowing. Seed yield was found maximum (1734 kg ha^{-1}) in BARI Jharsheem-1 in 15 November sowing which was statistically similar with same variety in 30 November sowing (1680 kg ha^{-1}) and also with BARI Jharsheem-2 on 15 November sowing. The lowest seed yield (421 kg ha^{-1}) was produced in BARI Jharsheem-2 from 30 December sowing and it was statistically similar with BARI Jharsheem-3 at the same date. The highest seed yield was obtained from November sowing might be due to favourable optimum temperature resulting better vegetative growth of plants which led to better flowering and pod setting that resulted in maximum seed yield of French bean. This finding is also in agreement with the findings of Mohanty et al., 2001. There was a trend of decreasing seed yield and yield components with the advancement of sowing date from 30 November sowing due to increased in temperature. The results obtained in the present study are conformity with Mouho-uche et al., 1998; Nielsen and Nelson, 1998; Ferreira et al., 2000.

Table 4. Seed yield and yield components of different French bean varieties under different sowing dates

Sowing date × variety	Plant height(cm)	Flowers plant ⁻¹ (no.)	Pods/ plant (no.)	Pods Abscission (%)	Seeds pod ⁻¹ (no.)	100-seed weight (g)	Seed yield (Kg ha ⁻¹)
D ₁ × V ₁	52.33	39.01	15.83	59.29	5.00	24.73	1734.00
D ₁ × V ₂	43.93	42.56	14.43	66.08	4.00	23.73	1620.33
D ₁ × V ₃	45.73	25.10	8.93	64.06	4.00	35.26	1501.67
D ₂ × V ₁	44.73	38.51	14.93	61.21	5.00	23.47	1680.67
D ₂ × V ₂	39.47	40.72	12.50	69.27	4.00	22.57	1460.67
D ₂ × V ₃	44.13	25.34	9.27	63.04	4.00	34.93	1354.67
D ₃ × V ₁	34.17	23.44	8.97	61.72	5.00	22.87	1567.33
D ₃ × V ₂	31.33	25.27	6.50	74.29	4.00	17.50	1114.33
D ₃ × V ₃	34.80	19.93	5.83	70.55	4.00	29.60	1164.33
D ₄ × V ₁	24.93	19.80	7.00	64.16	4.00	19.17	914.00
D ₄ × V ₂	22.33	15.03	3.47	76.93	3.00	16.45	421.00
D ₄ × V ₃	26.47	15.57	4.02	73.98	3.00	27.80	562.00
LSD (0.05)	2.59	2.98	1.25	2.94	0.37	1.73	143
CV (%)	4.03	6.26	7.76	4.50	4.89	4.03	6.57

V₁= BARI Jharsheem -1, V₂= BARI Jharsheem -2, V₃= BARI Jharsheem -3, D₁=15 November, D₂= 30 November, D₃= 15 December, D₄= 30 December

CONCLUSION

French bean varieties sown on November to December showed the variability with respect to the flowering, flowering duration, number of pods setting and seed yield. But 15 to 30 November sowing would be the optimum time for getting maximum seed yield of BARI Jharsheem-1 and this variety could be sown upto December with reasonable yield.

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PRODUCTION AND PRICE RELATIONSHIP FOR CHILLI IN BANGLADESH: AN EMPIRICAL ANALYSIS

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ABSTRACT

Chilli price fluctuates more in every year in Bangladesh. Therefore, this crop is faced with the highest risk and uncertainty. Due to that, prediction is not possible on production correctly. The study was conducted to aim at the determination of fluctuation, and production-price relationship of chilli in Bangladesh. The experiment was carried out by using chilli area, production and prices data from Bangladesh Bureau of Statistics (1985-2014). Data were analyzed using simplest method for fluctuation, and the Koyck model of distributed lag models. The study showed that the extent of annual price fluctuation of chilli was identified which was between -55 to 111 per cent. Ten several causes were identified which have been the reason of unstable price of chilli. Government price control, improvement of production technology and other identified measures are urgently needed for preventing price fluctuation. According to the results, chilli production in Bangladesh has been influenced by the lag value of average price formed in the market. The most striking result of the study is that the time required for the changes in the chilli prices in Bangladesh to have an effect on chilli production is 6.09 years. The value of coefficient indicated that the changes in lag values of the prices had a positive influence on production, this influence was getting smaller. To reduce the risk and uncertainty of the price of chilli which caused fluctuation more, sustainable chilli farming and establishment of an efficient marketing organization is necessity.

Keywords: Chilli, fluctuation, price and production

INTRODUCTION

Chilli, an important spice crop of Bangladesh is widely grown both in winter and summer seasons. Area under chilli cultivation was 93.55 thousand hectares producing about 102.25 thousand tons in the year 2012-13 (BBS 2015). Chilli is used in green and dried forms. It is especially liked for its pungency, spicy taste and the appealing colour, it adds to the food (Mathukrishnan et al., 1993). Chilli is used as

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pickles, sauces and other beverages (Purseglove et al., 1991). It has medicinal values also. It is a principal ingredient in the Bangladeshi kitchen as curry powder and paste.

Chilli is the crop in Bangladesh whose price fluctuates more in every year. Therefore, this crop is faced with the highest risk and uncertainty. Resulting this, prediction is not possible on production correctly. Besides, problems in chilli pricing also negatively affect the production since the prices of chilli produces are generally determined under market conditions. However, there is not an effective organization in the production and marketing of chilli in Bangladesh. Price of chilli is formed in domestic market conditions expanding upon the supply. Changes in the chilli prices result in fluctuations in the production. For this reason, farmers have to take the prices formed by the market as information and to make production plans accordingly. Prices in the market are formed based on supply and demand principles rather than production costs. The sensitivity of chilli farmers to prices in Bangladesh would be measured. Establishing the interaction between agricultural production and price via a distributed lag model, such a model can significantly contribute to the literature. Indeed, we have not come across with such an investigation into the subject.

Agricultural enterprises in Bangladesh are large. Farmers are not well organized in input and produce markets. Education level of farmers is low. All these factors make price uncertainties for the farmers in chilli market. Farmers consider the previous years' price when they plan to cultivate. Such a planning causes big price and production fluctuations in chilli markets. This is called Cobweb theory in economy literature, often encountered in agricultural production and has been the subject of the investigations about production amount and price relationships.

Because of this structural feature of chilli produces, relationship between the amount produced and the price can be studied using distributed lag model. In the regression models in which time series data were used, if the model uses not only the present values but also the delayed past values of the defining variable, this model is defined as distributed lag model (Gujarati, 2005). Two major problems arise in distributed lag models. One of them is multicollinearity and the other is the increasingly lowered degrees of freedom as lag length increases. In order to overcome these problems, Koyck model has been developed for the estimation of parameters in distributed lag models.

In a study conducted by Yurdakul (1998) in Turkey, relationship between the production and price of cotton crop in 1985-1997 periods was studied using Koyck approach. In another study by Dikmen (2005), production and price relationship for tobacco crop in 1982-2003 periods was analyzed using Koyck model. Eraktan et al. (2004) used Koyck model to investigate the relationship between Direct Income Support, a financial support paid by government to the farmers based on their agricultural land in Turkey, and value added produced. The study by Erdal (2006) dealt with the production and price in tomato crop in 1975-2004 period using Koyck

approach. Another study by Ozcelik and Ozer (2006) studied wheat production and price relationship in 1973-2004 period using Koyck approach. Study conducted by Erdal and Erdal (2008) analyzed the relationship between dry onion production and price in 1975-2006 period via Koyck approach.

Such type of study was not performed before on chilli crop in Bangladesh by using Koyck model. Therefore, the study was undertaken to aim at studying the price fluctuation and, the production amount and price relationship of chilli, a staple spices to a large extent in Bangladesh, using Koyck model.

The overall objective of the study is to determine production and price relationship caused by fluctuation of chilli production in Bangladesh.

The specific objectives of the study are:

- To study the fluctuation of price and production of chilli; and
- To determine the relationship between the prices and production amount of chilli

MATERIALS AND METHOD

Sources of data

Both primary and secondary data were used for the study. Secondary data were collected from several issues of Bangladesh Bureau of Statistics (BBS) and Department of Agricultural Marketing (DAM). For the collection of primary data, three districts namely Bogra, Sirajgong and Gaibandha were selected purposively depending upon the concentration of production and commercially marketing of winter chilli.

Sampling design and data collection

The study was considered for the time period of 1985 to 2014 for time series analysis. Purposive and simple random sampling techniques were used to pick a primary sample. A total of 92 farmers and traders were selected randomly from the three areas. Primary data were collected by face-to-face interview through pretested structured questionnaires.

Analytical technique

The collected data and information were reduced to tabular form which included classification of tables into meaningful results. Except this, the following analytical techniques were used for the study.

A. Fluctuation in price and production

The inter year fluctuation in prices were estimated by the simplest method as percentage change in price year by year. It is estimated as

$$\Delta P_t = \frac{P_t - P_{t-1}}{P_{t-1}} \times 100 \dots \dots \dots (1)$$

Where, ΔP =Percentage change in price in year t over the last period (year),
 P_t =Current year's price in year t, P_{t-1} =Previous year's price in year t-1

B. Distributed Lag Model

$$Y_t = \alpha + \beta_0 X_t + \beta_1 X_{t-1} + \beta_2 X_{t-2} + \dots + \beta_k X_{t-k} + u_t \dots \dots \dots (2)$$

Where, Y_t is chilli production in period t, X_t is chilli price in period t and X_{t-1} is chilli price in one period earlier than t.

C. Koyck Model

$$Y_t = \alpha (1 - \lambda) + \beta_0 X_t + \lambda Y_{t-1} + v_t \dots \dots \dots (3)$$

Where, Y_t is chilli production in period t, X_t is chilli price in period t and Y_{t-1} is chilli production in one period earlier than t.

RESULTS AND DISCUSSION

Estimation of fluctuation

The extent of annual price fluctuation of chilli was identified which was between -55 to 111 percent while the extend of fluctuation of area, production and yield ranged between -39 to 156, -27 to 161 and -17 to 55 percent respectively during the study period. The extreme fluctuation of price takes place in the year 1991-92 and 2005-06 (Table 1). Excessive fluctuation of area and production of chilli in Bangladesh occurred in 1998-99 (Table 1). This was mainly due to getting higher price in the previous year tends to the farmer to increases chilli cultivation. Yield variability is caused by weather fluctuation and also for the insect and pest infestation diseases.

Table 1. Fluctuation of price, area, production, yield of chilli in Bangladesh for the period of 1985-86 to 2013-14

Year	Price	Area (ha)	Production	Yield ha ⁻¹
1985-86				
1986-87	4.03	-4.21	-1.94	2.37
1987-88	-17.59	1.72	6.52	4.72
1988-89	60.62	0.28	4.03	3.73
1989-90	51.97	2.72	9.02	6.13
1990-91	2.24	0.56	0.10	-0.47
1991-92	-54.76	-4.00	-1.94	2.15
1992-93	53.17	-0.54	6.81	7.40

Year	Price	Area (ha)	Production	Yield ha ⁻¹
1993-94	31.17	-0.77	-0.98	-0.21
1994-95	9.13	-0.58	-2.86	-2.30
1995-96	-26.16	-0.26	-0.43	-0.18
1996-97	-27.52	-0.48	-0.91	-0.44
1997-98	101.31	-0.01	1.28	1.30
1998-99	-8.56	155.99	160.99	1.95
1999-00	-24.78	-0.01	0.62	0.63
2000-01	9.51	-0.22	-1.65	-1.44
2001-02	2.07	-3.32	-3.99	-0.69
2002-03	48.52	-0.06	1.01	1.08
2003-04	-24.22	-5.32	-2.64	2.83
2004-05	0.19	-5.44	40.24	48.30
2005-06	111.19	-8.59	-18.93	-11.31
2006-07	-7.14	-0.67	-0.33	0.34
2007-08	-33.30	-39.43	-27.04	20.46
2008-09	62.93	-2.87	-5.73	-2.94
2009-10	9.02	-1.96	0.15	2.16
2010-11	28.42	17.59	14.81	-2.37
2011-12	-15.61	-0.39	1.33	1.73
2012-13	5.45	-4.38	-21.09	-17.47
2013-14	7.53	-30.83	7.50	55.42
Extent of fluctuation	-55 to 111	-39 to 156	-27 to 161	-17 to 55

Average annual variability or random fluctuation of price of Bangladesh was measured by percentage deviation of actual prices from 3 years moving average. Average annual variability or random fluctuation of chilli prices was estimated; where 14 observations were found above the trend line (three years moving average) and 14 observations were found below the trend line out of 28 observations. The extent of random fluctuation was lower than the extent of annual fluctuation (Table 2). Lower price during the current year adversely affect next year's production and this uncertainty in supply causes price to be fluctuated to a great extent.

Causes of price fluctuation of chilli crop

Especially in the least developed countries, the agricultural commodities markets have frequently experienced extreme price fluctuations which often cause

Table 2. Random fluctuation of harvest price of chilli for the period from 1985-86 to 2013-14 in Bangladesh

Year	Harvest price	3 years moving average	% of deviation from actual price
1985-86	26869		
1986-87	27953	25953	7.71
1987-88	23036	29330	-21.46
1988-89	37001	38756	-4.53
1989-90	56230	50241	11.92
1990-91	57492	46577	23.43
1991-92	26010	41114	-36.74
1992-93	39840	39370	1.19
1993-94	52260	49710	5.13
1994-95	57030	50467	13.01
1995-96	42110	43220	-2.57
1996-97	30520	44690	-31.71
1997-98	61440	49380	24.42
1998-99	56180	53293	5.42
1999-00	42260	48240	-12.40
2000-01	46280	45260	2.25
2001-02	47240	54560	-13.42
2002-03	70160	56857	23.40
2003-04	53170	58867	-9.68
2004-05	53270	72980	-27.01
2005-06	112500	90080	24.89
2006-07	104470	95550	9.34
2007-08	69680	95893	-27.34
2008-09	113530	102327	10.95
2009-10	123770	132083	-6.29
2010-11	158950	138950	14.39
2011-12	134130	144840	-7.39
2012-13	141440	142553	-0.78
2013-14	152090	146765	3.63
Range of fluctuation	-	-	-37 to 25

severe supply problems. Based on that, the study explored to determine the various causes of price fluctuation of chilli products. The results are shown in figure 1.

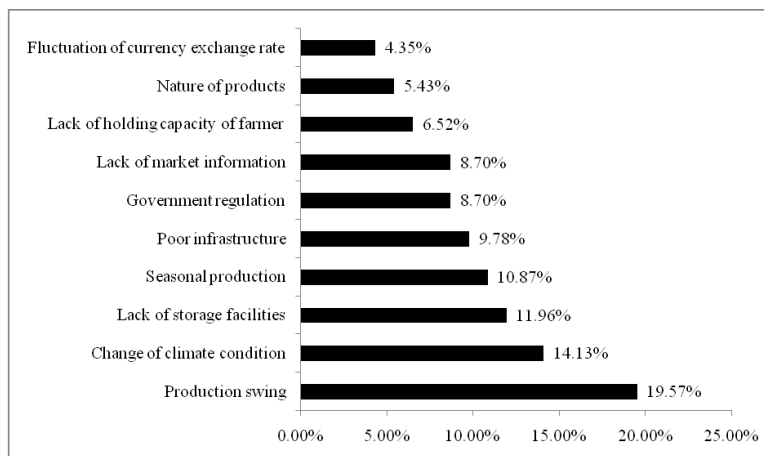


Figure1. Causes of price fluctuation of chilli in Bangladesh

Production swing: The main cause of price swing of chilli was occurred by production swing. Causes of low productions were performed not to use of improved variety and other production technologies by the farmers. When poor technology is use in production, price tends to raise compare to improve technologies. Used in improved technology reduces total cost of production. Due to low production, supply of chilli became low comparatively to the demand of consumer in the market. Resulting this, chilli price becomes high. About 19.57% respondent argued that, price fluctuation was performed by production swings.

Change in climate condition: Production of chilli depends on optimum rainfall and temperature. Adverse climatic condition reduces the yield of chilli drastically. Due to that, supply of chilli in the market becomes low and price tends to high. On the other hand, changes in optimum climatic condition enhance the huge supply of chilli and price tends to low. The reason of price fluctuation mentioned by 14.13% respondent due to change in climatic condition.

Lake of storage facility: It was argued by 11.96% respondent that the technological development of storage among small scale farmers is still poor and this adversely reduces the quality of chilli and the storage loss becomes high. When poor storage technology is used in production, supply reduces and price tends to raise compare to improved technologies.

Seasonal production: About 10.87% respondent pointed out that seasonal productions are also another causative factor for price fluctuation of chilli. Most agricultural products are seasonally produced which cause unequal balance of the availability of it and for this reason, price tend to be low during bumper production

period and to be high during scarcity time. For this reason, price of chilli became rise and fall in different periods.

Poor infrastructure: Furthermore, 9.78% respondent commented that poor infrastructure is one among the causes of price fluctuations. There are high differences in level of appropriateness of roads in this country which make the ever changing of prices for chilli from time to time around the year especially during the rainy season. In this season, most supply areas are not reached easily which causes price to rise.

Government regulation: It was also established by 8.70% respondent that Government regulation and policies does not provide a conducive environment to farmers, this is because when farmer harvests chilli then government allows importation of this product. Resulting this, importation badly affect the price sustainability of domestic price of chilli.

Lack of market information: Lack of market information is another cause for price fluctuation of chilli. 8.70% respondent argued that they do not know about the secondary and terminal market information. So a big gap of price was observed in farm gate and retailer price.

Lack of holding capacity of farmer: Small farmers are not able to hold their chilli up to higher price level in the market due to shortage of cash money and 6.52% of respondent opined that lack of holding capacity of farmer is a cause of price fluctuation.

Nature of products: Nature of products was also pointed out by 5.43% respondent as another cause of price fluctuations of chilli. It was established that most agricultural products are perishable in nature which influences the rise and fall of price over time. The perishability nature of some agricultural product makes it difficult to store during plenty in time hence farmers has to sell them even when the price is still low.

Fluctuation of currency exchange rates: It was argued that the unstable fuel prices cause everything else including farm products to fluctuate more often due to high cost of transporting the farm product from the farm area to the markets. Besides, fluctuation of currency exchange rates makes the import price unstable and market prices to fluctuate, 4.35% respondents opined it.

Measures to prevent price fluctuation of chilli products

There are various measures can be implemented to control price fluctuations in order to support the domestic price of chilli products as shown in figure 2 below. About 20% of the respondent reported that the government step is the major solution to resolving the problem of price fluctuations of chilli through the fixation of import time during the scarce period and avoiding import during harvest period, and by setting a minimum price that discourage imports and results domestic product which will ultimately fetch better prices. It was further argued that, when the government restricts selling of chilli outside, resulted into larger accumulation of stocks while

demand is low, this situation makes the price to go down. Improvement of farming and other infrastructures was identified by 12% respondents, small scale

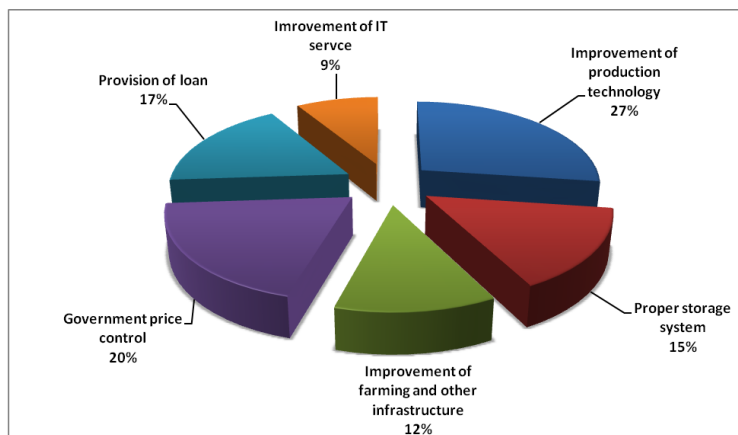


Figure 2. Measures to prevent price fluctuation of chilli in Bangladesh

farmers use poor farming tools which requires more labor force which results in high production costs. About 17% respondent commented that, provision of loan is very important in stabilizing prices and gaining control over the market and the economy of the country at larger. Again, 9% respondent gave opinion IT (Information Technology) service should be developed up to village. So, that the farmer and trader are able to know the market information easily. To control the excessive price of chilli, much production is needed. At the point of interview 27% respondent said improved variety and other production technology should be reached to the small and medium scale farmer. Lastly proper storage system was mentioned by 17%, having proper storage system farmers will be able to preserve their surplus and hence balance price will be maintained throughout the season.

Estimation of the relationship between chilli prices and production

In order to determine the relationship between chilli prices and production during the studied period, a correlation analysis was performed. A correlation coefficient of 0.70 was found, indicating a high level of relationship between the two variables. This result indicated that production amount and price relationship can be studied using Koyck model.

Distributed lag model was formed as follows:

$$Q_t = \alpha + \beta_0 P_t + \beta_1 P_{t-1} + \beta_2 P_{t-2} + \dots + \beta_k P_{t-k} + u_t \dots \dots \dots (4)$$

In the model, Q_t is chilli production (ton) in period t , P_t is chilli price in period t (Tk/ton). In order to form Koyck model, it is necessary to determine lag value of chilli price series lag length.

In a distributed lag model, Schwarz information criterion (SIC) was used to determine the lag length (Dikmen, 2005).

Schwarz proposes reduction of equation (5) to the lowest:

$$SIC = \ln \sigma^2 + m \ln n \dots\dots\dots (5)$$

Here, σ^2 is the highest probability estimate of $\sigma^2 = (RSS/n)$, m is length of the lag, n is the number of observations and RSS is the residual sum of square. In summary, a regression model is used along with some lag values ($=m$), and m value that makes the value of Schwarz criterion the lowest is selected (Gujarati, 2005). At this stage, without making any limitation to the form of the distributed lag, a very large k value length of the lag is used at the start. Then, when the duration of lag is shortened, whether the model goes wrong is checked (Davidson and Mackinnon, 1993).

Values for Schwarz criterion determined at different lag lengths for Equation (5) is given in table 3. As can be seen from the table, the lowest Schwarz value (24.25) was obtained from lag length $K=1$. Thus, the effect of chilli prices on chilli production is zero after one year. According to the determined lag lengths, the relationship between chilli production and price has been estimated using the classical least squares method given in Equation (4). The results of the model are given in table 4.

The results show that chilli prices in the period t , one ($t-1$) and two periods earlier ($t-2$) positively affected the chilli production while chilli prices three periods earlier ($t-3$) negatively affected the production. Partial regression coefficients in the model except (β_0) have been statistically insignificant. Model, as a whole, is also statistically significant. Multiple determination coefficient of the model is 0.62, which means that 62% of the changes in chilli production can be explained through changes in chilli price and its distributed lag values.

Although statistically significant as a whole, the model has to be questioned in terms of reliability for two points related to distributed lag models. The first is the multiple relationship problems as a result of the fact that lag values of price variable was used in the model. The second problem is the loss of observations occurred in lag value set. If the number of data in formed series is not large, estimated values can be inconsistent due to lags.

In order to overcome these two major problems, estimations were made using Koyck model. Estimation results of regression equation given in table 4 based on Koyck model are given in table 5.

The results in Koyck model given in table 5 show that one taka increase in chilli price increased the chilli production by 0.014 tons. But the coefficient was statistically insignificant. An increase of one tons of chilli production in the previous period increased the chilli production by 0.859 tons. According to mean lag number, the time required for changes in chilli prices to have a significant and detectable

effect on chilli production was 6.09 years. This result shows that Bangladesh farmers, who most often grow chilli as a staple spice crop, are very enthusiastic for growing chilli.

Table 3. Lag length values based on Schwarz criterion

Sl. No.	Lag length	Schwarz values
1.	K=1	24.25
2.	K=2	27.70
3.	K=3	31.09
4.	K=4	34.40
5.	K=5	37.62

Table 4. The results of distributed lag model

Items	$Q_t = 51879.09 + 0.327P_t + 0.196P_{t-1} + 0.027P_{t-2} - 0.142P_{t-3}$				
	Lag length				
	Constant	t	t-1	t-2	t-3
Coefficient	51879.09	0.327	0.196	0.027	-0.142
t-values	4.026	0.862	0.489	0.068	-0.357
Probability	0.000	0.396	0.629	0.946	0.724
$R^2=0.62$		$F=4.916$	$P=0.01$	$DW=0.349$	

Table 5. The results of Koyck model

Items	$Q_t = 11420.30 + 0.014P_t + 0.859Q_{t-1}$		
	Lag length		
	Constant	t	Q_{t-1}
Coefficient	$\alpha) 11420.30$	$\beta) 0.014$	$\lambda) 0.859$
t-values	1.542	0.152	8.549
Probability	0.134	0.880	0.000
$R^2 = 0.78$		$F = 52.07$	$P = 0.000$
Koyck Model: Mean lag	6.09		

Note: Q_t is chilli production in period t, P_t is chilli price in period t and Q_{t-1} is chilli production in one period earlier than t.

$$\text{Mean lag} = \frac{\lambda}{1 - \lambda}$$

Different crops the time required for the prices to have considerable changes is 1.19 years for tobacco (Dikmen, 2005), 18 years for tomato (Erdal, 2006), 0.83 years

for wheat (Ozcelik and Ozer, 2006) and 1.19 years for dry onion (Erdal and Erdal, 2008) in Turkey.

In Koyck model

$$Q_t = \alpha + \beta_0 P_t + \lambda Q_{t-1} + u_t \text{ and } \beta_k = \lambda^k \beta_0$$

Since $0 < \lambda < 1$, using the following calculations Equation (14) is reached;

$$\beta_0 = \lambda^0 \beta_0 = (0.859)^0 (0.014) = 0.0140$$

$$\beta_1 = \lambda^1 \beta_0 = (0.859)^1 (0.014) = 0.0120$$

$$\beta_2 = \lambda^2 \beta_0 = (0.859)^2 (0.014) = 0.0103$$

$$\beta_3 = \lambda^3 \beta_0 = (0.859)^3 (0.014) = 0.0089$$

$$\alpha_0 = \alpha / (1 - \lambda) = 11420.30 / (1 - 0.859) = 80995.04$$

When the regression formulae derived from Koyck model is rewritten using this results, equation (6) is obtained;

$$Q_t = 80995.04 + 0.0140P_t + 0.0120P_{t-1} + 0.0103P_{t-2} + 0.0089P_{t-3} \dots \dots \dots (6)$$

In Equation (6), which represents a distributed lag model derived from Koyck model, it is seen that lag chilli prices have a decreasing effect on chilli production, since $0 < \lambda < 1$. Decreasing effects of lag price parameters result from the fact that λ coefficient exerts an effect which was limited in the model.

According to Equation (6), a one-unit increase in chilli prices in Bangladesh increased the production by 0.0140 tons in that year while a one-unit increase in the previous year increased the production by 0.0120 tons. In addition, a one-unit price increase two years ago increased the production by 0.0103 tons. On the other hand, a one-unit price increase three years ago increased the production by 0.0089 tons. Although the changes in lag values of the prices had a positive influence on production, this influence was getting smaller.

CONCLUSIONS AND SUGGESTIONS

The results of the study depicted that a remarkable extent of price and production fluctuation was occurred in chilli. The study identified several causes which have been the reason of unstable price of chilli as change in production swing, climatic condition, lack of storage facilities, seasonal production, poor infrastructures, government regulation, lack of market information, lack of holding capacity of farmers, nature of products and fluctuations of currency exchange rate.

The relationship between amount of chilli produced and marketed, and price of chilli, all under domestic market economy conditions, were studied. There was a positive correlation of 70% between amount produced and the price. This coefficient showed that Koyck model was appropriate for studying the relationship between production amount and price of chilli crop.

For the estimation of unknown parameters in the model, lag length determined using Schwarz criterion was calculated as one. This means that chilli production is influenced by the prices of up to past one year in Bangladesh. According to Koyck model, the time required for the changes in chilli prices to have a significant and detectable effect on chilli production was calculated as 6.09 years.

For the studied period, one-unit increase in chilli prices increased the chilli production by 0.0140 tons in that year; while a one-unit increase in the previous year increased the production by 0.0120 tons. In addition, a one-unit increase in the prices of two years ago increased chilli production by 0.0103 tons. On the other hand, a one-unit price increase three years ago increased the production by 0.0089 tons. Thus, it can be said that each additional lag value results in a smaller effect on chilli production.

For sustainable chilli farming in Bangladesh, establishment of an efficient marketing organization is a necessity. The study also identified several measures of preventing price fluctuation viz., government price control, improvement of farming and other infrastructures, provision of loan and subsidies, improvement of IT service, improvement of production technology, proper storage system, etc.

For the chilli crop, it is necessary to conduct a contract based production system. Policies are needed to be developed for efficient, profitable and sustainable chilli farming. Thus, price uncertainties that the producers face can be overcome, and contribution of this major spice crop to national economy can be increased.

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NUTRIENT PROFILE OF INDIAN CLIMBING PERCH, *Anabas testudineus*

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ABSTRACT

The proximate composition, fatty acid, amino acid and fat soluble vitamins of Indian climbing perch, *Anabas testudineus*, locally called "Koi" were assayed in relation to its body weight. The fish samples were collected from different geographical locations and were grouped as small (10-50g) and big (52-150g) sizes. The proximate composition, essential amino acid (EAA) and non-essential amino acid (NEAA) contents in Koi did not differ significantly between the groups. The monounsaturated fatty acid (MUFA) content was significantly ($P<0.05$) higher in bigger sized Koi. The polyunsaturated fatty acid (PUFA) content was 23.67 ± 0.85 and 13.62 ± 1.02 (%) respectively in the small and big sizes of Koi, while the docosahexaenoic acid (DHA) was significantly higher in small Koi. The vitamin A content was 85.77 ± 0.35 and 93.90 ± 1.34 (I.U./100g) respectively in small and big Koi. Vitamin D content was significantly higher in small Koi compared to big one. Vitamin E and K were significantly ($P<0.05$) higher in big Koi. The results indicated that *Anabas testudineus* is a good source of protein, fat, vitamins, amino acids and fatty acids.

Keywords: *Anabas testudineus*, Proximate composition, vitamins, amino acid profile and fatty acid profile

INTRODUCTION

Fish protein occupies an important position in human nutrition (Nargis 2006). Fish is consumed by human being for centuries and is preferred as a perfect diet not only due to its taste and high digestibility but also because of having higher proportions of unsaturated fatty acids, essential amino acids and vitamins and minerals (Kumar, 1992). The high nutritional value of fish is mainly related to their readily digested proteins which are good source of essential amino acid (Mohanty et

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al., 2014). Fish is also a good source of polyunsaturated fatty acids (PUFAs), viz., the ω -3 and ω -6 PUFAs, which are well known for beneficial effect to human health (Das et al., 2009; Paul et al., 2015; Mohanty et al., 2016 and Giri et al., 2010). Fat-soluble vitamins viz., Vitamin A, D, E and K act as essential nutrients in important biological processes of the human body (Sau and Paul, 2004).

Anabas testudineus, commonly called as climbing perch or Koi, is a small fish widely distributed throughout south and south-east Asia. This is a highly priced fish owing to its consumer preference compared to carps. There are few reports available on nutrition and aquaculture of *A. testudineus* (Mukhopadhyay and Paul, 1996; Kumar et al., 2013 and Bungas et al., 2013). Also, not much information is available on the nutrient profile of Indian climbing perch (Wimalasena and Jayasuriya 1996 and Bogard et al., 2015). Keeping in view the importance as food fish; the nutrient profile of Indian climbing perch, *A. testudineus* was determined to document the information of amino acid and fatty acid profiles, along with some selected vitamins.

MATERIALS AND METHODS

Collection of samples

The samples of *Anabas testudineus* (n=40) were collected from West Bengal, Odisha and Andhra Pradesh. The collected fish samples were categorized into two groups as per their bodyweight i.e., small (10-50g) and big (52-150g). The fish samples were eviscerated and the head was removed. The representative portion of the edible part was taken and homogenized in a mixer for further analysis as per the methodology reported earlier (Sankar et al., 2010). The proximate composition of fish tissue samples was done as per 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) in methanol from lipids fraction as per Metcalfe et al. (1966). The fatty acid methyl esters were quantified by injecting 1 μ L (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 and 300°C. Identification of individual FA was identified by comparison of retention times to those of standards (SUPELCO, Cat. No. 47885-U) and quantified by comparing with respective areas, following "Total Chrome" software of Perkin Elmer.

Amino acid analysis

The amino acid analysis was done as per the method of Ishida et al., (1981) and Paul et al. (2016a). For amino-acid analysis phenylisothiocyanate (PITC) was used

for pre-column derivatization, while reversed-phase gradient elution high-performance liquid chromatography (HPLC) separates the phenylthiocarbamyl (PTC) derivatives which are detected by their UV absorbance of Pico Tag method of Waters Associates.

Vitamin analysis

The fat soluble vitamins Retinol (Vitamin A), Cholecalciferol (Vitamin D), α -Tocopherol (Vitamin E) and Vitamin K were analyzed in high performance liquid chromatography system. Fish tissue (30g) was grinded with anhydrous sodium sulfate. Then extracted the oil using 2:1 chloroform: methanol after adding BHA as antioxidants (Folch et al., 1957). The sample preparation and vitamin analysis was done as per Sankar et al. (2010). To about 2.0 g oil in a round bottom flask, added 25 ml alcohol, and 1.5 ml of 150% KOH. Reflux in water bath for 30 min. Transfer the contents in to a 250 ml separating funnel after cooling; wash the flask with 50 ml petroleum ether and add to the separating funnel; shake the contents thoroughly and allowed to separate. Extract the aqueous layer twice more and pool the solvent layer. Wash the solvent layer with 20 % of water (v/v) to make it alkali free. Concentrate non-saponifiable matter in the ether extract fraction using a flash evaporator at 30-40° C to a definite volume. The non saponifiable matter is filtered through 0.45 μ syringe filter and stored under refrigerator. Then the fat soluble vitamins were analyzed by injecting 20 μ L of sample in HPLC equipped with C₁₈ Bondapack column. The mobile phase of HPLC consists of water (HPLC grade) solvent A and acetonitrile as solvent B with 1% TFA. A gradient system was used (solvent A/solvent B), starting from 50/50, 80/20 to 100/0 at the rate of 1 mL/min for 20 min. The fat soluble vitamins were identified and quantified by comparing with the retention times and peak area of respective vitamins standards.

The data were analyzed using t-test as per Snedecor and Cochran (1968) and have been presented as Mean \pm S.E.

RESULTS AND DISCUSSION

The proximate composition of Koi is presented in table 1. The moisture, crude protein, crude fat and ash content did not differ significantly between the size groups. The protein and fat content were 16.47 \pm 0.11 and 16.91 \pm 0.59; 6.68 \pm 1.35 and 6.98 \pm 1.49 (%), respectively in small and big Koi. The moisture content of Koi in the present study was lower than the freshwater Eel (*Mastacembelus armatus*) and *Anguila bengalensis bengalensis* as reported by Pal and Ghosh (2013). Fat content of Koi was higher in the present study in comparison to that of many freshwater fish species (Pal and Ghosh, 2013; Swapna et al., 2010, Ackman, 2002 and Paul et al., 2016b). Fat content of Koi of the present study is in agreement with earlier report by Nargis (2006). The protein content of Koi was lower than other freshwater fish (Pal and Ghosh, 2013). Generally an inverse relationship between tissue moisture and lipid content is observed with the increase of age and body weight of fish (Wheeler

and Morrissey, 2003; Jankowska et al., 2007), which was not observed in the present case. The amino acid contents of Koi of two weight ranges are presented in table 2. The essential amino acid contents (EAA) are 44.67 ± 1.79 and 44.65 ± 1.54 g/100g whole body protein, respectively in small and big size groups. The Leucine content was maximum among EAA. The non essential amino acid (NEAA) content was 54.48 ± 0.26 and 55.80 ± 0.91 g/100g protein, respectively in small and big size of Koi; where Glycine was maximum among NEAA in small size range group. However both the groups contain a good proportion of the essential as well as non-essential amino acids with non significant mean values.

Table 1. Proximate composition (% as such basis) of Koi (*Anabas testudineus*) of different body size.

Particulars	Small	Big
Moisture	68.65 ± 0.68	68.00 ± 1.77
Crude Protein	16.47 ± 0.11	16.91 ± 0.59
Crude Fat	6.68 ± 1.35	6.98 ± 1.49
Ash	5.03 ± 0.04	5.50 ± 0.49

Data are presented as Mean \pm S.E. (n=50)

The present study for the amino acid content of Koi shows that Isoleucine, Leucine, Valine and Phenylalanine among the essential amino acids (EAA) and Aspartate, Glutamine, Glycine and Alanine among the non essential amino acids (NEAA) are higher in quantity. However, Iwasake 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). Our study denotes that Koi of both sizes contain glutamine about 13.14-14.41 (%) which is very effective for human health. It is observed that the EAA/NEAA ratio is 0.82 and 0.80 in the small and big Koi. Wessilnova (2000) reported the variation in amino acid content of fish with season and location. However, in this study there was no significant variation between the two size groups of Koi.

The fat soluble vitamin content in Koi of different body weights are presented in table 3. The vitamin A content was 85.77 ± 0.35 and 93.90 ± 1.34 (I.U/100g) in two weight groups, which did not differ significantly. The vitamin D content in Koi is significantly ($P < 0.05$) higher in smaller Koi. The Koi of bigger size (50-152 g) contains significantly higher vitamin E (1.27 I.U/100g) and vitamin K (1.15 μ g/100g) than the Koi of smaller size.

Table 2. Amino acid profile (g/100g protein) of Koi (*Anabas testudineus*) of different body weights

Particulars	Small	Big
Essential Amino Acids (EAA)		
Arg	2.10±0.32	2.01±0.29
His	3.99±0.44	4.12±0.39
Ile	5.36±0.10	5.58±0.46
Leu	8.13±0.25	8.42±0.66
Lys	3.02±0.40	2.53±0.24
Met	1.60±0.06	1.44±0.05
Phe	6.26±0.29	5.95±0.31
Thr	5.48±0.46	5.88±0.23
Try	1.39±0.50	1.09±0.08
Val	7.32±0.06	7.61±0.28
ΣEAA	44.67±1.79	44.65±1.54
Non-Essential Amino Acids (NEAA)		
Asp	10.84±0.69	10.95±0.38
Ser	5.07±0.43	5.30±0.13
Glu	13.14±0.07	14.41±0.30
Pro	1.63±0.32	1.42±0.03
Gly	15.29±0.56	14.90±0.18
Ala	7.26±0.44	7.77±0.96
Cys	0.22±0.07	0.14±0.00
Tyr	1.01±0.07	0.89±0.38
ΣNEAA	54.48±0.26	55.80±0.91
EAA/NEAA	0.82	0.80

Data are presented as Mean ± S.E. (n=8)

Fish is a good source of fat soluble vitamins. Vitamin A content from fish is more readily available to the body than from plant sources (Liu, 2003). Vitamin A is responsible for normal vision and bone growth is well known and its derivative retinoic acid regulates gene expression in the development of epithelial tissue (Roos et al., 2003). Vitamin D functions to activate the innate and dampen the adaptive immune systems (Hewison, 2011). As Koi contains a good amount of vitamin D, it plays a major role for immune system. Vitamin E is an indispensable nutrient

required to maintain flesh quality, immunity, normal resistance of red blood corpuscles to haemolysis, permeability of capillaries and heart muscles (Halver and Hardy, 2002). Vitamin E content in Koi ranges from 0.70-1.27 (I.U/100g). The Koi contains 0.53-1.15 ($\mu\text{g}/100\text{g}$) of vitamin K. Vitamin E also functions as lipid soluble antioxidants and protects biological membranes, lipoproteins and lipids against oxidation (Hamre, 1998 and Sau et al., 2004). The human body needs vitamin K for post translational modifications of certain proteins required for blood coagulation and in metabolic pathways in bone and other tissue (Halver and Hardy, 2002).

Table 3. Vitamin content of Koi (*Anabas testudineus*) of different body weights

Particulars	Small	Big
A (I.U/100g)	85.77 \pm 0.35	93.90 \pm 1.34
D (I.U/100g)	85.60 ^b \pm 1.29	43.12 ^a \pm 1.03
E (I.U/100g)	0.70 ^a \pm 0.04	1.27 ^b \pm 0.03
K ($\mu\text{g}/100\text{g}$)	0.53 ^a \pm 0.03	1.15 ^b \pm 0.02

Superscript ^{a,b} in a row differs significantly ($P<0.05$). Data are presented as Mean \pm S.E. (n=8)

The fatty acid profile of Koi is presented in table 4. The saturated fatty acids (SFA) were 66.19 \pm 3.33 and 60.73 \pm 1.25 (%) respectively in small and big Koi. Among SFA the palmitic and stearic acid are found to be higher in both the groups. The palmitic acid is significantly ($P<0.05$) higher in big Koi compared to small one; conversely stearic acid was higher in small Koi than big one. Other SFAs viz., pentadecanoic, heptadecanoic and arachidonic acid were significantly ($P<0.05$) higher in small size of Koi.

The monounsaturated fatty acid (MUFA) contents were 10.39 \pm 0.51 and 25.70 \pm 0.89 respectively in small and big groups. Palmitoleic acid was significantly ($P<0.05$) higher in big size of Koi. The MUFA content is significantly ($P<0.05$) higher in big Koi. The polyunsaturated fatty acids (PUFA) are 23.67 \pm 0.85 and 13.62 \pm 1.02 respectively in the small and big koi. The PUFA content is significantly higher in small group compared to the bigger group. Among PUFA, α -linolenic acid is significantly ($P<0.05$) higher in small Koi. The docosahexaenoic acid (DHA) is also significantly ($P<0.05$) higher in smaller group. $\Sigma\omega 3$ PUFA are significantly ($P<0.05$) higher in smaller Koi but $\Sigma\omega 6$ PUFA are significantly ($P<0.05$) higher in the bigger size of Koi. It is also observed that SFA and MUFA are higher in bigger sized Koi whereas the PUFA content is higher in Koi of smaller size.

Fatty acid composition of aquatic animals is 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

Table 4. Fatty acid profile (% of total fatty acid) of Koi (*Anabas testudineus*) of different body weights

Particulars	Small	Big
C12:0 (Lauric Acid)	0.43±0.03	0.68±0.05
C14:0 (Myristic Acid)	1.33±0.18	1.35±0.13
C15:0 (Pentadecanoic Acid)	1.27 ^b ±0.08	0.48 ^a ±0.09
C16:0 (Palmitic Acid)	40.56 ^a ±1.60	52.56 ^b ±2.12
C17:0 (Heptadecanoic Acid)	2.70 ^b ±0.28	0.11 ^a ±0.02
C18:0 (Stearic Acid)	15.32 ^b ±2.04	5.19 ^a ±0.64
C20:0 (Arachidic Acid)	0.71 ^b ±0.06	0.31 ^a ±0.03
C21:0 (Heneicosanoic Acid)	3.21±0.21	ND
Other	0.66 ^b ±0.03	0.05 ^a ±0.01
Σ SFA	66.19±3.33	60.73±1.25
C15:1 (Pentadecenoic Acid)	0.51±0.03	0.91±0.06
C16:1 (Palmitoleic Acid)	8.56 ^b ±0.46	3.27 ^a ±0.18
C17:1 (Heptadecanoic Acid)	0.66±0.05	0.46±0.11
C18:1n9c (Oleic Acid)	ND	2.49±0.11
C18:1n9t (Elaidic Acid)	ND	18.13±0.88
C20:1n9 (Eicisanoic Acid)	0.66±0.03	0.44±0.05
Σ MUFA	10.39 ^a ±0.51	25.70 ^b ±0.89
C18:2n6c (Linoleic Acid)	ND	8.17±0.96
C18:3n3(α Linolenic Acid)	17.83 ^b ±0.85	1.91 ^a ±0.06
C18:3n6 (γ Linolenic Acid)	0.37±0.04	0.11±0.01
C20:4n6 (Arachidonic Acid)	1.24±0.11	ND
C20:5n3 (Eicosapenta enoic Acid)	0.38±0.04	ND
C22:6n3 (Docosahexaenoic Acid)	2.67 ^b ±0.16	1.26 ^a ±0.07
Other	1.18±0.09	2.18±0.11
Σ PUFA	23.67 ^b ±0.85 ^b	13.62 ^a ±1.02
Σω-3 PUFA	20.88 ^b ±0.66	4.11 ^a ±0.03
Σω-6 PUFA	1.61 ^a ±0.12	8.30 ^b ±0.20
ω-3: ω-6 PUFA	1.16 ^b ±0.12	0.49 ^a ±0.02

Superscript in row ^{a,b} differs significantly (P<0.05). Data are represented as Mean ± S.E. (n=8)

et al., 1995; Sener et al., 2005). Fatty acids in fishes are derived from two main sources, namely, biosynthesis and diet (Hearn et al., 1987, Morris et al., 1995, Kamler et al., 2001). Palmitic acid content among the SFA is maximum in big size Koi which is in agreement with earlier report (Kaya et al., 2008). The SFA content is higher in freshwater fish (Indian Major Carp) as reported by Paul et al., (2015) which is in agreement with the present result. The palmitic acid is considered to be a key to many metabolic processes in fish and other aquatic animals (Ackman and Eaton, 1966). Fish oils are known to be rich source of essential PUFA of the omega-3 family (Kenari et al., 2009). In Koi PUFA content varies from 13.62-23.67% and DHA ranges from 1.26-2.67%. This is in agreement with earlier report (Kenari et al., 2009). 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). These fatty acids have beneficial effect in the prevention of cardiovascular and inflammatory diseases (Gebauer et al., 2006) and neurodegenerative syndromes, such as Alzheimer's disease (Moyad, 2005). The consumption of fish and fish oils containing omega-3 fatty acids are beneficial for a number of biological factors like cardiovascular diseases, rheumatoid arthritis, psoriasis (Paul et al., 2016a).

CONCLUSION

The results indicated that Koi is a good source of essential amino acids, protein, fat and ash. Among the fatty acids, palmitic and stearic acid were dominant in SFA and palmitoleic acid was predominant in MUFA. Among the PUFA, linolenic and docosahexaenoic acids were found in higher contents. Vitamin A and D were also present in good quantity in Koi. Irrespective of the size groups, the nutrient profile reflected that the fish was enriched with fat, protein, fatty acids, essential amino acids and vitamins.

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EVALUATION OF EXISTING SLAUGHTER ACT AND WAY FORWARD TO ITS DEVELOPMENT IN BANGLADESH

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ABSTRACT

This study was undertaken for the evaluation of existing animal slaughter and meat quality control act 2011 and way forward to its development in Bangladesh. Published data, SWOT analyses, structured questionnaires survey, Focus Group Discussions (FGDs) and Key Informant Interviews (KIIs) were carried out for critical analyses and explore the different stakeholder's views. The study was carried out in seven divisions of Bangladesh namely Dhaka, Chittagong, Rajshahi, Khulna, Sylhet, Barisal and Rangpur. Total 285 representative samples (covering all stakeholders like farmers, butchers, meat processors, ULO/VS/PDO) were interviewed. Four FGDs and 50 KIIs were administered with different stakeholders. Descriptive statistics like percentage, mean, ranks and Binary logistic regression and Z test were used for data analyses. Findings show that the authority is not functioning properly due to lack of action plan and monitoring cell. Lack of certification authority, sanitary and meat inspector visit, restricted day, pre-slaughter and post-slaughter examination is visible. Act should be updated time to time to address the field based need. The study reveals that to implement the slaughter act Department of Livestock Services (DLS) as authority should have action plan and monitoring cell.

Keywords: Slaughter act, quality, safe meat, action plan, implementation

INTRODUCTION

According to the estimate of the Department of Livestock Services, the present population of cattle, goat, buffalo and poultry is about 23.44 million, 25.61 million, 1.45 million and 307.47 million, respectively in 2012-13 (DLS, 2014). There is a huge gap between the demand and supply of nutritious foods for the human population. An adult people require at least 250 ml milk, 120 g meat daily, but supply

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is about 44 ml milk and 20.6 g meat per day (DLS, 2009) which indicates that we are in serious shortage of milk and meat. According to DLS report (2010), annual deficiency of milk, meat and egg is 82.28%, 80.22%, and 62.20%, respectively.

The livestock sub-sector in Bangladesh is currently governed by the following legislation: Bengal Cruelty to Animals Act, 1920, Prevention of Cruelty to Animals Ordinance, 1962, Bangladesh Veterinary Practitioners Ordinance, 1982, Animal Disease Act, 2005, Bangladesh Animal and Animal Product Quarantine Act, 2005, National Livestock Development Policy, 2007, National Poultry Development Policy, 2008, Avian Influenza Compensation Strategy and Guidelines, 2008, Bangladesh Zoo Act, 2009 (Draft), Animals Slaughter and Meat Control Act, 2011, Fish Feed and Animal Feed Act, 2010, National Livestock extension policy, 2012 (<http://www.dls.gov.bd/livestockdevpolicy.php>). These pieces of legislation will continue to be updated from time to time in accordance with the policy changes and production trends. In any given country, it is the role of a Government Ministry to develop appropriate policy guidelines for development of the sector the Ministry is involved in. However, development of such policies must involve all the key stakeholders for the guidelines to adequately address real problems and gaps that hinder the development of a sector.

All the attempts in livestock sector development are running to increase production in respect of milk, meat and egg. No activity has yet been taken giving special emphasis for producing safe and healthy food in relation to Hazards Analysis and Critical Control Point (HACCP), Good Management Practices (GMP) and Sanitary and Phytosanitary (SPS). In spite of having increased production of animal protein the consumers are not getting safe food as well as the animal industrialists can not export their products as per World Trade Organization (WTO) regulations. The dynamic potential of this emerging sub-sector thus requires critical policy attention. Although gains have been achieved in the sector to some extent, several gaps still exist that have not been addressed in the previous policies. There is need to give direction to the industry by putting in place a policy that will guide the development of the livestock sub-sector in Bangladesh. The policy needs to be reviewed from time to time, taking into account the dynamics of the domestic, regional and global socio-political and economic environment.

With above facts and circumstances to gain new explanatory insight a holistic survey analysis was carried out having different stakeholders on existing animal slaughter act, with the following objectives:

- (i) Critical analysis of the existing slaughter act in Bangladesh.
- (ii) To explore the implementation status of slaughter act with different stakeholders.
- (iii) To identify the gaps between existing slaughter act and expectations from the perspective of stakeholders on the implementation.

- (iv) To recommend the output of the study to the concerned policy makers of the Government of Bangladesh.

MATERIALS AND METHODS

Selection of the study area

The study was carried out in seven divisions namely Dhaka, Chittagong, Rajshahi, Khulna, Sylhet, Barisal and Rangpur. These seven divisions were chosen as these locations are contributing significantly in livestock and poultry sector of Bangladesh.

Selection of sample and sampling technique

Table 1 shows the sampling technique of the study. Total 285 representative samples (covering all stakeholders) were interviewed from selected seven divisions consisting 116 farmers, 64 butchers, 66 Meat processors, 39 Government officials of livestock sector (Table 1). In addition, four FGDs (Focus Group Discussions) at BAU, DLS, BLRI, field level and 50 Key Informant Interviews (KII) were administered with different stakeholders.

Table 1. Population, Sample Size and Sampling Techniques

Objectives	Data Source	Divisions	Population	Sample
One	Secondary sources: desk review, BBS, DLS, BER, Scientific articles, Internet, Compendium, SWOT analyses	-	-	Animal slaughter act
Two	FGDs (Four), KIIs (50), Questionnaire survey	Seven divisions, BAU, DLS, BLRI	Livestock owners, meat processors, butchers, DLS personnel, academicians, researchers & consumers	10-12 persons in each FGD, 50 KIIs
Three	Questionnaire survey, KIIs, FGD	Seven divisions	Livestock owners, meat processors, butchers, DLS personnel, academicians, researchers & consumers	285
Four	Previous sources	-	-	-

SWOT Analysis

A SWOT analysis was carried out to critically analyze the existing animal slaughter act. This tool was used for policy-review approach aiming at Strengths,

Weaknesses, Opportunities and Threats (SWOT) analysis of the existing slaughter act in Bangladesh (Pearce et al., 2012).

Preparation of survey instruments and pre-testing

To collect the required data, four types of questionnaires were prepared in accordance with the objectives set for the study. Questionnaires then pre-tested in the field among some stakeholders before final data collection. After pre-testing, the final questionnaires were prepared after making necessary corrections, modifications and adjustment.

Data collection and processing

The research relies on both primary and secondary data. Secondary information were sought from various publications, DLS reports, previous survey reports, compendium and the strategic plan documents of the Ministry of Livestock and Fisheries. Primary data were collected by the trained enumerator including the researcher himself through face to face interview.

Data analyses

Descriptive statistics like percentage, mean and ranks were used for different variables to describe the present status of different stakeholders. Binary logistic regression was used to identify the variables influencing the slaughter act. Most of these analyses were conducted by using the statistical package named SPSS 20.

RESULTS AND DISCUSSION

Study on SWOT analyses of animal slaughter act, 2011

Animal slaughter and meat quality control act, 2011 enacted with effect from 20 September 2011. The strength, weakness, opportunities and threats of the animal slaughter act are summarized in table 2.

Table 2. Result of SWOT analysis of the animal slaughter act 2011

No.	Strength	Weakness	Opportunities	Threats
1	Restriction of slaughtering outside slaughterhouse defined in section 3 (1-2).	Butchers are slaughtering animal outside the slaughterhouse.	To verify whether animal is being slaughter outside or not.	Non-cooperation from butchers and meat inspectors.
2	Slaughtering restricted animal is prohibited prescribed in section 4 (1-2).	Pregnant, lactating and under aged animal may be slaughtered.	Govt may add provision to ban the slaughtering of restricted animals. Identified.	Threats from animal supplier, butchers etc.
3	Examination of health of pre-slaughter and post-slaughter of animal and carcass is well defined in section 5 (1-2).	Lack of pre-slaughter and post-slaughter of animal and carcass.	Defect of provision can be found out	Non-cooperation from corrupt vet. and from other stakeholders.
4	Environment of slaughterhouse in section 6.	Lack of hygienic environment and	To find out the defect slaughterhouse.	Pressure may create unlawfully from traders.

No.	Strength	Weakness	Opportunities	Threats
		quality of slaughterhouse.		
5	Setting up of slaughterhouse, meat selling and processing plant is shown in section 7.	Does not follow the provision under the act. Lack of monitoring.	To find out the provision duly applied in setting up.	Non-cooperation and discourage to implement the provision from DLS.
6.	License for slaughterhouse, meat selling and processing plant is given in section 8 (1-2).	Without license holders it may be practiced.	To check whether meat stakeholders are licensed holders or not.	Humiliation and physical assaults.
7.	Process of licensing shown in section in 9 (1).	Might have fraudulence.	To find out defect in the process of application of licensing.	Non-cooperation from the authority.
8.	Period and renewal of license stipulated in section 10 (1-3).	Period of license is very minimal.	To find out application filed after termination of license or 60 days before termination.	Non-cooperation and demand for bribe.
9.	License held up and cancellation defined in section 11.	Defective condition in the processing of licensing.	To check of whether license holders are convicted for any offense or violating any condition.	Offender may attack on authority.
10.	Power of entry and inspection stated in section 12 (1-2).	Provision or act not formulated in applying judicial mind.	To verify irrelevance in the provision of act in setting up slaughterhouse related plant.	Obstacle and confinement.
11.	Health status of employees working in slaughterhouse, meat processing and selling centre is shown in section 13.	Infected employee without medical treatment may practice.	Whether employee is infected or free from contagious disease.	Contagious disease may be spread.
12.	Transport of animal, meat and meat products stipulated in section 14 (1-2).	Lack of appropriate provision.	To find out whether provision is being followed in transporting.	Threats may come from stakeholders in confiscating, disposing and destructing.
13.	Restriction day for slaughtering and selling mentioned in section 15.	Not issuance of gazette notification in time.	To find out the restriction day followed by butchers.	Physical assaults or injury may come from butchers.
14.	Emergency slaughtering stated in section 16.	Sick animal can be slaughtered.	To examine the health of animals before slaughtering.	Authority can be corrupted.
15.	Declaration to non-edible meat stated in section 17.	May have non-edible meat for eating whole or part of carcass.	To examine the whole or part of carcass as edible or non-edible.	Without examination of carcass non-edible meat may enter to food chain.
16.	Direction of destruction or disposal of non-edible part of whole or part of carcass and offal explained in section 18.	Non-edible part of whole or part of carcass and offal may be edible.	Whether non-edible part of whole or part of carcass and offal is disposed of or destructed.	Diseases can be spread.
17.	Direction to send the sample at the laboratory stated in section 19.	Laboratory test may not be performed.	To verify whether sample may be tested or not in proper way.	Test might be done in fake laboratory.

No.	Strength	Weakness	Opportunities	Threats
18.	Disposal of slaughter wastes mentioned in section 20.	Irrelevant provision might be formulated.	Whether wastes are disposed of according to provision.	Environment may be polluted.
19.	Standardization mentioned in section 24 (1-2).	Might have residual affect of hormones, antibiotics, preservatives, poisonous metals, harmful organism in meat.	Whether safe meat is being sold or not..	Hides, skin and meat export may be difficult and questionable..
20.	Seizure and disposal of meat and meat products mention in section 22.	Might not practice.	To explore about seizure and disposal of meat and meat products accordingly.	Authorities will get scared execute the power.
21.	Offence and trial stated in section 23 (1-2).	Lack of soundness of mobile court practice.	Mobile court act 2009 whether being honestly practiced or not.	Executive magistrate can work for Govt.
22.	Punishment stated in section 24 (1-2).	Criminals may escape.	Punishment should be increased.	Criminal may commit offense repeatedly.
23.	Appeal stated in section 25.	Criminals may be acquitted.	To increase punishment as well as fine.	Slaughter act may not be effective due to lack of punishment.
24.	Delivery of power explained in section 26.	Animal production professionals avoided in this act.	To bring back AH graduate for the purpose of meat quality and control.	Veterinary council and vet are dead against to recruit the AH graduates.
25.	Power of formulation of provision mentioned in 27.	Dictator non elected and non-participatory Govt. may promulgate gazette notification.	Pro-people Govt can promulgate provision.	Repression, oppression, death threat come from constitutionally illegitimate Govt.
26.	Banning and sustaining the act mentioned in section 28 (1-2).	Lapse and gaps of the existing act.	Latest animal slaughter act is well up-to-date or not.	Govt. might not be interested.

Farmers' knowledge about animal slaughter act

Different variables in relation to farmer's knowledge about slaughter act are mentioned in table 3. There is a positive relationship between release of blood and bleeding time. Islam (2015) stated that time required for complete bleeding ranged from 9.13 to 16.35 minutes. Mobile court not conducted as per law. Adzitey et al. (2011) described that poor animal handling has adverse effects on the animal, carcass and meat quality.

Table 3. Farmers knowledge about animal slaughter act, 2011

Farmers knowledge	Yes (%)	No (%)	Ranks
Knowledge about the irregular shape and flay-cuts	53	47	1
Proper bleeding of animal done by the butcher	47	53	2
Knowledge about the slaughter act	42	58	3
Butcher follow the age, sex, pregnancy and lactating considering during slaughtering the animal	40	60	4
Knowledge about the restricted day of slaughtering animal	38	62	5
Live animal and carcass examined to know healthy status at pre slaughter and post slaughter time	30	70	6
Mobile court conduct for prohibiting the crime related to slaughter act or not	25	75	7
The butcher follow hang and pull system of flaying or not	25	75	8
Know without slaughterhouse no animal can be slaughtered except for holly greatest festival and family feast	23	77	9
Whether the existing slaughterhouse is environment friendly or not	23	77	10
Sanitary inspector visit the slaughterhouse or not	14	85	11

Knowledge and perception of butcher about animal slaughter act, 2011

Table 4 reveals that more than 80% butchers sell their by-products like blood, bone, kidney, liver, stomach; have proper bleeding knowledge; slaughter should be done in slaughter house except festival. These all sorts of positive answer is a good which aligned to good management practices for Halal and safe meat production. During beef cattle fattening there is a report (Islam et al., 2012) wherein stated farmers having 2-5 heads of cattle are highly fattened using growth promoters like Oradexon (Glucocorticoid steroid), Decason (Glucocorticoid steroid), Dexavet (Synthetic Steroid), Tredexanol (Synthetic Steroid), Pednivet (Steroids). Sixty nine per cent butchers are license holders. It is mandatory to have license in slaughter act. Slaughterhouse and meat processing centre are not set up scientifically as per rules and provisions of the act. Most meat is handled in unhygienic sanitary conditions in both rural and urban areas in Bangladesh. Enforcement of legislation relating to slaughtering or meat inspection is weak (Murshed, 2014). Mobile court should be consisted of livestock expertise along with executive magistrate who is well versed in slaughter act. Food animals such as cattle, buffalo, sheep, and goats are brought to these slaughterhouses from long distances usually by driving or on the hoof. Since there is no lairage, animals generally do not receive *ante mortem* care (Rahman, 2001).

Table 4. Knowledge and perception of butcher about slaughter act

Particulars	Yes (%)	No (%)	Rank
Selling of by-products	91	9	1
Requirement of proper bleeding knowledge	88	12	2
Slaughter should be done in slaughter house except festival	86	14	3
The license must be renewed after one year is known to	84	16	4
Know 4-5 minutes is needed to have <i>halal</i> meat for proper bleeding	80	20	5
Heard about the restricted day of slaughtering animal or not	75	25	6
Visit by meat inspector to give seal on carcass or not	70	30	7
Taken licenses to set up slaughterhouse, meat processing and sale center from licensing authority or not	69	31	8
Slaughterhouse, meat processing center and plant set up as per prescribed size and available facilities or not	67	31	9
Visit slaughter house by sanitary inspector	64	36	10
Any irregular shape and flay-cuts or not	58	42	11
Introduce hang and pull system of flaying or not	58	42	12
Cut on hides and skin during flaying or not	55	45	13
Care environmental safety (air, water) during slaughtering the animal or not	50	50	14
The live animal and carcass has been examined to know healthy status at pre slaughter and post slaughter time	47	53	15
Follow binding age for animal for slaughter or not	47	53	16
Follow flaying and preservation of hides and skin as per provision or not	39	61	17
Mobile court conducted for prohibiting the crime related to slaughter act or not	37	63	18
Animal has been kept at stockyard before slaughtering or not	34	66	19
Follow the provision of animal slaughter and waste disposal as per Act or not	23	77	20
Has the meat contained the tolerable level of antibiotic, preservative, hormone, poisons substance, heavy metal and micro-organism	19	81	21
Whether your slaughterhouse is environment friendly or not	17	83	22
The carcass, meat, consumable meat offal, used water and ice collected for sample testing is examined by the veterinary public health and microbiology laboratory or not	11	89	23

Knowledge and perception of meat processor about slaughter act

Table 5 shows that 74% meat processors used clean water in processing plant. Islam (2015) cited that 71.43% butchers clean their slaughter house/meat selling centre which is in agreement with the present findings. It reveals that 30% selling center are not certified by the authority which is the violation of the slaughter act. In many developing countries, regulations concerning meat inspection and/or control are inadequate or non-existent allowing consumers to be exposed to pathogens including zoonotic parasites (Adzitey and Huda, 2012). Perez et al. (2002) observed that a lairage period of two to three hours is necessary to recover from transport stress, because of reduced meat quality with shorter lairage times.

Table 5. Knowledge and perception of meat processor about slaughter act

Particulars	Yes (%)	No (%)	Rank
Clean water is used in your processing plant or not	74	26	1
Whether the selling center is certified by the designated authority or not	70	30	2
Whether the persons involved in meat processing are free from infectious and contagious diseases or not	62	38	3
Certified by registered physician in getting cured from aforesaid diseases or not	39	61	4
The DG or authorized officer inspects your meat processing center/plant or not	36	64	5
Follow the provision of transport and marketing of meat and meat products as per Act or not	35	65	6
Whether your slaughterhouse is environment friendly or not	30	70	7
Manager or owner or responsible person keep the medical certificate of employees of processing plant/center, slaughterhouse or not	27	73	8
Show the health certificate while veterinary surgeon inspect or not	23	77	9
There are modern facilities in your processing plant/center or not	18	82	10
Importing meat based on animal and animal products quarantine act-2005 or not	10	90	11

Knowledge and perception of ULO/DLO about slaughter act

Table 6 shows that 82% Govt. officials does not follow the animal slaughter act. Farmers are in loss in livestock business due to lack of proper marketing and value of the product.

Table 6. Knowledge and perception of ULO/DLO about slaughter act

Particulars	Yes (%)	No (%)	Rank
Follow the animal slaughter act or not	18	82	1
Working for entrepreneurship development or exporting livestock products or not	46	54	2
In case of animal importing quarantine facilities is created or not	56	44	3
Advise farmer for maintaining cool chain method in selling dressed poultry	56	44	4
Get encourage and assist to set up disease diagnostic lab at private level	69	31	5
Maintain withdrawal period of drugs in prescription or not	74	26	6
Live bird marketing, bio-security in city corporation and Pouroshava are mentioned in policy is known to it or not	77	23	7
Residual effect of medicine must not have in the poultry product are maintaining or not	77	13	8
Long term planning in preventing and controlling diseases such as bird flu and other infectious disease	77	23	10
Following international standard in case of using probiotic and antibiotic	77	23	11
Registered veterinary surgeon or not	80	20	12
Bio-security protocol reached to the farmer or not	82	18	13
Taking initiative on epidemiology, disease reporting and recording system	85	15	14
Taking any initiative on disease control extension program	85	15	15
Giving advice to the farmer not to sell live birds in case of	85	15	16
Getting encourage the farmer to sell dressed broiler and poultry meat	87	13	17
While setting up farm, registration, control of food value, disease control and others follow the act/provision/ordinance or not	90	10	18
Followed the veterinary practitioner act or not	92	8	19
Non-registered veterinary surgeon not allowed to practice is known or not	92	8	20
The veterinary practitioner act is known or not	95	5	21
Any involvement in poultry disease surveillance at Govt. and private level farm or not	95	5	22
The animal slaughter act is known or not	97	3	23

Binary logistic regression for butchers about slaughter act

From binary logistic regression it reveals that none of the endogenous variables have significant effect on the knowledge of farmers about slaughter act. From proportion test (Z test) it reveals that less than 50% of farmers have knowledge about the slaughter act and less than 40% of processors are known about the slaughter act.

Qualitative results on Focus Group Discussion (FGD) and KIIs

We had four FGDs and KIIs with DLS personnel, University teachers and BLRI scientist. From the study it revealed that authority should have appropriate implementation strategy. Scientific slaughter house, chilling facility of fresh meat, important role of academician and researcher, health status of workers of slaughter house and processing plant, consumer's exploitation by butchers should be maintained. Policy should be implemented on step by step basis targeting a period. There should have sophisticated and accredited laboratories to carry out chemical analysis of feeds (de Jonge & Jackson, 2013).

During illegal cross border traffic there is a possibility of transmission of trans-boundary diseases, loss of foreign currencies and threatens of livestock breeding policies and border killings (The Daily Star, August 10, 2015). If this illegal entry is blocked there will be an opportunity to increase own livestock resources through small scale and large scale livestock farming resulting in poverty reduction in the country (Ali and Hossain, 2014).

CONCLUSION

The study reveals that to implement the slaughter act authority should have action plan and monitoring cell in order to implement the slaughter act; establishment of modern scientific slaughterhouse; slaughtering license process should be transparent; cattle smuggling from neighboring countries should be controlled; act should be updated time to time to address the field based need.

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DNA FINGERPRINTING AND GENETIC DIVERSITIES IN SOME BANGLADESHI AUS RICE (*Oryza sativa* L.) GENOTYPES

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ABSTRACT

The allelic diversity and relationship among 120 Aus rice landraces were determined through DNA fingerprinting using microsatellite (SSR) markers. A total of 85 SSR markers were used to characterize and discriminate all tested Aus rice genotypes, 45 of which were polymorphic for different chromosome numbers. The number of alleles per locus varied from 6 alleles (RM484 and RM541) to 30 alleles (RM519) with an average of 13 alleles per locus. The polymorphic information content (PIC) values varied ranged from 0.5211 (RM536) to 0.9369 (RM519) with an average 0.8217. The highest PIC value (0.9369) was obtained for RM519 followed by RM286 (0.9357). The genetic distance-based results seen in the unrooted neighbor-joining tree clustering revealed nine genetic groups. Being grouped into distant clusters and with highest genetic distance, eleven genotypes viz., Atithi dhan, Kadar chap, Pankiraj, Japanese-7, Jamri saity, Logi jota, Joba, Lada moni, Manik Mondal-2, Boilum and Brmulka-2 could be selected as potential parents for crop improvement for their distinctive characters. Panchash and Parija had closest distance in the SSR based CS-Chord distance (0.000) might have same genetic background. The highest genetic dissimilarity (1.000) was found among the nineteen Aus genotypes combinations followed by the second highest (0.9778) among 94 Aus rice combinations. Whereas lowest genetic dissimilarity was found between Kala and Kalo Hizli (0.1778) followed by Holat and Holae (0.2667). This information will be useful in the selection of diverse parents, background selection during backcross breeding programs and assist in broadening germplasm-based rice breeding programs in the near future.

Keywords: Aus rice, genetic diversity, microsatellite markers, DNA fingerprinting

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INTRODUCTION

Rice (*Oryza sativa*) is one of the most important food crops and a primary source of food for more than half of the world's population (Khush, 2005). According to the United Nations (UN) estimates, the current world population 6.1 billion is expected to reach 8.0 billion by 2025. Most of this increase (93%) will take place in the developing world. Global rice production must reach 800 million tones of paddy rice to meet projected demand in 2025 (Peng et al., 1999) which is about 200 million tones more than rice production in 2006. This additional rice must come mainly from irrigated land in Asia, because improving rice yield in most rainfed regions is constrained by drought, flooding and poor soil quality (Cassman, 1999). Bangladesh is already under pressure both from huge and increasing demands for food, and from problems of agricultural land and water resources depletion. Bangladesh needs to increase the rice yield in order to meet the growing demand for food emanating from population growth.

For the study of genetic diversity, the plant scientists have used generally morphological, physiological as well as chemical features of plant. The number of scoreable morphological characters is varying as compared to the biological active genes. Moreover in most cases, plant genomes have large amount of repetitive DNA which are not expressed and do not contribute to the physiological or morphological appearance of plants. In the case of very closely related plant varieties, there are very few morphological differences, which as a matter of fact do not represent the true genetic differences at DNA level. So, there is always a need to study polymorphism at DNA level, which can be an indicative of genetic diversity. Several types of molecular markers viz., RFLP, RAPD, AFLP, microsatellites and SNP have been developed. PCR-based markers such as microsatellites are co-dominant, hyper variable, abundant and well distributed throughout the rice genome (Temnykh et al., 2001). Microsatellites have shown great promise in genetic diversity, genome mapping, gene tagging and marker-assisted selection (MAS) because they are technically simple, time saving, highly informative and require small amount of DNA. Abundance of microsatellite markers is now available through the published high-density linkage map (McCouch et al., 2002; IRGSP 2005) or public database. A study was conducted on 234 rice landraces in Plant breeding division, Cornell University and they identified five distinct groups corresponding to *indica*, *aus*, *aromatic*, *temperate japonica* and *tropical japonica* rice (Amanda et al., 2004). They also have very high diversity with 98% of loci polymorphic in Aus groups. Despite of their drought tolerance and early maturity, the group has received less attention compared to *indica* and *japonica* group.

There are four distinct ecotypes of rice-Boro, Aus, Transplanted aman and Deep water aman in Bangladesh. Bangladesh has a good source of indigenous rice cultivars. About 4000 T. Aman, 2500 Boro and 1500 Aus landraces are present in BRRRI rice germplasm gene bank. Only a few decades ago large numbers of farmers

were growing local cultivars as their main crop. Those cultivars have good adaptation but are poor yielder. Actually cultivation of these landraces was gradually replaced by high yielding varieties during last twenty years. These landraces adapted in different parts of this country, some of which have very nice quality, fineness, aroma, taste and high protein contents (Dutta et al., 1998). After establishment of BRRI, DNA fingerprinting has been done only for a small number of local germplasm. Indigenous crop landraces were characterized at both molecular and phenotypic level by many countries. Such types of characterization have been done for keeping the crop identity and searching for new genes for further crop improvement. But information on the genetic diversity of local landraces particularly for Aus rice is very scanty. Precise information on the extent of genetic diversity among population is crucial in any crop improvement program, as selection of plants based on genetic diversity has become successful in several crops (Ananda and Rawat, 1984; De et al., 1988). That's why, the present investigation has been undertaken in order to find out the genetic diversities among Aus genotypes at the molecular level.

MATERIALS AND METHODS

Plant materials and genomic DNA isolation

One hundred and twenty genotypes, including twelve BRRI released Aus genotypes were used in this study (Table 1). Genomic DNA was isolated from young leaves from 21 days old plants with minor modification of CTAB method. The concentration of extracted DNA was estimated by DNA confirmation test by (1.5%) agarose gel electrophoresis with lambda DNA (50ng/μl).

Table 1. List of one hundred and twenty Aus rice genotypes

SL#	Name of Genotypes	Acc No.	Origin	Collection	SL #	Name of Genotypes	Acc No.	Origin	Collection
1	Ajab Bett	1546	Chittagong	BRRI	61	Kheri Jamri	4029	Kushtia	BRRI
2	Agun Ban	1770	Jessore	BRRI	62	Khamar Mundu	4040	Meharpur	BRRI
3	Atithi dhan	4568	Dhaka	BRRI	63	Kaika	4041	Meharpur	BRRI
4	Aalo Sate	4752	Feni	BRRI	64	Kadar Chap	4042	Meharpur	BRRI
5	Begun Bahar	651	Comilla	BRRI	65	Laksmi lofa	1211	Faridpur	BRRI
6	Boilum	1205	Faridpur	BRRI	66	Lada Moni	1286	Kushtia	BRRI
7	Barmulka-2	1212	Faridpur	BRRI	67	Lagi jota	1768	Jessore	BRRI
8	Benaful	1529	Dinajpur	BRRI	68	Manik Modu	1323	Kushtia	BRRI
9	Benaful	1773	Jessore	BRRI	69	Mary satia	1626	Comilla	BRRI
10	Bathuri	1550	Chittagong	BRRI	70	Manik Mondal-1	1692	Faridpur	BRRI
11	Baisamugur	1696	Faridpur	BRRI	71	Manik Mondol-2	1765	Jessore	BRRI
12	Baismuguria	1701	Faridpur	BRRI	72	Madhu Mala	1737	Khulna	BRRI
13	Bawoi	1721	Khulna	BRRI	73	Maraka Migichak	2316	Dhaka	BRRI

SL#	Name of Genotypes	Acc No.	Origin	Collection	SL #	Name of Genotypes	Acc No.	Origin	Collection
14	Beri	1751	Khulna	BRR1	74	Mazra	4019	Faridpur	BRR1
15	Beni muri	1767	Jessore	BRR1	75	Magi Sarsa	4033	Jessore	BRR1
16	BR319-1-HR-12	3843	Dhaka	BRR1	76	Moush Doll	4036	Jessore	BRR1
17	Bora dhan	4020	Kustia	BRR1	77	Morich Boti	4043	Meharpur	BRR1
18	Baisha Muri	4026	Faridpur	BRR1	78	Mi-Mandi	4573	Dhaka	BRR1
19	Bar Pa	4027	Jhenaidhah	BRR1	79	Mi-mandisarang	4574	Dhaka	BRR1
20	Balion	4032	Faridpur	BRR1	80	Matia	4596	Noakhali	BRR1
21	Bil Kalae	4038	Jessore	BRR1	81	Nayan Tara	654	Comilla	BRR1
22	Balam	4045	Kustia	BRR1	82	Nusha Ratoi	4046	Khulna	BRR1
23	Bhatkarari	4551	Dhaka	BRR1	83	Nordi	4751	Jessore	BRR1
24	Boailla	4559	Dhaka	BRR1	84	Porangi 7	1216	Faridpur	BRR1
25	Borga Dhan	4565	Dhaka	BRR1	85	Parangi	1689	Faridpur	BRR1
26	Bali Bokri	4587	Dhaka	BRR1	86	Paik jota	1528	Dinajpur	BRR1
27	Chenri	808	Sylhet	BRR1	87	Pankirajs	1700	Faridpur	BRR1
28	Chamka	1549	Chittagong	BRR1	88	Pipre Sail	1723	Khulna	BRR1
29	Chiknal	1642	Noakhali	BRR1	89	Panburi	1730	Khulna	BRR1
30	Chitri	2081	Dhaka	BRR1	90	Padma Moni	1782	Jessore	BRR1
31	Chapila	4571	Dhaka	BRR1	91	Padha Moidu	4039	Meharpur	BRR1
32	Chakulya	4575	Dhaka	BRR1	92	Panchash	4566	Dhaka	BRR1
33	Darial	649	Comilla	BRR1	93	Parija	4588	Feni	BRR1
34	Goreswar	953	Faridpur	BRR1	94	Ratol	1772	Jessore	BRR1
35	Gutle	1774	Jessore	BRR1	95	Rathail	4047	Bagarhat	BRR1
36	Hidi 2	1289	Kushtia	BRR1	96	Saita	1681	Faridpur	BRR1
37	Holat	1551	Chittagong	BRR1	97	Sribalium	1699	Faridpur	BRR1
38	Holae	1656	Dhaka	BRR1	98	Soloi	1720	Khulna	BRR1
39	Haita saita	1691	Faridpur	BRR1	99	Sodai Soru	1725	Khulna	BRR1
40	Honuman jota	1739	Khulna	BRR1	100	Saribail	1756	Jessore	BRR1
41	Hijoli Aus	4048	Pabna	BRR1	101	Soda	1762	Jessore	BRR1
42	Haji Sail	4564	Dhaka	BRR1	102	Sail bogi	2077	Dhaka	BRR1
43	Hati Bajor	4766	Bagerhat	BRR1	103	Tarabali	811	Sylhet	BRR1
44	IR19746-28-2-2	3821	Dhaka	BRR1	104	Tepakain	1532	Dinajpur	BRR1
45	Jamri saity	1317	Kushtia	BRR1	105	Tapa sail	1752	Khulna	BRR1
46	Jamurus	1525	Dinajpur	BRR1	106	Tusha	4567	Dhaka	BRR1
47	Jagli	1761	Jessore	BRR1	107	Udobali	4572	Dhaka	BRR1
48	Japanese #7	3611	Japanese	BRR1	108	Zamir Saita	4044	Meharpur	BRR1
49	Japanese #3	3617	Japanese	BRR1	109	BR1(chandina)	MV*	-	BRR1
50	Joba	4030	Kushtia	BRR1	110	BR2 (Mala)	MV	-	BRR1
51	Korcha Muri	948	Khulna	BRR1	111	BR3(Biplob)	MV	-	BRR1
52	Khushni	952	Khulna	BRR1	112	BR6	MV	-	BRR1

SL#	Name of Genotypes	Acc No.	Origin	Collection	SL #	Name of Genotypes	Acc No.	Origin	Collection
53	Katar	1632	Chittagong	BRR1	113	BR7 (Brr1 Balam)	MV	-	BRR1
54	Kali Bori	1633	Jessore	BRR1	114	BR8 (Aasa)	MV	-	BRR1
55	Kamani sail	1662	Chitta. H. T	BRR1	115	BR9 (Sufala)	MV	-	BRR1
56	Kali Boro	1707	Faridpur	BRR1	116	BR12 (Mayana)	MV	-	BRR1
57	Koblerash	1728	Khulna	BRR1	117	BR15 (Mohinye)	MV	-	BRR1
58	Korcha	1755	Jessore	BRR1	118	BR16 (Shahi Balam)	MV	-	BRR1
59	Kala	4023	Jessore	BRR1	119	BR20 (Nizamy)	MV	-	BRR1
60	Kalo Hizli	4025	Kushtia	BRR1	120	BR21 (Niamat)	MV	-	BRR1

* = Modern BRR1 released Aus Variety; # = SI No. 7-94 local Aus landraces

SSR primers analysis

A total of 45 primers were selected (Table 2) to detect polymorphic DNA alleles for discriminating the tested Aus rice genotypes. Each PCR was carried out in a 10 µl reaction volume containing 1 µl of MgCl₂ free 10X PCR buffer with (NH₄)₂SO₄, 1.2 µl of 25 mM MgCl₂, 0.2 µl of 10 mM dNTPs, 0.2 µl of 5 U/µl Taq DNA polymerase, 0.5 µl of 10 µM forward and reverse primers and 3 µl (10ng) of DNA using a 96 well thermal cycler. The mixture was overlaid with one drop (3 µl) of mineral oil to prevent evaporation. The temperature profile used for PCR amplification comprised 94° C for 5 minutes (initial denaturation) followed by 35 cycles of 94° C for 1 minute (denaturation), 55° C for 1 minute (annealing), 72° C for 2 minutes (extension) with a final extension for 7 minutes at 72° C at the end of 35 cycles. The annealing temperatures were adjusted based on the specific requirements of each primer combination. The PCR products were mixed with gel loading dye (bromophenol blue, xylene cyanol and sucrose) and electrophoresed in 8% polyacrylamide gel using vertical poly acrylamide gels for high throughput manual genotyping. Three-Four µl of amplification products were resolved by running gel in 1X TBE buffer for 1.5 hrs to 2.5 hrs depending upon the allele size at around 90 volts and 500 mA electricity. The gels were stained in 1 µg/ml ethidium bromide and were documented using UVPRO (Uvipro Platinum, EU) gel documentation unit.

Data analysis

Size for each amplified allele was measured in base pair using Alpha-EaseFC 5.0 software. The summary statistics including the number of alleles per locus, major allele frequency, gene diversity, Polymorphism Information Content (PIC) values were determined using Power Marker version 3.25 (Liu and Muse, 2005). The allele frequency data from Power Marker was used to export in binary format (allele

presence=1 and allele absence=0) for analysis with NTSYS-pc version 2.1 (Rohlf, 2002). A similarity matrix was calculated with the Simqual subprogram using the Dice coefficient, followed by cluster analysis with the SAHN subprogram using the UPGMA clustering method as implemented in NTSYS-pc.

Table 2. Selected primers, their sequence and chromosome number

Primer code	Chr No	Position (cM)	Product Size(bp)	Forward primer sequence (5' to 3')	Reverse primer sequence (5' to 3')
RM1	1	29.7	113	GCGAAAACACAATGCAAAAA	GCGTTGGTTGGACCTGAC
RM283	1	31.4	151	GTCTACATGTACCCTTGTGGG	CGGCATGAGAGTCTGTGATG
RM237	1	115.2	130	CAAATCCCGACTGCTGTCC	TGGGAAGAGAGCACTACAGC
RM259	1	54.2	162	TGGAGTTTGAGAGGAGGG	CTTGTTGCATGGTGCCATGT
RM431	1	178.3	251	TCCTGCGAACTGAAGAGTTG	AGAGCAAAACCCTGGTTCAC
RM452	2	58.4	209	CTGATCGAGAGCGTTAAGGG	GGGATCAAACCACGTTTCTG
RM154	2	4.8	183	ACCCTCTCCGCTCGCTCCTC	CTCCTCCTCCTGCGACCGCTCC
RM327	2	67.45	213	CTACTCCTCTGTCCCTCCTCTC	CCAGCTAGACACAATCGAGC
RM514	3	216.4	259	AGATTGATCTCCATTCCCC	CACGAGCATATTACTAGTGG
RM489	3	29.2	271	ACTTGAGACGATCGGACACC	TCACCCATGGATGTTGTCAG
RM85	3	231	107	CCAAAGATGAAACCTGGATTG	GCACAAGGTGAGCAGTCC
RM307	4	0	174	GTACTACCGACCTACCGTTCAC	CTGCTATGCATGAACTGCTC
RM252	4	99	216	TTCGCTGACGTGATAGGTTG	ATGACTTGATCCCCGAGAACG
RM119	4	76.1	166	CATCCCCCTGCTGTGCTGCTG	CGCCGGATGTGTGGGACTAGCG
RM178	5	118.8	117	CAGTGGGCGAGCATAGGAG	ATCCTTTTCTCCCTCTCTCG
RM413	5	26.7	79	GGCGATTCTTGATGAAGAG	TCCCCACCAATCTTGCTCTC
RM169	5	57.9	167	TGGCTGGCTCCGTGGGTAGCTG	TCCCGTTGCCGTTTATCCCTCC
RM153	5	0-2.3	201	ACCAACGCCAAAAGCTACTG	TACTCGCCCTGCATGAGC
RM122	5	6.4	227	GAGTCGATGTAATGTCATCAGTGC	GAAGGAGGTATCGCTTTGTTGGAC
RM161	5	96.9	187	AAACTGTTTTACCCTGGCC	ATCCCCTTCTGCGGTAAC
RM541	6	75.5	158	TATAACCGACCTCAGTGCCC	CCTTACTCCCATGCCATGAG
RM204	6	25.1	169	GTGACTGACTTGATCATAGGG	GCTAGCCATGCTCTCGTACC
RM217	6	28.6	133	ATCGCAGCAATGCCTCGT	GGGTGTGAACAAAGACAC
RM11	7	47	140	TCTCCTCTTCCCCGATC	ATAGCGGGCGAGGCTTAG
RM18	7	90.4	157	TTCCCTCTCATGAGCTCCAT	GAGTGCCGCGCTGTAC
RM134	7	73.2	93	ACAAGCCGCGAGAGGATTCCG	GCTCTCCGGTGGCTCCGATTGG
RM25	8	52.2	146	GGAAAGAATGATCTTTTCATGG	CTACCATCAAAACCAATGTTT
RM44	8	60.9	99	ACGGGCAATCCGAACAACC	TCGGGAAAACCTACCCTACC
RM105	9	32.1	134	GTCGTCGACCCATCGGAGCCAC	TGGTCGAGGTGGGGATCGGGTC
RM215	9	99.4	148	CAAAATGGAGCAGCAAGAGC	TGAGCACCTCCTTCTCTGTAG
RM219	9	11.7	202	CGTCGGATGATGTAAAGCCT	CATATCGGCATTCGCCTG
RM171	10	58.1	328	AACGCGAGGACACGTAATTAC	ACGAGATACGTACGCCTTTG
RM147	10	99.8	97	TACGGCTTCGGCGGCTGATTCC	CCCCGAATCCCATCGAAACCC
RM484	10	97.3	299	TCTCCTCCTCACCATTGTC	TGCTGCCCTCTCTCTCTCTC
RM216	10	17.6	146	GCATGGCCGATGGTAAAG	TGTATAAAACCACACGGCCA
RM536	11	55.1	243	TCTCCTCTTGTGGCTC	ACACACCAACACGACCACAC

Primer code	Chr No	Position (cM)	Product Size(bp)	Forward primer sequence (5' to 3')	Reverse primer sequence (5' to 3')
RM209	11	73.9	134	ATATGAGTTGCTGTCGTGCG	CAACTTGCATCCTCCCCTCC
RM167	11	73.9	128	GATCCAGCGTGAGGAACACGT	AGTCCGACCACAAGGTGCGTTGTC
RM206	11	102.9	147	CCCATGCGTTAACTATTCT	CGTCCATCGATCCGTATGG
RM286	11	0	110	GGCTTCATCTTTGGCGAC	CCGGATTCACGAGATAAACTC
RM144	11	123.2	237	TGCCCTGGCGCAAATTTGATCC	GCTAGAGGAGATCAGATGGTAGTGC ATG
RM287	11	68.6	118	TTCCTGTAAAGAGAGAAATC	GTGTATTTGGTGAAAGCAAC
RM20	12	0	234	ATCTTGTCCTGCAGGTCAT	GAAACAGAGGCACATTTTCATTG
RM519	12	62.6	122	AGAGAGCCCCTAAATTTCCG	AGGTACGCTCACCTGTGGAC
RM277	12	57.2	124	CGGTCAAATCATCACCTGAC	CAAGGCTTGAAGGGAAG

RESULTS

Overall microsatellite diversity

One hundred and twenty Aus genotypes were assessed for genetic variability using 45 polymorphic Microsatellite DNA markers. A total of 228 alleles were detected at the loci of 45 microsatellite markers across the Aus rice genotypes.

Table 3. Data on the number of alleles, allele size range, highest frequency allele and polymorphism information content (PIC)

Marker	Chr No	Position (cM)	Motif*	Allele no.	Size range (bp)	Highest frequency allele		PIC Value
						Size (bp)	Freq (%)	
RM1	1	29.7	(GA)26	19	70-121	79	16	0.9052
RM283	1	31.4	(GA)18	13	146-170	155	33	0.8172
RM237	1	115.2	(CT)18	7	136-150	140	36	0.7146
RM259	1	54.2	(CT)17	16	152-175	168	23	0.8672
RM431	1	178.3	(AG)16	17	235-270	262	13	0.9154
RM452	2	58.4	(GTC)9	12	185-217	190, 197	17	0.8760
RM154	2	4.8	(GA)21	25	160-220	190	15	0.9161
RM327	2	67.45	(CAT)11(CTT)5	14	193-216	215	16	0.8890
RM514	3	216.4	(AC)12	9	245-262	245, 252	18	0.8475
RM489	3	29.2	(ATA)8	18	236-271	254	18	0.8753
RM85	3	231	(TGG)5(TCT)12	7	89-117	107	35	0.7062
RM307	4	0	(AT)14(GT)21	18	129-186	148	23	0.8880
RM252	4	99	(CT)19	16	198-245	203	21	0.8829
RM119	4	76.1	(GTC)6	10	160-174	160	18	0.8508
RM178	5	118.8	(GA)5(AG)8	9	117-124	124	33	0.7682
RM413	5	26.7	(AG)11	14	70-101	82	19	0.8705
RM169	5	57.9	(GA)12	19	163-219	204	12	0.9211
RM153	5	0-2.3	(GAA)9	25	177-230	218	14	0.9332

Marker	Chr No	Position (cM)	Motif*	Allele no.	Size range (bp)	Highest frequency allele		PIC Value
						Size (bp)	Freq (%)	
RM122	5	6.4	(GA)7A(GA)2A(GA)11	8	223-238	227	28	0.8032
RM161	5	96.9	(AG)20	8	165-186	172	46	0.6917
RM541	6	75.5	(TC)16	6	158-170	168	28	0.7671
RM204	6	25.1	(CT)44	16	106-155	115	22	0.8639
RM217	6	28.6	(CT)20	11	124-155	134,137	23	0.8080
RM11	7	47	(GA)17	8	123-147	124	48	0.6227
RM18	7	90.4	(GA)4AA(GA)(AG)16	11	153-171	160	32	0.7831
RM134	7	73.2	(CCA)7	7	82-94	93	21	0.8272
RM25	8	52.2	(GA)18	14	124-158	135	29	0.8477
RM44	8	60.9	(GA)16	14	104-120	111	13	0.8907
RM105	9	32.1	(CCT)6	13	130-144	135	21	0.8539
RM215	9	99.4	(CT)16	11	144-168	147	25	0.8120
RM219	9	11.7	(CT)17	14	195-230	210	17	0.8834
RM171	10	58.1	(GATG)5	18	289-334	317	15	0.8907
RM147	10	99.8	(TTCC)5(GGT)5	7	93-99	95	37	0.7036
RM484	10	97.3	(AT)9	6	290-319	299	48	0.6233
RM216	10	17.6	(CT)18	12	122-147	128	37	0.7112
RM536	11	55.1	(CT)16	9	238-252	250	62	0.5211
RM209	11	73.9	(CT)18	17	122-160	154	18	0.8779
RM167	11	73.9	(CT)18	10	124-159	147	28	0.8056
RM206	11	102.9	(CT)21	14	126-171	132	24	0.8478
RM286	11	0	(GA)16	25	96-130	117	13	0.9357
RM144	11	123.2	(ATT)11	24	214-261	241	15	0.9218
RM287	11	68.6	(GA)21	14	96-119	103	28	0.8382
RM20	12	0	(ATT)14	9	155-191	165	25	0.8078
RM519	12	62.6	(AAG)8	30	117-150	129	10	0.9369
RM277	12	57.2	(GA)11	7	116-124	124	37	0.6556
				13			25	0.8217

* Motif of the SSR and number of repeats as previously published (<http://www.gramene.org>)

The highest amplicon size was produced by RM171 (334 bp) and the lowest by RM1 (70 bp). The number of alleles per locus ranged from 6 alleles (RM484 and RM541) to 30 alleles (RM519), with an average of 13 alleles across the 45 loci (Table 3). The frequency of the most common allele at each locus ranged from 10% (RM519) to 62% (RM536). On average, 25% of the 120 Aus rice genotypes shared a common major allele at any given locus. The polymorphic information content (PIC)

values were ranged 0.5211 (RM536) to 0.9369 (RM519) with an average 0.8217. The highest PIC value (0.9369) was obtained for RM519 followed by RM286 (0.9357), RM153 (0.9332), RM144 (0.9218) and RM169 (0.9211), respectively. PIC value revealed that RM519 and RM286 were considered as the best marker for 120 Aus genotypes. The gel pictures of figure 1 showed amplified fragment using primer designed for the SSR marker RM519 for all 120 genotypes.

Table 4. 100% dissimilarity of the nineteen Aus genotypes

Sl #	Combinations
1	IR19746-28-2-2 × Tapa sail
2	Holae × Sail bogi
3	IR19746-28-2-2XUdobali
4	IR19746-28-2-2 × Zamir Saita
5	Jamri saity × BR6
6	Boilum × BR6
7	Barmulka-2 × Soda
8	Mi-Mandi × Baisamugur
9	Baismuguria X Lagi jota
10	Beni muri × Lakhi Lata
11	Bar Pa × Noroi
12	Balam × Noroi
13	Bil Kalae × Padha Moidu
14	Bil Kalae × Panchash
15	Balam × Padha Moidu
16	Balam × Panchash
17	Darial × Parija
18	Holae × Parija
19	Hati Bajor × Sodai Soru

Genetic distance-based analysis

An unrooted neighbor-joining tree showing the genetic relationships among 120 Aus rice genotypes of Bangladesh was constructed based on the alleles detected by 45 microsatellite markers. The genetic distance-based results seen in the unrooted neighbor-joining tree revealed nine groups in the 120 genotypes (Figure 2). Aus genotypes of BIRRI released modern varieties were clustered in the same group in the cluster IX. All Aus landraces were distributed into different cluster but Panchash (sl no.92, acc. no. 4039) were not found in any cluster, it may be duplicate with Parija (sl

no. 93, acc. no.4566). Cluster number VIII contain highest number of genotypes (23) and cluster no IV contain only one genotypes, it was Jamri saity. Furthermore, the two Aus landraces viz., Madhu Mala (73) and Khushni (52) were clustered in the same group (cluster II). Three Aus landraces (Hati Bajor, Haji Sail, and IR19746-28-2-2) were clustered distinctly in the same group (cluster VII). Cluster III and Cluster V contains same number of genotypes (21) on the other hand cluster I and cluster VI contain 17 and 19 number of genotypes, respectively. Genetic dissimilarity coefficient was recognized between every two genotypes based on DNA profile. The highest genetic dissimilarity (1.000) was found among the nineteen Aus genotypes combinations (Table 4.) Followed by the second highest (0.9778) ninety four Aus rice combinations (Table 5). Whereas lowest (0.1778) genetic dissimilarity was found Kala and Kalo Hizli.

Table 5. 98% dissimilarity found in 98 combinations of Aus genotypes

Sl #	Combinations	Sl #	Combinations
1	Bathuri × BR6	48	Bil Kalae × Mary satia
2	Bathuri × BR12 (Mayana)	49	Boailla × Lagi jota
3	Baisamugur X Soda	50	Beri × Mazra
4	Soda × BR2 (Mala)	51	Moush Doll × Bawoi
5	Beni muri × BR319-1-HR-12	52	Mazra × Morich Boti
6	Beni muri × Bora dhan	53	Mazra × Mi-mandisarang
7	Beni muri × Agun Ban	54	Mazra × Beri
8	Beni muri × Bil Kalae	55	Mi-Mandi × Nordi
9	Beni muri × Borga Dhan	56	Mi-Mandi × BR319-1-HR-12
10	Beni muri × Bali Bokri	57	Mi-Mandi × Bar Pa
11	Beni muri × Soda	58	Mary satia × Bil Kalae
12	BR319-1-HR-12 × BR1(chandina)	59	Noroi × Bil Kalae
13	Borga Dhan × Zamir Saita	60	Lagi jota × Boailla
14	Chamka × Panchash	61	Noroi × Boailla
15	Chamka × BR1(chandina)	62	Nordi × Boailla
16	Chamka × BR2 (Mala)	63	Padha Moidu × BR15 (Mohinye)
17	Boailla × BR6	64	Panchash × BR15 (Mohinye)
18	Bhatkarari × BR7 (Bri Balam)	65	Sodai Soru × BR15 (Mohinye)
19	Atithi dhan × Soda	66	Beni muri × Padma Moni
20	Gutle × Soda	67	Beni muri × Rathail
21	Darial × Sail bogi	68	BR319-1-HR-12 × Pankliiras
22	Holat × Sail bogi	69	Bora dhan × Padma Moni

Sl #	Combinations	Sl #	Combinations
23	Darial × Tapa sail	70	Bora dhan × Sodai Soru
24	Gutle × Tapa sail	71	Balion × Padha Moidu
25	Benaful × Tarabali	72	Balion × Panchash
26	Holat × Zamir Saita	73	Boailla × Parija
27	Hijoli Aus × Zamir Saita	74	Boailla × Rathail
28	Hati Bajor × Tapa sail	75	Khushni × Chitri
29	Hati Bajor × Zamir Saita	76	Bali Bokri × Mary satia
30	Jamri saity × Zamir Saita	77	Pankliiras × Borga Dhan
31	Jagli × Soda	78	Borga Dhan X Rathail
32	Kali Bori × Soda	79	Chakulya × Parangi
33	Katar × BR8 (Aasa)	80	Darial × Pankliiras
34	Baisamugur × Kalo Hizli	81	Ratol × Rathail
35	Baisamugur × Kheri Jamri	82	Ratol × Darial
36	Lagi jota × Baisamugur	83	Barmulka-2 × Japanese #3
37	Manik Mondol-2 × BR9 (Sufala),	84	Benaful × Joba
38	Padha Moidu × Panchash	85	Panburi × Japanese #3
39	Padha Moidu × Tepakain	86	Soloi, × Haji Sail
40	Bil Kalae × Balam	87	Soloi × Hati Bajor
41	Bil Kalae × Boailla	88	Soloi × Japanese #7
42	Bil Kalae × BR16 (Sahya Balam)	89	Benaful × Moush Doll
43	Jamri saity × BR15 (Mohinye)	90	Morich Boti × Benaful
44	Jamurus × BR16 (Sahya Balam)	91	Morich Boti × Benaful
45	Japanese #3 × Baismuguria	92	Benaful × Matia
46	Khamar Mundu × Baismuguria	93	Benaful × Porangi 7
47	Bawoi × Laksmi lofa	94	Benaful × Parangi

DISCUSSIONS

In crop improvement breeding program these genetically diverse genotypes could be chosen as parents for crossing program to create genetic variability and produce transgressive segregants. It was also recognized that two Aus landraces (Panchash and Parija) were sorted out as exactly same genotypes in this analysis (zero dissimilarity) might possess same genetic background. Hence, microsatellite marker based molecular fingerprinting could serve as a potential basis in the identification of genetically distant accessions as well as in duplicate sorting of the morphologically close accessions.

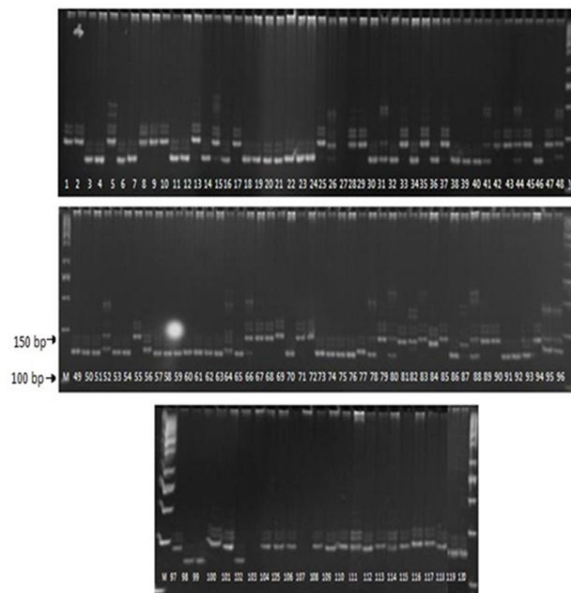


Figure 1: DNA profile of 120 Aus genotypes (108 landraces and 12 BRRI released Aus variety) with the SSR marker RM519

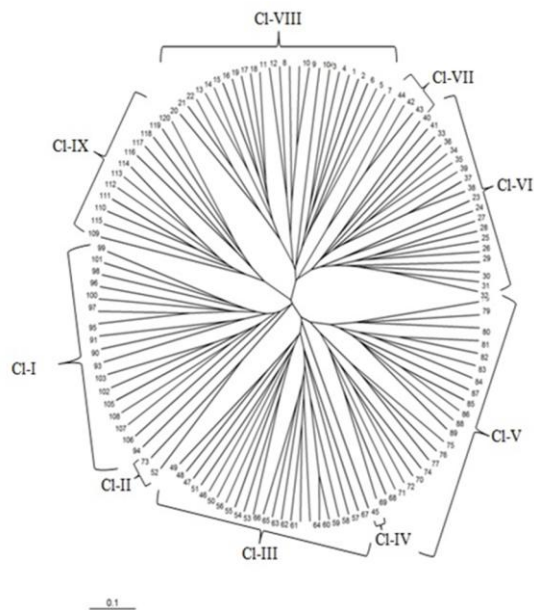


Figure 2: An unrooted neighbor-joining tree showing the genetic relationships among 120 Aus landraces based on the alleles detected by 45 microsatellite markers

In contrast, DNA-based molecular markers have proven to be powerful tools in the assessment of genetic variation and in the elucidation of genetic relationships within and among species, characterized by abundance and untouched by environmental influence (Powell et al. 1996). Ravi et al. (2003) also generated unique SSR profiles in rice by using a few primers that covered all 12 chromosomes. In the present investigation, SSR marker loci generated by 45 primers were used to assess the genetic diversity among 120 Aus rice genotypes. The SSR primers generated 228 alleles with the number of alleles per locus varying from 6 to 30. Similar number of microsatellite markers was previously used as subset for genetic diversity analysis of *O. sativa* (Garris et al.; 2005 Chakrabarthia and Naravaneni, 2006). The average number of alleles per locus was 13.0, indicating a greater magnitude of diversity among the plant materials included in this investigation. This value is comparable to 4 alleles (RM484) to 31 alleles (RM474), with an average of 13.0 alleles across the 30 loci (Thomson et al., 2007). The polymorphic information content (PIC) values were ranged 0.5211 (RM536) to 0.9369 (RM519) with an average 0.8217. The PIC values observed, are comparable to three previous estimates of microsatellite analysis in rice viz., 0.67-0.88 (Gohain et al., 2006), 0.20-0.90 with an average 0.560 (Jain et al., 2003). Many studies have also reported significant differences in allelic diversity among various microsatellite loci (Ravi et al., 2003). The alleles revealed by markers showed a high degree of polymorphism. The mean PIC value observed in this study was higher than the PIC value of 0.578 recorded by Ravi et al. (2003) in an earlier study among rice cultivars, landraces and wild relatives. The findings indicated that the genotypes used in the present study were more diversified due to differences in origin, ecotype and speciation. Panaud et al. (1996) studied using SSR markers in rice, described similarly high genetic similarity among landraces of common geographic origin and low similarity among landraces of diverse geographic origins.

The efficient use of SSR markers to discriminate between *Oryza* species with various genomes was also demonstrated by Cai and Morishima (2002). The multi allelic nature of SSR markers has the unambiguous advantage of discriminating between the genotypes more precisely. The Unrooted neighbor-joining tree cluster analysis of the SSR-based genetic similarity matrix resulted in the classification of Aus genotypes into separate clusters. Moreover, varietal profiling based on SSR markers will be more reliable as compared to profiling based on other markers, since SSR markers detect finer levels of variations among closely related lines. Cluster I was obtained as largest constellation and included 23 genotypes.

CONCLUSION

The allelic diversity revealed by 45 SSR primers was sufficient enough to distinguish among the tested Aus rice genotypes. The allelic variation was lower within the genotypes group than the other genotypes, indicating the possibility to exploit distant relatives to broaden the genetic base of rice.

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RESPONSE OF OPTIMUM NITROGEN RATE IN MAIZE WITH LEGUME INTERCROPPING SYSTEM

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ABSTRACT

A field experiment was conducted at S.V. Agricultural College farm (ANGRAU), Tirupati, India during *rabi*, 2009-10. The experiment was consisted of five system treatments (sole maize at 60 x 20 cm sowing, sole maize in skipped row sowing, maize in skipped row + greengram, maize in skipped row + blackgram and maize in skipped row + cluster bean) and three nitrogen doses (100, 75 and 50% recommended dose of nitrogen). The treatment combinations were laid out in factorial randomized block design with three replications. Yield attributes and grain yield of maize were maximum with sole maize at 60x20cm spacing but at par with maize + cluster bean, maize + blackgram and maize + greengram intercropping system. The lowest parameters were observed with sole maize sown in skipped rows. All the parameters were maximum with the application of 100% recommended dose of nitrogen to maize, but comparable with that of 75%. The maize equivalent yield, land equivalent ratio and benefit cost ratio were higher with maize + cluster bean intercropping as compared to other treatments. The result revealed that among the different maize intercropping systems tested, maize in skipped rows + cluster bean with 75% recommended dose of nitrogen to maize was found economically profitable.

Keywords: Intercropping, legume, LER, maize, nitrogen doses, yield and BCR

INTRODUCTION

The sustainable productivity of crops is the need in the present Indian farming. Hence, possibility for crop intensification with sustainable nutrition for achieving the sustainability is urgently required. Hybrid maize being an exhaustive crop, requires high quantity of nutrients particularly nitrogen. The recent maize hybrids are responding to more than 240 kg N ha⁻¹. But, due to high cost and rate of fertilizer specially nitrogen, the farmers do not apply adequate quantity. In the present day's concern about environmental degradation coupled with high cost of nitrogen, there is

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a need to find out supplemental alternative sources. Legumes, if associated with maize can minimize nitrogen requirement to some extent, besides maintaining soil health. Hence, the present study was designed to explore the possibilities of intercropping of short duration legumes under varied doses of nitrogen to maize.

MATERIALS AND METHODS

A field experiment was conducted at S.V. Agricultural College farm (ANGRAU), Tirupati, India during *rabi*, 2009-10. The experiment consisted of five system treatments (sole maize at 60x20 cm sowing, sole maize in skipped row sowing, maize in skipped row + greengram, maize in skipped row + blackgram and maize in skipped row + cluster bean) and three nitrogen doses (100, 75 and 50% recommended dose of nitrogen). The treatment combinations were laid out in factorial randomized block design with three replications. The results of physico-chemical analysis of soil revealed that the soil was sandy clay loam in texture, near neutral in soil reaction, low in organic carbon and available nitrogen and medium in available phosphorus and potassium. The data pertaining to different weather parameters during the crop growth recorded at the meteorological observatory, S.V. Agricultural College Farm, Tirupati are presented in table 1. The test varieties of maize, greengram, blackgram and cluster bean were DHM-117, LGG-460, LBG-623 and RGM-112, respectively. Healthy seeds of maize and intercrops greengram, blackgram and cluster bean were treated with Mancozeb @ 3 g kg⁻¹ of seed and sown @ 2 seeds hill⁻¹ on 6 November 2009. Intercrops were sown at a spacing of 60 x 5 cm. Uniform dose of 60 kg P₂O₅ and 50 kg K₂O ha⁻¹ through Single super phosphate and Muriate of potash, respectively were applied as basal to the maize in all the plots. Nitrogen was applied as per the treatments in three equal splits viz., basal, knee height and at tasseling stage. Recommended dose of nitrogen applied to maize was 240 kg ha⁻¹. For the intercrops of greengram, blackgram and cluster bean 20, 50 and 40 kg N, P₂O₅ and K₂O ha⁻¹ respectively, were applied as basal. Gap filling was done at one week after sowing, to maintain desired population as per treatments. Thinning was carried out at one week after sowing, to retain one seedling hill⁻¹. Weeding was done twice at 15 and 30 days after sowing with rotary weeder followed by hand hoeing to keep the crop free from weeds. Spraying of chlorpyrifos @ 2.5 ml l⁻¹ once and thiodicarb @ 1g l⁻¹ water was sprayed twice to protect the crops from *Spodoptera litura*. Maize and intercrops were harvested when the sheaths of the cobs and pods were completely dried, respectively. Date of harvests of greengram, blackgram, clusterbean and maize were 7 January 2010, 19 January 2010, 20 January 2010 and 25 February 2010 respectively. The cobs of maize and pods of intercrops from net plot area were sun dried, threshed, cleaned and weighed separately. The total cost of cultivation was calculated for each treatment on the basis of inputs used. Gross returns were calculated based on the prevailing market price output. Net returns were arrived at by subtracting the cost of cultivation of respective treatment from gross returns of the corresponding treatment. Benefit cost ratio was calculated by dividing

the gross returns with cost of cultivation of the respective treatments. The data was statistically analyzed by following the method of analysis of variance as suggested by Panse and Sukhatme (1985). Critical difference was worked out at 5 % level of probability, where ever the treatmental differences were significant.

RESULTS AND DISCUSSION

Yield attributes of maize

Cob length: The length of the dehusked cobs of maize was significantly influenced by maize + legume intercropping and dose of nitrogen application to maize (Table 2). The longest cobs was observed in sole maize sown at 60 x 20 cm which was closely followed by cob lengths recorded in maize + cluster bean, maize + blackgram and maize + greengram intercropping systems. The shortest cobs were recorded when sole maize sown in skipped rows. Among the nitrogen management, application of recommended dose of nitrogen to maize resulted in longest cob length which was comparable with that of 75 per cent recommended dose of nitrogen application and both were significantly superior to application of nitrogen at 50 per cent recommended dose.

Table 1. Weekly meteorological data during the crop growth period (2009 - 2010)

Standard week	Date and month	Temperature (°C)				Relative humidity (%)		Rainfall (mm)		No. of rainy days		Mean evaporation (mm)		Mean bright sunshine (hours day ⁻¹)	
		Maximum		Minimum		A	DN	A	DN	A	DN	A	DN	A	DN
		A	DN	A	DN	A	DN	A	DN	A	DN	A	DN	A	DN
45	05-11 Nov	27.2	-2.6	22.5	1.5	85.2	11.5	111.4	84.4	5.0	3.3	1.6	-1.8	1.1	-4.2
46	12-18 Nov	29.9	0.0	23.3	3.5	74.9	6.6	22.4	8.8	2.0	0.9	2.6	-1.6	3.1	-3.8
47	19-25 Nov	31.6	3.6	22.2	2.0	71.0	-2.0	15.0	-29.9	1.0	-0.9	3.2	-0.3	6.1	0.7
48	26-2 Dec	29.4	1.4	20.4	1.3	66.8	-4.8	0.0	-49.2	0.0	-1.6	4.3	0.7	6.5	0.5
49	3-9 Dec	28.7	0.4	19.6	1.6	71.5	1.4	14.2	-8.8	2.0	1.0	2.5	-1.2	3.9	-3.3
50	10-16 Dec	28.7	0.4	21.0	3.2	67.4	-1.3	33.2	29.8	1.0	0.6	2.6	-1.3	4.3	-2.3
51	17-23 Dec	27.5	-0.8	19.3	1.8	72.3	2.4	14.7	-15.5	3.0	2.2	2.2	-1.5	2.4	-3.9
52	24-31 Dec	28.1	-0.8	18.3	0.9	67.6	-2.0	0.0	-4.5	0.0	-0.5	2.6	-1.4	4.2	-2.6
1	1-7 Jan	29.1	0.7	17.0	-0.5	68.8	0.8	0.0	-4.2	0.0	-0.2	3.5	-0.6	5.3	-2.1
2	8-14 Jan	29.8	0.9	20.6	4.0	67.4	-0.6	0.0	-0.5	0.0	0.0	3.4	-1.1	4.7	-2.8
3	15-21 Jan	30.0	0.3	18.4	2.4	61.6	-4.3	0.0	0.0	0.0	0.0	4.5	-0.3	6.8	-1.5
4	22-28 Jan	29.5	-1.4	16.6	-1.3	61.3	-2.1	0.0	0.0	0.0	0.0	4.5	-0.4	8.1	0.2
5	29-4 Feb	30.1	-0.8	15.7	-2.5	60.4	-4.9	0.0	-0.6	0.0	-0.2	4.4	-0.5	7.9	0.2
6	5-11 Feb	30.7	-0.9	16.3	-1.7	58.6	-2.0	0.0	-1.5	0.0	-0.1	5.4	-0.1	8.3	0.1
7	12-18 Feb	32.1	-0.4	19.8	1.6	59.8	-0.5	0.0	0.0	0.0	0.0	4.9	-1.0	7.3	-1.6
8	19-25 Feb	35.0	1.8	22.0	3.8	52.8	-3.9	0.0	-2.9	0.0	-0.1	5.4	-0.8	8.7	0.0

A- Actual DN- Deviation from decennial mean

Cob girth: The maize + legume intercropping and nitrogen management practices influenced the cob girth significantly (Table 2). Maximum cob girth was noticed when the sole maize was sown at 60 x 20 cm which was in parity with girth of the cob recorded in maize + cluster bean, maize + blackgram, maize + greengram intercropping systems. The lowest cob girth was recorded when sole maize was sown in skipped rows. However, it was on par with maize + greengram, maize + blackgram treatments and significantly lower to other treatments. Among the nitrogen management, application of recommended dose of nitrogen to maize resulted in highest cob girth which was comparable with that of 75 per cent recommended dose of nitrogen application and both were significantly superior to application of nitrogen at 50 per cent recommended dose.

Number of grains per cob: The number of grains per cob was influenced by maize + legume intercropping and nitrogen management practices (Table 2). Maximum number of grains per cob was noticed when the sole maize was sown at 60 x 20 cm which was in parity with grain number recorded in maize + cluster bean, maize + blackgram and maize + greengram intercropping systems. The lowest number of grains per cob was recorded when sole maize was sown in skipped rows. Among the nitrogen management, application of recommended dose of nitrogen to maize resulted in highest number of grains per cob which was comparable with 75 per cent recommended dose of nitrogen application and both were significantly superior to application of nitrogen at 50 per cent recommended dose.

Hundred grain weight: The hundred grain weight of maize was significantly influenced by maize + legume intercropping and doses of nitrogen application to maize (Table 2). Highest hundred grain weight was noticed when the sole maize was sown at 60 x 20 cm which was similar with hundred grain weight recorded in maize + cluster bean, maize + blackgram and maize + greengram intercropping systems. The lowest number of grains per cob was recorded when sole maize was sown in skipped rows. However, it is on par with maize + greengram treatment and significantly lower to all other treatments. Among the nitrogen management, application of recommended dose of nitrogen to maize resulted in highest hundred grain weight which was comparable with 75 per cent recommended dose of nitrogen application. Significantly lowest hundred grain weight was recorded when nitrogen was applied at 50 per cent recommended dose.

Grain yield and stover yield of maize

The grain and stover yield of maize as influenced either by maize + legume intercropping or nitrogen management was found similar varying only in quantity (Table 2). The maximum grain and stover yield was recorded when sole maize was sown at 60 x 20 cm, which was statistically on par with the yields recorded with maize + cluster bean, maize + blackgram and maize + greengram intercropping in the order of decrease. The grain and stover yield recorded with sole maize in skipped rows was significantly lower to all other treatments. Among the nitrogen

management, application of recommended dose of nitrogen to maize resulted in highest grain and stover yield which was comparable with that of 75 per cent recommended dose of nitrogen application. Significantly lowest yield of grain and stover was recorded when 50 per cent recommended dose of nitrogen applied to maize.

Harvest index of maize

Regarding harvest index of maize was concerned neither maize + legume intercropping nor nitrogen management did not exert any significant influence. Numerically higher value of harvest index was found when sole maize was sown in skipped rows. Among nitrogen management it was higher with 75 per cent recommended dose of nitrogen application to maize.

Table 2. Yield attributes and yield of maize as influenced by maize + legume intercropping and nitrogen management

Treatment	Cob length (cm)	Cob girth (cm)	No. of grains per cob	100 grain weight (g)	Grain yield (kg ha ⁻¹)	Stover yield (kg ha ⁻¹)	Harvest index
Crop combination							
T ₁ : Maize 60 x 20 cm	14.4	14.7	333	28.8	4009	5921	40.4
T ₂ : Maize skipped row	12.1	12.9	252	23.8	3071	4277	41.8
T ₃ : T ₂ + Greengram	13.7	13.4	297	26.4	3791	5685	40.0
T ₄ : T ₂ + Blackgram	13.9	14.4	299	27.5	3864	5771	40.1
T ₅ : T ₂ + Cluster bean	14.4	14.6	324	27.7	3905	5825	40.1
SEm ±	0.74	0.54	26.83	1.15	86.5	93.7	1.23
CD (P=0.05)	2.1	1.6	77	3.3	250	271	NS
Nitrogen management							
N ₁ : 100 % Rec. N to maize	14.8	14.6	333	28.1	3838	5809	39.7
N ₂ : 75 % Rec. N to Maize	14.5	14.3	324	27.7	3791	5694	40.0
N ₃ : 50 % Rec. N to Maize	12.3	13.0	245	24.7	3055	4984	38.0
SEm ±	0.57	0.42	20.78	0.88	79.2	83.4	1.01
CD (P=0.05)	1.6	1.2	60	2.5	229	241	NS

Yield attributes viz., cob length and girth, number of grains cob⁻¹ and 100 grain weight and grain yield of maize were maximum with sole maize at 60x20cm spacing, which were at par with maize + cluster bean, maize + blackgram and maize + greengram intercropping. The lowest parameters were observed with sole maize

sown in skipped rows (Table.2). The superiority of 60 x 20cm was due to minimal competition of available resources leading to better plant growth, as reported by Singh and Singh (2001). The skipped row method of planting resulted in grain yield reduction to the extent of 23.3 % compared to 60x20 cm spacing might be due to intraspecific competition under closer intra row spacing. Similar findings were reported by Ramaswamy et al. (1996), Moses et al. (2000), Asmat Ullah et al. (2007) and Sahoo and Mahapatra (2004). The yield attributes achieved with maize + cluster bean, maize + blackgram and maize + greengram were as par with sole maize at 60x20 cm spacing. In cereal legume intercropping, legume crops are capable of fixing atmospheric nitrogen which might have resulted in enhancing growth of the intercropped maize. Among the crop combinations, intercropping of legumes in skipped rows of maize increased the main crop yield to the tune of 22.2 to 15.5 %, compared to skipped rows of maize as sole crops. Similar results have been reported by Dasaraddi et al. (2002), Rana et al. (2006) and Sharma et al. (2008). Among the intercropped legumes, yield attributes and yields were numerically higher in cluster bean followed by blackgram and greengram. It indicates that cluster bean was more compatible with less competition to maize.

Table 3. Yield attributes and yield of different intercrops as influenced by maize + legume intercropping and nitrogen management

Crop combination		Yield attributes				Yield	
		No. of clusters per plant	No. of pods per cluster	No. of seeds per pod	1000 seed weight (g)	Seed yield (kg ha ⁻¹)	Haulm yield (kg ha ⁻¹)
Maize with 100 % RDF (N ₁) +	Greengram	11.0	5.3	5.5	37.5	418	860
	Blackgram	6.2	12.6	3.6	46.6	378	609
	Cluster bean	5.0	16.8	5.9	42.9	1063	2900
Maize with 75 % RDF (N ₂) +	Greengram	10.8	4.9	5.2	37.1	394	854
	Blackgram	5.3	12.3	4.3	46.0	362	538
	Cluster bean	5.0	16.3	5.3	35.7	985	2510
Maize with 50 % RDF (N ₃) +	Greengram	9.6	5.4	4.9	36.1	384	851
	Blackgram	5.2	12.0	4.1	45.7	358	514
	Cluster bean	5.0	15.6	4.8	35.1	969	2370

The parameters studied were highest with the application of 100 % recommended dose of nitrogen to maize, but at par with 75% nitrogen to maize. Nitrogen being the major constituent of chlorophyll, whose intensity is known to increase with added nitrogen supply, might have promoted the plant growth. Analysis of grain yield data showed that increase in grain yield of sole crop of maize was due to positive effect of nitrogen, whereas that of maize + legume should be attributed to

combined effect of nitrogen as well as complementary effect of legume association with maize. The findings of the present study are in accordance with those to Sharma (1994) and Khandkar and Nigam (1996).

Yield attributes and yield of intercrops

Yield attributes and yield of three intercrops viz., greengram, blackgram and cluster bean were highest with respective sole crops followed by application of 100, 75 and 50 % recommended dose of nitrogen to maize in the order of descent (Table 3). But the above said parameters of intercropped legumes did not deviate much from the respective sole crops. Shah et al. (1991) already found similar results. However, when the nitrogen level to maize was gradually decreased the yield attributes and yield of intercropped legumes was also found decreased. Green gram, blackgram and cluster bean suffered yield reduction to the extent of 4.0, 4.2, 7.3 and 8.1, 5.3, 8.8 % with the application of 75 and 50 % nitrogen recommended dose to maize. This shows that legumes are capable to extend their positive effect at low fertility levels in association with non legumes. These results are in accordance with Barik (1997) and Halikatti and Banarasilal (1998).

Table 4. Maize equivalent yield (kg ha⁻¹), land equivalent ratio, gross returns, net returns and BCR of maize as influenced by maize + legume intercropping and nitrogen management

Treatment	Maize grain equivalent yield	Land equivalent ratio	Gross returns (Rs ha ⁻¹)	Net returns (Rs ha ⁻¹)	BCR
Crop combination					
T ₁ : Maize 60 x 20 cm	4009	1.000	37993	21654	2.32
T ₂ : Maize skipped row	3071	1.000	28845	12506	1.76
T ₃ : T ₂ + Greengram	5535	1.883	49965	31541	2.71
T ₄ : T ₂ + Blackgram	5465	1.833	49491	31067	2.68
T ₅ : T ₂ + Cluster bean	5790	1.938	52145	33721	2.83
SEm ±	39.45	0.039	314.1	298.2	0.04
CD (P=0.05)	114	0.113	907	861	0.82
Nitrogen management					
N ₁ : 100 % Rec. N to maize	5663	1.916	51113	32871	2.80
N ₂ : 75 % Rec. N to maize	5509	1.854	49766	32176	2.83
N ₃ : 50 % Rec. N to maize	4742	1.655	42920	25982	2.53
SEm ±	37.13	0.038	307.2	278.1	0.04
CD (P=0.05)	107	0.110	888	803	0.12

Maize equivalent yield and land equivalent ratio

As indicated in table 4, the maize equivalent yield and land equivalent ratio was higher with maize + cluster bean intercropping followed by maize + greengram and maize + blackgram. With regards to nitrogen management to maize, significant superiority of maize equivalent yield and land equivalent ratio was found with application of 100 % recommended dose of nitrogen to maize.

Gross returns, net returns and B.C ratio

Gross and net returns were significantly influenced by maize+legume intercropping as well as nitrogen management (Table 4). The highest gross and net returns were realized with the maize + cluster bean intercropping which was significantly superior to all other treatments. The gross and net returns estimated with maize + greengram and maize + blackgram intercropping were statistically on par with each other but significantly higher to that of sole maize at 60x20 cm. Significantly, lowest gross and net returns was recorded when sole maize was planted in skipped rows.

As regards the nitrogen management, graded decrease in recommended dose of nitrogen to maize gradually decreased the gross and net returns with significant disparity between 100, 75 and 50 per cent recommended dose of nitrogen.

Maximization of income from the farm produce is most desirable criterion for any peasant. Intercropping in maize improved the monetary value of the total productivity compared to the respective sole crops, which might be due to additional yield of intercrops. The benefit cost ratio was significantly higher with maize + cluster bean intercropping as compared to other treatments. This was due to the benefits realized with high seed yield of cluster bean and maize, with lower cost of cultivation. These are in accordance with those of Parvender et al. (2009), and Dilip and Nepalia (2009). The respective lowest values were associated with sole maize in skipped rows. Regarding nitrogen management to maize, application of 100 % recommended dose of nitrogen to maize resulted in higher benefit cost ratio but at par with application of 75 % recommended dose of nitrogen to maize.

Nitrogen uptake and dynamics of soil available nitrogen

Sole maize at 60x20cm and application of 100% recommended dose of nitrogen to maize resulted in highest uptake of nitrogen followed by maize + greengram, maize + black gram and maize+ cluster bean (Table 5).The highest values with sole maize at 60x20 cm was due to minimum competition from the intercrops and as a result the available nitrogen utilized effectively. Intercropped maize with associated legumes have created better microbial environment and maintained better nitrogen dynamics in soil, assist in better uptake of nutrients. Lowest nitrogen uptake was estimated with sole maize sown in skipped rows and with 50% recommended dose of nitrogen to maize. The net gain of post harvest soil available nitrogen was higher with maize + greengram, with the application of 100% recommended dose of

nitrogen to maize and the net loss of soil available nitrogen was with sole maize sown in skipped rows at 50% recommended dose of nitrogen. Thus, inclusion of legume with maize either improved or maintained the available nitrogen status of soil due to its root nodulation. Among the three legumes tested, the improvement in soil available nitrogen was maximum with greengram followed by blackgram and cluster bean.

Table 5. Soil available nitrogen balance (kg ha^{-1}) as influenced by maize + legume intercropping and nitrogen management

Treatment	Initial nitrogen	Added nitrogen	Removed nitrogen	Computed balance	Post experiment nitrogen in soil	Net gain or loss
	1	2	3	(2-3)	4	(4-1)
T ₁ N ₁	168	240	124	116	181	13
T ₁ N ₂	168	180	115	65	162	-6
T ₁ N ₃	168	120	80	40	143	-25
T ₂ N ₁	168	240	96	114	136	-32
T ₂ N ₂	168	180	87	93	124	-44
T ₂ N ₃	168	120	73	47	114	-54
T ₃ N ₁	168	240+20*	114+21**	125	196	28
T ₃ N ₂	168	180+20*	96+20**	84	174	6
T ₃ N ₃	168	120+20*	86+18**	36	160	-8
T ₄ N ₁	168	240+20*	121+29**	110	187	19
T ₄ N ₂	168	180+20*	89+24**	87	169	1
T ₄ N ₃	168	120+20*	80+22**	38	154	-14
T ₅ N ₁	168	240+20*	101+34**	125	183	15
T ₅ N ₂	168	180+20*	93+29**	78	170	2
T ₅ N ₃	168	120+20*	85+25**	30	150	-18

* Nitrogen added to intercrop

** Nitrogen removed by intercrop

In conclusion, the result revealed that among the different maize intercropping tested, maize in skipped rows + cluster bean with 75% recommended dose of nitrogen to maize was found higher grain maize equivalent yield as well as profitable to the farmers, besides sustaining the soil fertility.

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EFFECTS OF PLANT GROWTH REGULATORS ON GROWTH AND YIELD OF PRE-BASIC SEED POTATO PRODUCTION UNDER GLASSHOUSE CONDITION

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ABSTRACT

A glasshouse experiment was carried out to study the effect of Plant Growth Regulators (PGRs) on the production of pre-basic seed (PBS) potatoes, cv. Janakdev at Khumaltar, (1360 masl) Lalitpur, Nepal during autumn and spring seasons of 2014 and 2015 respectively. Four - week old virus free in vitro plantlets grown in tissue culture laboratory were planted with 20 cm distance between rows and 10 cm between plantlets under glasshouse in completely randomized design. Three growth regulators, paclobutrazol (50 ppm), daminozide (100 ppm), chlormequat chloride (Cycocel) (200 ppm) and simple water as control were used for single foliar spray 6 weeks after transplanting with four replications. Analysis showed that there were significant differences on growth parameters; plant survival, plant uniformity, plant height and yield components; number of minitubers per plant and per square meter in both the seasons. Significantly maximum number of potato minitubers was recorded in growth retardants treated plants as compared to control. Experimental results showed that maximum number of minituber (22.9/plant and 668.2/m²) in autumn and (23.7/plant and 1185.0/m²) in spring season were gained from paclobutrazole spray, which were statistically differed from those of other growth regulators and control. Plants without PGR and treated with daminozide produced the least amount of minituber per unit area. Plant height decreased in all growth regulators over control due to their inhibiting effect on growth of the plants. paclobutrazol significantly reduced plant height but produced larger and heavier minitubers compared to other treatments. Hence, the best PGR for optimum enhancement of minituber number and size was paclobutrazol. The results suggested that paclobutrazol could be used in pre-basic seed potato production, leading to maximum minituber production which ultimately gave higher returns. The study concluded that spraying growth retarding chemicals at grand growth stage checked the excessive vegetative growth and increased the number and size of minituber.

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Keywords: Daminozide, chlormequat chloride, glasshouse, minituber, paclobutrazol, potato, plant growth regulators, pre-basic seed

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most important crops in Nepal. It is used as a major vegetables in terai and mid-hills and as a staple food in the high hills and mountains. According to recent statistics (ABPSD, 2015), it ranks fifth in area coverage (197,037 ha), second in quantity of production (25, 86, 287 tons) and first in productivity (13.1 t ha⁻¹) compared with the main staple food crops of rice, maize, wheat and finger millet grown in Nepal. Despite its importance in food security and cash generation, the average productivity is very low which has been considered mainly due to unavailability of high quality seed potato tubers and several other factors.

Potato, being vegetatively propagated crop, is very prone to seed degeneration as several potato viruses accumulate to the seed tubers overtime resulting in its reduced yield potential. So far, six major potato viruses, namely PLRV, PVS, PVX, PVY, PVA and PVM had been reported to infect potato crops in Nepal (Akius and Kloos, 1990; Ranjit et al., 1994). Seed potatoes should be replaced by high quality seed potatoes at regular intervals to overcome seed degeneration (Sakha and Rai, 2004). A continuous source of high quality seed potatoes is, therefore, necessary in the country.

Pre-basic seed (PBS) potatoes are disease free potato minitubers produced by transplanting pathogen free *in vitro* potato plantlets under protected condition in aphid-proof glasshouse and/or screen house. Since 1990, National Potato Research Program (NPRP) has been producing about 2,00,000 number of pre-basic seeds annually, out of which approximately 35% PBS were small sized (<0.5 g size), which are difficult for direct planting in the farmer's field condition (NPRP, 2016). Those farmers who are involved in potato seed production are always demanding for bigger size (>1 g size) of PBS.

PBS production practice is to increase productivity by replacing old degenerated seed potatoes from the major seed potato production pocket areas of the country. PBS production per plant is very low (1:5-6), so it needs to increase at least by 1:9-10. PBS demand was about 0.4 million in the year 2011 (NPDP, 2011) while it increased up to 1 million in 2015 (NPRP, 2015). Compared to the country demand of about 1 million, supply of about 0.2 million tuberlets is far below and private seed industries are also in their infant stage. Intervention of efficient technologies to increase productivity of PBS potato minituber under glass/screen house is an urgent need to fulfil this demand.

Recently minitubers productions become popular worldwide. In this system, *in vitro* plantlets are produced that can be used for further rapid multiplication (*in vitro*),

micro tuber production (*in vitro*), minituber production (in the greenhouse) (Struik and Lommen, 1990). The size of minitubers is from 5-25 mm. and their weight range is from 0.1-10 g or even more (Struik, 2007). Minitubers have the least amount of pathogens (Balali et al., 2008). Minitubers production from *in vitro* plantlets allows a faster multiplication rate in seed tubers (Imma and Mingo-Castel, 2006).

One of the major constraints in the productivity of potato minituber is excessive growth of haulm at the cost of stolon formation, tuberization & tuber bulking under glass house condition. There is also a major challenge to increase the size of potato minituber. These twin objectives could be achieved by spraying growth retarding chemicals at grand growth phase, thereby checking the excessive vegetative growth and enhancing more translocation of photosynthates to the tuber for increasing the number and size of tuber. There are many plant growth regulators (PGRs) especially plant growth retardants used for inhibiting excessive vegetative growth of plants including potato. These PGRs can also be used for long term conservation of potato germplasm under different conditions. The PGRs are used in potato production for changing different characters of the plant such as: numbers of tubers, secondary growth in field and length time of plantlet and microtuber production *in vitro* (Struik and Wiersema, 1999).

Some reports reported that Coumarin (EL-Sawy et al., 2007), Etephon (Mele et al., 1982) and Paclobutrazole (Davis et al., 1988) often showed inhibiting growth effect. With using other exogenous cytokinin, the number of tubers has been increased most of times (Ewing and Struik, 1992). One of these growth regulators is Thidiazuron that acts the same as cytokinins and has cytokinin like effects (Pavlista and Gall, 2010). The application of plant growth regulators for tuberization, is a componential process. Therefore, there are several methods that produce a balance in PGRs for inducing tuberization (Tovar et al., 1985). The main goal of this research is exploring the effects of plant growth regulators under glasshouse on the production of minituber and diverse effects of these PGRs on number and size of minitubers.

MATERIALS AND METHODS

The study was carried-out during 2013-14 to 2014-15 under glasshouse conditions at NPRP, Khumaltar, Lalitpur, Nepal.

Plant materials

For conducting the experiment, virus-free *in vitro* plantlets of cv. Janakdev were propagated in NPRP Laboratory by single node cuttings (SNCs) on MS medium and incubated for four weeks (Dhital and Lim, 2004). Thermotherapy cum meristem tip excision technique was used to eliminate potato viruses. Clones regenerated from meristem culture were utilized for virus testing by DAS-ELISA (Double Antibody Sandwiched Enzyme Linked Immunosorbant Assay) technology. Virus-free *in vitro* plantlets were rapidly propagated by single nodal cuttings on modified MS solid media supplemented with 2 mg l⁻¹ calcium pantothenate. With subsequent

subcultures, desired numbers of *in vitro* plantlets of required cultivar Janakdev were obtained for transplantation. The cultures were incubated in growth chambers with culture conditions of 16 h photo period, 2000 lux light intensity and 20 ± 2 °C temperatures.

Hardening

Before using plantlet in glasshouse we did hardening of plantlets. This operation was necessary for optimum growth of plantlets in glasshouse. In general, four week old plantlets were acclimatized and planted in bench prepared under glasshouse.

Planting in glasshouse for minituber production

Four week old *in vitro* plantlets were transplanted into sterile mixture of 2:1 sand and soil substrate under aphid-proof glasshouse for pre-basic seed production. Special cultivation techniques such as planting by making 1.5 -2 inch deep hole in trench (furrow) @ spacing of 10 cm between two 20 cm spaced ridges, irrigation with UV sterilized water, fertilizer application, earthing up, plant protection and haulm pulling were used between transplantation and harvest. Chemical fertilizers were applied at the rate of 200:200:120 NPK kg ha⁻¹. Maintained day temperatures of <25 °C and relative humidity >75% by applying sprinkle irrigation at 2 days interval and manually operated other cooling system were applied to reduce the temperature. Media contained two parts sand and one part soil dried faster so, plants need frequent irrigation under naturally ventilated glasshouse.

Spraying plant growth regulators

Three growth regulators, paclobutrazol (50 ppm), daminozide (100 ppm) and chlormequat chloride (Cycocel) (200 ppm) were used for single foliar spray and control i.e., water spray. PGRs used in experiment were from Flukar SIGMA-ALORICH Company. The spraying of PGRs was done 6 weeks after planting.

Minituber harvesting

Irrigation was stopped at least two weeks before harvest and haulms pulled a week ahead of harvesting. After 110 days, minituber were harvested and kept in shade in room temperature for a week. After that the number, size and weight of minituber per plot was recorded to assess the direct effects of PGRs on tuberization.

Data recording

During growth period, the plant growth traits such as plant survival, plant uniformity (1-5 scale), ground coverage (%), number of stems plant⁻¹ (no.) and plant height (cm) were observed. After harvesting, minitubers were graded as NPRP system (>5 g, 1-5 g, 0.5-1 g, 0.25-0.5 g and < 0.25 g). Minituber number plant⁻¹, plot⁻¹, m²⁻¹ and average size of minituber were recorded.

Experimental design and statistical analysis

The experiment was carried out in a completely randomized design (CRD) with four replications. Four treatments included three PGRs and one control i.e., water spray. The individual plot size was 2m x 1.4m with ten rows and 140 *in-vitro* plantlets. The data were statistically analyzed using computer software package MSTAT-C, version 1.3 and DMRT of MSTATC was also used for mean comparison.

RESULTS

Autumn Season

Results showed that maximum plant survival was gained from paclobutrazol and Cycocel (100%) which were statistically different from two other treatments. Maximum plant uniformity (5) and number of stems per plant (7.3) were recorded from spraying paclobutrazol as compared to other treatments. It was observed that all the growth parameters studied except ground coverage was statistically significant with respect to applied treatments (Table 1). There was a significant reduction in plant height due to application of PGRs (growth retardants), where maximum plant height (68.4 cm) was observed in control (no PGRs spray) and minimum was in paclobutrazol (30.5 cm). Generally, Cycocel (200 ppm) and daminozide (100ppm) registered higher values of plant height as compared to paclobutrazol indicating that superiority of these chemicals in bringing about enhanced growth (Table 1).

Table 1. Effects of PGR on plant survival and growth parameters of PBS potato production under glasshouse condition during autumn season at Khumaltar, Lalitpur, 2014

Treatments	Plant survival (%)	Plant uniformity (1-5)	Ground Cover (%)	Stems plant ⁻¹ (No)	Plant height (cm)
Paclobutrazol @50 ppm	100.0 a	5.0 a	95.0	7.3 a	30.5 b
Daminozide @100 ppm	95.0 b	4.0 bc	90.0	5.9 ab	60.7 a
Chlormequat chloride @200 ppm	100.0 a	3.0 c	90.0	4.3 b	58.0 a
Control(no PGRs spray)	96.0 b	4.0 ab	90.0	4.7 b	68.4 a
P value	0.0250	0.0014	0.0728	0.0264	0.0002
Significance	*	**	NS	*	***
CV(%)	2.38	9.61	15.22	23.36	15.22

NS=not significantly different, *significant at <0.05, **highly significant at <0.01 and *** highly significant at <0.001 levels, respectively.

In column figures with similar small letter(s) do not differ significantly by DMRT at 0.05 level, Plant uniformity: 1=very poor, 2=poor,

3=fair, 4=good and 5=excellent

The effect of PGRs on minituber plant⁻¹ was highly significant ($P < 0.001$). Plants sprayed with paclobutrazol produced significantly more number of PBS (22.9) plant⁻¹ compared with the other two PGRs (Figure 1). The least minituber number plant⁻¹ (14.5) was produced with control treatment, while daminozide and Cycocel slightly increased the number of minituber however, differences was at par statistically (Table 2).

The effect of PGRs on minituber size (>5g) and (0.5-1g) was highly significant ($P < 0.001$) and ($P < 0.01$) respectively. Paclobutrazol produced maximum number (208.0) of the largest minituber plot⁻¹ followed by Cycocel (201.8). PGRs had no significant effect on the production of PBS size (1-5 g, 0.25-0.5 g and <0.25 g). Results showed that, maximum number of minituber (668.2 m²⁻¹) produced with the application of Paclobutrazol while minimum number (469.3 m²⁻¹) was recorded from control treatment (Table 2).

Table 2. Effects of PGR on yield characters of PBS potato production under glasshouse condition during autumn season at Khumaltar, Lalitpur, 2014

Treatments	Minituber plant ⁻¹ (No)	PBS size distribution by weight(g) & (No)					Total Minituber Plot ⁻¹ (No)	Minituber m ² ⁻¹ (No)
		>5 g	1-5 g	0.5-1 g	0.25-0.5 g	<0.25 g		
Paclobutrazol@50ppm	22.9 a	208.0 a	794.0	616.0 a	173.5	79.5	1871.0 a	668.2 a
Daminozide@100	16.3 b	95.5 b	629.3	555.5 a	187.0	79.8	1547.0 ab	552.5 ab
Chlormequat chloride@200ppm	16.6 b	201.8 a	583.3	510.3 a	185.5	93.3	1574.0 ab	562.1 ab
Control(no PGRs spray)	14.5 c	170.8 a	687.0	293.3 b	108.8	54.3	1314.0 b	469.3 b
P value	0.0000	0.0001	0.1053	0.0044	0.1526	0.4143	0.0332	0.0332
Significance	***	***	NS	**	NS	NS	*	*
CV(%)	5.79	14.31	16.93	20.821	31.24	41.85	14.39	14.39

NS=not significantly different, *significant at <0.05, **highly significant at <0.01 and *** highly significant at <0.001 levels, respectively.

In column figures with similar small letter(s) do not differ significantly by DMRT at 0.05 level.

Spring Season

Plant survival of potato plants was not significantly affected with the application of PGRs. Other growth parameters like plant uniformity, ground coverage and plant height had the significant response of PGRs (Table 3). The number of stems per plant had no significant effect of PGRs. The data indicated that there was significant decrease in the plant height of potato plants which were subjected to the foliar application of PGRs compared with control where only water was sprayed and same trend was found in case of autumn season. The lowest plant height (22.6 cm) was observed in the application of paclobutrazol as compared to other two PGRs and the highest plant height (69.3 cm) was recorded with water spray (control).

Table 3. Effects of PGR on plant survival and growth parameters of PBS potato production under glasshouse condition during spring season at Khumaltar, Lalitpur, 2015

Treatments	Plant survival (%)	Plant uniformity (1-5)	Ground Cover (%)	Stems Plant ⁻¹ (No)	Plant height (cm)
Paclobutrazol @50 ppm	98.8	5.0 a	95.0 c	5.4	22.6 c
Daminozide @ 100 ppm	97.5	4.0 b	97.5 b	5.4	39.8 b
Chlormequat chloride @200 ppm	97.5	4.5 ab	100.0 a	4.7	44.9 b
Control(no PGRs spray)	97.5	4.5 ab	100.0 a	4.3	69.3 a
P value	-	0.0346	0.0009	0.2225	0.0000
Significance	NS	*	**	NS	***
CV(%)	3.54	9.07	1.47	16.57	17.70

NS=not significantly different, * significant at <0.05 , **highly significant at <0.01 and *** highly significant at <0.001 levels, respectively.

In column figures with similar small letter(s) do not differ significantly by DMRT at 0.05 level, Plant uniformity: 1=very poor, 2=poor,

3=fair, 4=good and 5=excellent

Table 4. Effects of PGR on yield characters of PBS potato production under glasshouse condition during spring season at Khumaltar, Lalitpur, 2015

Treatments	Minituber plant ⁻¹ (No)	PBS size distribution by weight(g) & (No)					Total Minituber Plot ⁻¹ (No)	Minituber m ² ⁻¹ (No)
		>5 g	1-5 g	0.5-1 g	0.25-0.5 g	<0.25 g		
Paclobutrazol @50 ppm	23.7 a	32.3 a	417.3 a	1203.5	832.5	832.5 b	3318.0 a	1185.0 a
Daminozide @100 ppm	20.9 a	15.0 b	281.0 b	1044.0	621.0	960.0 ab	2921.0 a	1043.2 a
Chlormequat chloride @200ppm	22.2 a	12.0 b	379.5 a	1057.5	540.0	1122.5 a	3111.5 a	1111.3 a
Control(no PGRs spray)	11.5 b	1.5 c	167.0 c	676.0	367.0	400.0 c	1611.5 b	575.5 b
P value	0.0105	0.000	0.0000	0.0804	0.1076	0.0002	0.0105	0.0105
Significance	*	***	***	NS	NS	***	*	*
CV(%)	23.19	27.66	11.25	26.64	41.28	19.17	23.18	23.18

NS=not significantly different, *significant at <0.05 , **highly significant at <0.01 and *** highly significant at <0.001 levels, respectively.

In column figures with similar small letter(s) do not differ significantly by DMRT at 0.05 level

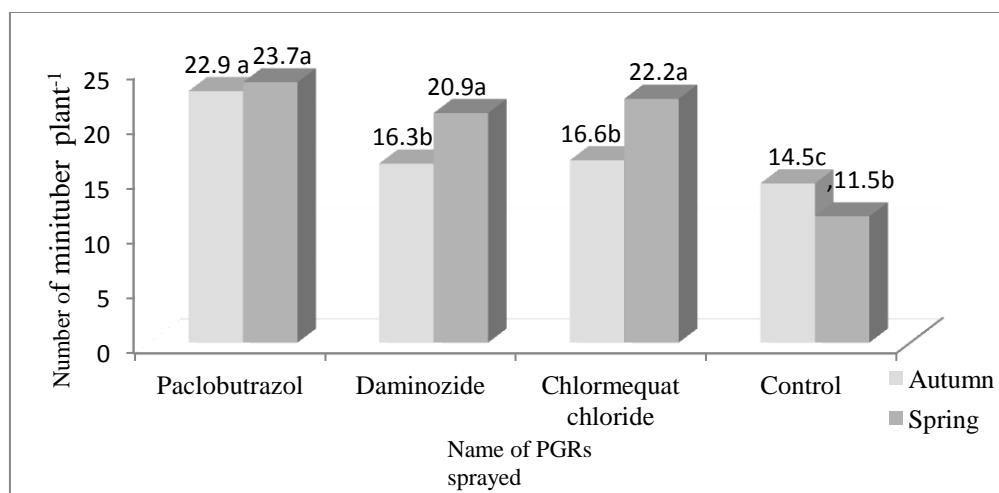


Figure 1. The comparison of the PGRs on production of minituber number plant⁻¹ during autumn and spring season

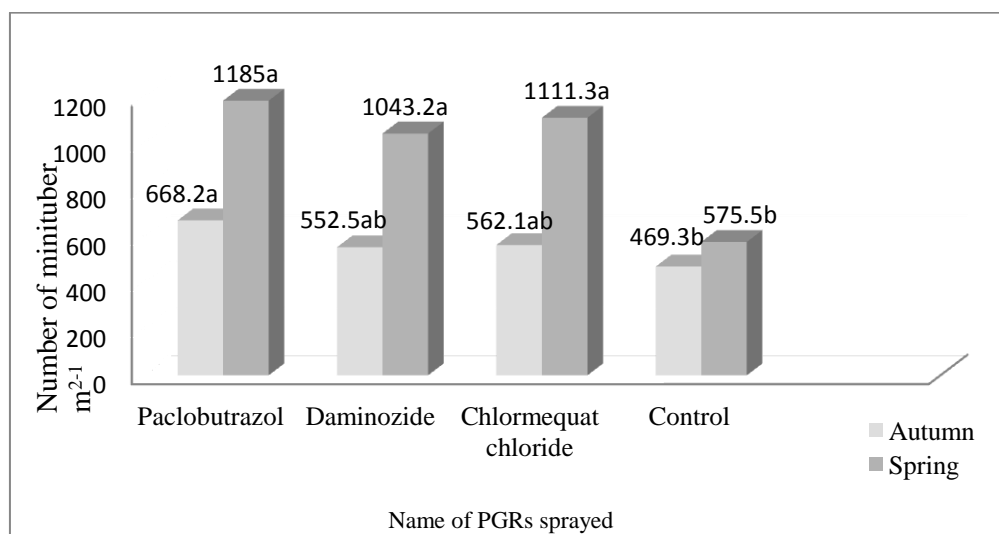


Figure 2. The comparison of the PGRs on production of minituber number m⁻² during autumn and spring season

The spraying of PGRs gave significantly higher number of minituber plant⁻¹ than control treatment (Table 4). Among the various PGRs, the number of minituber was maximum (23.7) with paclobutrazol followed by Cycocel (22.2) as compared to control (11.5) (Figure 1). Minituber size was also affected by the application of

PGRs. The results revealed that PBS size (>5 g, 1-5 g and <0.25 g) was highly significant ($P<0.001$) due to the applications of PGRs. Maximum number of large size minituber was produced by the application of paclobutrazol which were 32.3 plot^{-1} (>5g) and 417.3 plot^{-1} (1-5g). The results showed that control treatment reduced the number of big size minituber. Total number of minituber m^{2-1} was the highest in paclobutrazol (1185.0) which were statistically differed from control (575.5) and at par with Cycocel (1111.3) and daminozide (1043.2) (Table 4).

DISCUSSION

It is evident from the data presented in the preceding chapter that PGRs had significant effect on vegetative growth parameters like plant uniformity, number of stems plant^{-1} and plant height during autumn season while percentage of ground coverage was not significantly differed among the studied treatments (Table 1). Plant survival also differed with each other. In case of spring season, plant survival and number of stems plant^{-1} was not significantly affected by the application of PGRs, but growth parameters plant height, plant uniformity and ground coverage were significantly influenced (Table 3). Trend of low ground coverage percentage was noticed in the plants subjected to the foliar application of growth retardants as compared to control treatment indicating that application of PGRs reduced leaf area of potato plant. Though, there was an increase in the number of stems plant^{-1} , but there was decrease in ground coverage due to the application of growth retardants, which could be mainly attributed not only to decrease in the plant height but due to decreased cell division and cell expansion. The present findings are also consistent with the results of Prakash et al. (2001) in potato. Studies indicated that leaf area was reduced due to the application of growth retardants (Govindakrishna and Sahota, 1984; Madalageri, 1996).

The data indicated that there was a significant decrease in plant height due to the application of growth retardants (Table 1&3). The height declined due to inhibiting effect of PGRs. There was the highest number of stems per plant but low plant height with the application of paclobutrazol which might be due to growth retarding properties which increased the minituber number rather than maximum vegetative growth of the plants (Table 3). Results showed that paclobutrazol was more effective to reduce the plant height as compared to other two growth retardants. Similar results were reported by Bandara and Tanino (1995) in potato. The inhibitory effect of paclobutrazol on vegetative growth in potatoes was also reported by Simco (1991) *in vitro* and by Balamani and Poovaiah (1985) under greenhouse condition. Daminozide was used by the most bedding plant producers for height control of vegetable transplants for many years (Cantliffe, 2009).

The results (Table 2&4) revealed that there was a significant difference in number of minituber production plant^{-1} with the application of PGRs. The spraying of PGRs increased minituber number plant^{-1} and m^{2-1} . Among the various PGRs used,

paclobutrazol gave the highest minituber number as compared to other two PGRs while, control treatment produced minimum minituber number (Figure 1&2). Treatment with paclobutrazol promoted tuberization in potatoes under *in vitro* (Harvey et al., 1991; Simco, 1991) and under greenhouse conditions (Balamani and Poovaiah, 1985; Bandara and Tanino, 1995). Banerjee and Das (1984) also reported an increased tuber yield and yield components due to the application of growth retardants. PBS size was also significantly influenced by the application of PGRs. Paclobutrazol produced the maximum number of heavier and larger minituber than other PGRs (Table 2&4). The results of the present investigation are in agreement with the results of Kianmehr et al. (2012) and Bandara et al. (1998). Paclobutrazol is a triazole compound which inhibits extension growth in many plant species and blocks GA biosynthesis in plants (Davis et al., 1988). It has also been shown to inhibit shoot growth in a wide range of plant species (Barrett and Bartuska, 1982; Child et al., 1993) including potatoes (Balamani and Poovaiah, 1985; Pelacho et al., 1994).

CONCLUSION

From the study, it is concluded that spraying growth retardants gave significantly higher number and larger size of potato minituber than control. Hence, the best PGR for optimum enhancement of minituber number and size was paclobutrazol with implication for quality seed potato. The results suggest that paclobutrazol @50 ppm could be used in pre-basic seed potato production, leading to maximum minituber production under glasshouse.

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Status Paper

**OILSEEDS SECTOR OF BANGLADESH:
CHALLENGES AND OPPORTUNITIES**

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ABSTRACT

The imports of edible oils and oilseeds are increasing year after year to fulfill the increasing demand of Bangladesh. Appropriate policy measures are needed to increase oilseed production. Therefore, an in-depth investigation was carried out to assess the challenges and opportunities in the oilseeds sector of Bangladesh. The study analyzes data generated from a sample of 1980 households spread over 11 oilseed growing districts. In addition, eleven focus group discussions (FGDs) were also conducted. The study shows that there are ample opportunities to increase both area and productivity of oilseeds because of availability of short-duration improved varieties and suitable agro-climatic conditions. Also at the production and post-harvest processing levels, there is some potential for mechanical interventions that might add to the current oilseed production processes, and allow farmers to earn more from oilseed cultivation. There are also challenges such as climate variability, competition with other crops for scarce resources, and high infestation of insects & diseases.

Keywords: Oilseeds, challenges, opportunities, Bangladesh

INTRODUCTION

Edible oils play vital roles in human nutrition by providing calories and aiding in digestion of several fat soluble vitamins, for example Vitamin A (National Research Council, 1989). The per capita recommended dietary allowance of oil is 6 gm/day for a diet with 2700 Kcal (BNNC, 1984). At least 15% (405 kcal) of the total calories must come from visible and invisible oils or fats for maintaining good health. Some oilseeds are also a source of good quality protein, vitamins, and fuel. Oilcake is also an important manure for crop production and livestock feed.

Bangladesh has to spend a huge amount of foreign exchange on imports of edible oils and oilseeds to meet the increasing demand of its population. The value of imports is increasing year after year (Figure 1). The values of imported edible oils

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and oilseeds were USD1574 million and USD354 million in 2014-2015, respectively (Bangladesh Bank, 2016).

The area under oilseeds cultivation is decreasing over the years due to various economic and technical reasons. However, the area under mustard (major oilseed crop in Bangladesh) has started increasing from 2010 onwards (Miah et al., 2014). Bangladesh government has given due importance for research and development (R&D) of oilseed crops and invests a lot for attaining self-sufficiency in edible oils. Bangladesh Agricultural Research Institute (BARI) and Bangladesh Institute of Nuclear Agriculture (BINA) have released a good number of improved varieties of oilseeds. The rate of adoption of these improved varieties at farm level is encouraging (Miah et al., 2015b; Miah et al., 2015c) and have created positive impact and saved foreign exchange for the country (Miah et al., 2015a).

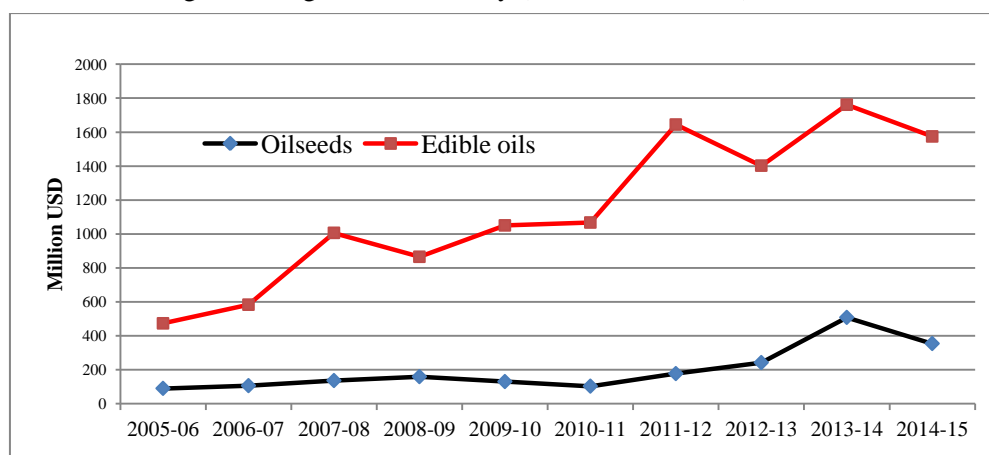


Figure 1. Trend of import value of oilseeds and edible oils

Policy-makers and research managers need overall information on oilseed crops to formulate suitable policy guidelines on oilseeds. However, an in-depth analysis is needed to explore the causes of low adoption and find out the ways for the expansion of oilseed cultivation. This study explores the challenges and opportunities in the oilseeds sector of Bangladesh.

MATERIALS AND METHODS

Study area selection and sampling technique

Four major oilseed crops namely mustard, sesame, groundnut, and soybean were taken into consideration in this study. Multi-stages sampling procedure was followed to select sample farmers. In the first stage of sampling, study areas were selected based on the area coverage of the aforesaid oilseed crops during the cropping season 2008-2009. Thus, three districts consisting high (covered $\geq 10\%$ of the total

area), medium (covered <10% area), and low (covered <5% area) growing areas were chosen for studying each type of oilseed crop. The selected districts were Manikgonj, Faridpur, Tangail, Mymensingh, Rajshahi, Pabna, Dinajpur, Noakhali, Luxmipur, Comilla, and Jessore. In the case of mustard and groundnut, the numbers of districts under high, medium and low growing areas were 3, 3, and 16, respectively, while the respective numbers were 3, 6, and 14 for sesame. For soybean, about 96% area was under Noakhali and Luxmipur. In the second stage, three suitable (in terms of data availability, accessibility, and logistic supports) Upazilas from each district were selected for each crop. Thirdly, three agricultural blocks were also selected in consultation with Agricultural Officer of the respective Upazila for collecting primary data from each oilseed growers. Finally, a total of 540 households (3 districts \times 3 Upazilas \times 60 HHs) for each type of crop (improved & local varieties) were randomly selected from a complete list of selected oilseed growing farmers for interview to collect primary data. Thus, a total of 2160 (540 HHs \times 4 crops) oilseeds cultivating farmers were interviewed for the study. But, the total sample size was only 1980, because no third district has sufficient area (>1%) under soybean cultivation to consider for the study.

Data collection and study period

Both qualitative and quantitative techniques of data collection were adopted in the present study. The researchers and trained enumerators collected data and information for this study. Data and information were collected during the period from October 2011 to October 2012. Quantitative data and information were gathered through personal interview with selected oilseed farmers using a pre-tested structured interview schedule. Qualitative technique was based on Focus Group Discussion (FGD). In total 11 FGD at Upazila level and one FGD at scientist level were conducted for this study. The focused group at Upazila level was formed with different sections of people such as Agriculture Officer (1 no.), Sub-Assistant Agricultural Officer (2 nos.), oilseed farmers (2-3 nos.), local influential persons (1-2 nos.), and oilseed traders (1-2 nos.).

Analysing adoption and profitability of oilseed cultivation

An attempt was made to estimate the adoption rate and profitability (financial and relative) of local and improved oilseed varieties in Bangladesh. The financial profitability of improved oilseeds production over their local varieties was calculated using simple accounting procedures. It was examined on the basis of gross return, gross margin and benefit cost ratio analysis. Besides, the opportunity costs of family supplied labour and cultivated land were taken into consideration in estimating total cost. Land use cost was calculated on the basis of lease value of land. In estimating relative profitability, the financial profitability of different competing crops was also estimated and compared with selected oilseed crops. Again, the costs and returns of improved oilseed variety were also compared with the respective costs and returns of local oilseed variety. Hence, data relating to input use for the production of selected

oilseeds and their competing crops, and their market prices were collected. Besides, data on outputs and their prices were also gathered for the study.

RESULTS AND DISCUSSION

Challenges: Based on the survey studies it was observed that the challenges involved in the oilseeds sector of Bangladesh are related to climatic variability, competition with other crops, production and post-harvest management and market access. These issues are briefly discussed below.

Climate variability: Farm level information revealed that more than 12.4% mustard growing farmers, 7.2% sesame farmers, and 4.2% soybean farmers faced unsuitable weather problem during cultivation (Table 1). The temperature and rainfall pattern are very irregular in Bangladesh which is harmful for mustard and soybean production. Water logging is a problem for sesame cultivation. Flash flood during April-May in the coastal region is a problem for groundnut cultivation. Therefore, weather variability could have detrimental effects on the yields of oilseeds. Besides, Bangladesh is also facing a problem of salinity which also hinders expansion of oilseeds area (FGD, 2013).

Lack of availability of improved short-duration oilseed varieties: There is a demand for improved and short-duration oilseed varieties. Most of the mustard farmers opine that they want to cultivate *boro* rice just after harvesting of oilseed crops. The gap between *T. Aman* and *boro* rice cultivation is very small (80-90 days). Therefore, they need short duration varieties of mustard, groundnut, and soybean. BARI and BINA has developed a number of improved oilseed varieties, but the non-availability of such oilseed varieties at farm level compels farmers to use local or BARI released old varieties (Figure 2). Most of the BARI released varieties of oilseeds are of longer duration that cannot be fit in the cropping pattern *T. Aman* – Oilseeds- *boro* rice (FGD, 2013). Therefore, the lack of short-duration hybrids of oilseeds is also a constraint to expand oilseed area in Bangladesh. Again, the existing seed companies or NGOs working with farmers are not interested from business point of view to produce and market improved seeds of oilseeds (FGD, 2013). They usually want more profit from their business.

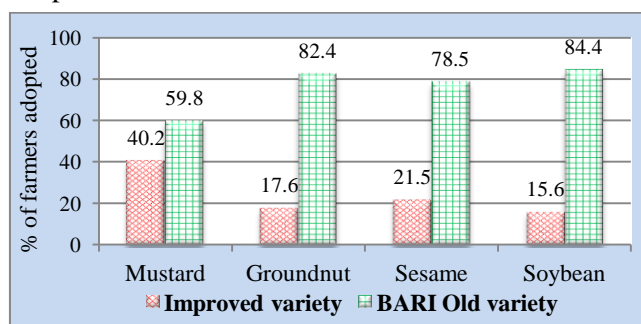


Figure 2. Overall adoptions of improved oilseeds at farm levels, 2012

Competition with other crops: Most of the farmers in Bangladesh are poor and the main cereal food items for them are rice and wheat. They are always interested in growing *boro* rice in the winter season. Nevertheless, a variety of high value crops are also grown in the winter season. As a result, oilseeds have to face serious competition with these high value crops in terms of crop choice by the farmers.

Lack of stress tolerant varieties: Many areas of Bangladesh are prone to various biotic and abiotic stresses. The prominent abiotic stresses are heat, drought, water logging, and excess moisture. The water logging problem is mainly associated with *Kharif-1* oilseed production. The farmers who have low lying land normally face loss due to water logged oilseed plants. But, Bangladesh does not have suitable oilseed varieties for such situation.

Lack of short-duration rice variety: Short-duration rice variety, especially *T. Aman* or *boro* rice is very much important for the expansion of oilseeds cultivation in Bangladesh. They need short duration varieties of *T. Aman* and *boro* rice so that they can successfully cultivate oilseed crops in between two rice crops. But, due to the unavailability of short-duration rice varieties (i.e., BINAdhan-7, BRRIdhan-33), farmers cannot cultivate mustard at the desired level. About 40% of soybean and groundnut farmers, 26.9% mustard, and 22.2% sesame farmers have reported lack of short duration rice varieties as a crucial problem (Table 1).

Infestation of insects and diseases: With the expansion of modern variety, pest management from 'seed to seed' is getting increasing importance. Infestations of different insects and diseases are reported to be the problems in oilseed cultivation. The important insects are pod borer, jassid, stem borer, leaf eater, leaf hopper, caterpillar, and hoq moth. About 56% soybean farmers have mentioned insect infestation problem, which followed by groundnut (43%), sesame (37.8%), and mustard (19.4%) farmers.

Oilseed farmers also report various disease symptoms during oilseed cultivation. The symptoms are black spots on leaf and siliqua (pod), leaf becomes yellow or curl or white, white spot on leaf, flowers fall off, and plant becomes dry. About 20% groundnut farmers and 13.9% mustard farmers encountered this disease problem during cultivation (Table 1).

Lack of cash and access to financial institutions: The cost of cultivation is a burden to many small and marginal farmers. They do not have enough access to state owned financial institutions due to complicated rules and regulations. Therefore, lack of liquid cash and lack of access to finance leads to inferior input purchases and improper post-harvest management resulting in low income. Usually, they borrow money from informal sources (e.g. moneylenders, friends, relatives, local traders, etc.) at very high interest rates. The moneylender's interest rate varies considerably with a mean of 103.3% with minimum 10% and maximum 240% (Mallick, 2009). The highest percentage of soybean (38.9%) and groundnut farmers (25.9%) expressed this as a major problem (Table 1).

Table 1. Problems and constraints to oilseed production in the study areas

Problem and constraint	Mustard (n=540)	Groundnut (n=540)	Sesame (n=540)	Soybean (n=360)
1. Lack of short duration rice variety	26.9	39.8	22.2	40.8
2. Incidence of insects ^a	19.4	43.0	37.8	55.8
3. Infestation of diseases ^b	13.9	19.6	--	1.9
4. Foggy/unsuitable weather	12.4	2.2	7.2	4.2
5. Lack of cash/access to credit	1.7	25.9	8.9	32.2
6. Damage by birds and foxes	8.1	6.3	9.4	--
7. Scarcity of power tiller	0.7	4.1	1.7	7.5
8. Lack of technical knowledge	3.3	3.7	0.6	--
9. Marketing problem ^c	9.3	15.4	1.9	3.3
10. Other problems ^d	2.8	19.6	6.9	28.9

Note: ^aPod borer, jassid, stem borer, leaf eater, leaf hopper, caterpillar, and hoq moth.

^bAlternaria leaf spot, white mould on leaf, leaf becomes yellow in color, flowers are fallen off, leaf becomes white, and plant dried.

^cLack of transport facility and its higher cost, high market charge, low price of produces, lack of marketing facilities

^dProblems related to adulteration of fertilizers, water logging, salinity, storage, nodule formation in the stem, and lack of suitable cultivable land.

Attack by birds and animals: Oilseed farmers also faced the problems of birds and foxes. Foxes generally dig the soil beds erratically which is harmful to groundnut production. Again, birds like pigeon, crow, and *Shalik* were reported to be harmful to mustard and sesame crops (Table 1).

Lack of availability of power tiller: Most of the tillage operations in the study areas are done by power tiller (PT). The well-off farmers generally buy PT for their own use as well as use as custom hiring basis. In the peak period of cultivation (generally winter season), some small and marginal oilseed farmers cannot plough their lands timely due to lack of availability of PT (Table 1).

Lack of proper technical knowledge: Although technical knowledge on planting time, seed rate, plant spacing, fertilization, irrigation plays a significant role in getting higher seed yield, many farmers still are not aware of the improved methods of oilseed cultivation leading to lower yield. They cultivate oilseeds based on their experience because they have no formal training on oilseed cultivation. Lack of oilseed related technical knowledge dissemination to oilseed farmers is also a problem (Table 1).

Marketing: A small number of oilseed farmers have also mentioned marketing problems. The major problem of oilseed marketing is the lack of transports facilities. Owing to this problem, they sometimes are compelled to sell their produces at farm gate and at the local market at a low price. Sometimes, they cannot take advantage of the higher prices prevailing at the distant markets due to lack of transportation and the higher cost of transports. The other problems observed are higher marketing charges demanded by lease holders, lower price of the produces, and inadequate marketing facility (Table 1).

Farmers' interest: The sample farmers were asked to mention the possibility of expanding their cultivated area for improved oilseed crops. Owing to higher profitability and assured markets of oilseeds, most farmers are interested to cultivate oilseed crops in future. In the case of improved variety adopters, 92.63% groundnut farmers and 51.78% mustard farmers have shown interest to increase oilseed cultivation in the next year. In the case of non-adopters, 85.53% soybean farmers and 46.63 mustard farmers have interest to expand their cultivable area (Table 2). They mentioned various reasons behind their eagerness to increase oilseed cultivation in future. These include higher yield, good price of the produces, low cost but high profit, easy cultivation, less labour, and available lands for oilseed cultivation.

Table 2. Willingness of farmers to increase oilseed cultivation in the next year

(Figures in %)

Particulars	Mustard	Groundnut	Sesame	Soybean
A. Adopter	<i>n</i> =197	<i>n</i> =95	<i>n</i> =116	<i>n</i> = 56
1. Increase	51.78	92.63	77.59	85.71
2. Not increase	47.72	3.16	17.24	7.14
3. Decrease	0.51	4.21	5.17	7.14
B. Non-adopter	<i>n</i> =343	<i>n</i> =445	<i>n</i> =424	<i>n</i> = 304
1. Increase	46.65	81.12	80.90	85.53
2. Not increase	51.60	16.18	14.62	7.89
3. Decrease	1.75	2.70	4.48	6.58
C. All category	<i>n</i> =540	<i>n</i> =540	<i>n</i> =540	<i>n</i> =360
1. Increase	48.52	83.15	80.19	85.56
2. Not increase	50.19	14.07	15.19	7.78
3. Decrease	1.30	2.78	4.63	6.67

Opportunities

The opportunities exist in oilseeds sector are related to research capacity, availability of input, availability of production technology, profitability, farmers' interest, and extension facilities. All these issues are briefly discussed below.

Suitability and availability of lands for oilseeds cultivation: Suitable land for growing a variety of crops including oilseeds is a gift of nature in Bangladesh. Most of the areas of Bangladesh are suitable for rice and oilseeds cultivation. Huge potential lies in areas under fallow i.e., after *T. Aman* harvest, *Char* (0.82 million ha), low-lying, dried-up riverbeds, and saline (0.85 million ha) areas of Bangladesh, where oilseeds can be grown successfully (Baniket al., 2011). In different parts of Bangladesh (e.g. Comilla, Manikgonj, Sherpur, Jamalpur, Netrokona, Tangail, Dinajpur, etc.), a huge amount of lands are kept fallow after *T. Aman* harvest. These fallow lands can be easily used by growing short-duration improved mustard. *Char* areas and dried-up riverbeds can also be utilized by growing different crops including different oilseeds (Financial Express, 2012). The major cropping pattern found in most of the coastal areas is *T. Aman*-Fallow-Fallow. Salt tolerant groundnut varieties can be grown in these coastal areas successfully. However, if the aforesaid potential areas can be brought under oilseeds cultivation, the country can fulfill its increasing demand by producing huge amount of oilseeds.

Dissemination of promising oilseed varieties: Technology related to oilseed cultivation has been changed to some extent due to the efforts of BARI and BINA scientists. BARI has developed some good varieties of mustard for farm level cultivation. Among these varieties, BARI mustard-14 and -15 are reported to be very much promising. These two varieties are good yielder ($1.4-1.6 \text{ t ha}^{-1}$) and short-duration (75-85 days). BINA has also developed three improved varieties of mustard (i.e., Agrani, Binasarisha-3 & -4) which are high yielding ($1.75-2.50 \text{ t ha}^{-1}$) and short-duration (83-85 days). The production of mustard can be increased manifold without increasing its area, by replacing Tori-7 and *MaghiSarisa* (local variety) with these high yielding varieties. Among short duration varieties, BARI Jhingabadam, BINACHINABADAM-2, and BINACHINABADAM-3 are potential varieties for farm level cultivation. BARI/BINA has also developed improved varieties of sesame that need to be disseminated. Therefore, a vast opportunity exists to popularize these improved oilseed varieties among farmers.

Higher productivity of improved oilseeds: Higher productivity is one of the major strengths of BARI and BINA released improved oilseed varieties in Bangladesh. The yield advantage of improved varieties of oilseeds ranged from 5-49% (Figure 3).

Higher profitability: The cultivation of improved oilseeds is highly profitable to the farmers (Table 2). The profitability of improved oilseeds is much higher than that of its traditional variety. It is also reported that the profitability of improved mustard cultivation is higher compared to its competing crops like cabbage, carrot, maize, onion, potato, and wheat. Again, the profitability of improved groundnut cultivation is much higher than that of its competing crops, such as pulses, chilli, brinjal, wheat and onion. Sesame cultivation is also more remunerative than *Aus* rice cultivation (Miah et al. 2015d). Dey et al. (2013) analysed the profitability of mustard production and estimated average net return and BCR as Tk.14,649/ha and 1.36

respectively. Kawser (1993) estimated the net return and BCR of groundnut cultivation Tk. 2,030/ha and 1.11, respectively. Again, Akter et al. (2010) found soybean as the second most profitable crop in Noakhali and Laxmipur districts. Thus, there is an ample opportunity to expand oilseeds cultivation in Bangladesh.

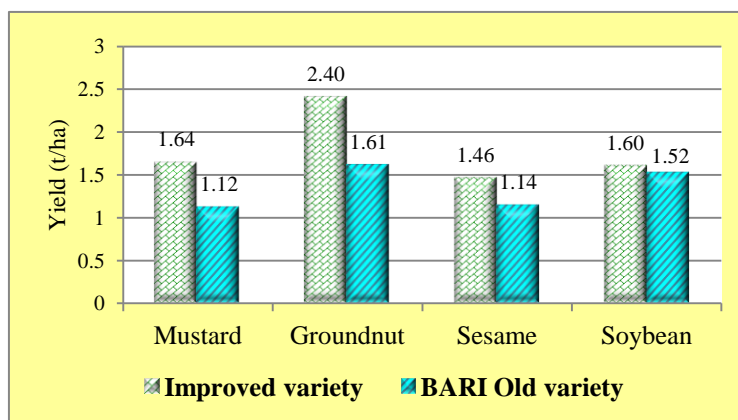


Figure 3. Yield of local and improved oilseeds at farm levels

Introduction of mechanized seeder and raised bed technology: There is an ample scope of introducing mechanized seeder and raised bed technology in oilseed, especially soybean, groundnut and sesame cultivation to increase the yield. The turnaround period between *T. Aman* rice and oilseed crops is very short. After ploughing the land, farmers can use mechanized seeder for sowing soybean, sesame, and groundnut seed quickly. The system of sowing seeds on raised bed is relatively new in Bangladesh and is very much useful for sesame and groundnut cultivation (FGD, 2013)

Improved post-harvest management practices: Improved post-harvest management practices are very much important especially for groundnut, sesame and soybean. Many oilseed farmers experience low yield due to poor storage of seeds and lack of knowledge on post-harvest practices. Providing adequate knowledge on post-harvest management practices to the farmers and creation of adequate storage facility both can play an important role in this aspect (FGD, 2013).

Private sector involvement: Private sector in Bangladesh is interested in promoting hybrid seeds of different crops (e.g. maize, wheat, rice, vegetables, etc.). Currently, ACI Seed and CCDB have extended their hands to promote BARI mustard 14 and 15 varieties among their stakeholders. Besides, the oilseed scientists of BARI have opined that higher demand for branded mustard oil is being created by the expansion of manufacturing capacities of the private oil mills in the country (FGD, 2013). Therefore, there are ample opportunities for private sectors for doing business with different improved varieties and products of oilseeds.

Table 3. Profitability of improved oilseeds cultivation in the study area

Particular	Mustard (n=217)	Groundnut (n=95)	Sesame (n=425)	Soybean (n=56)
Varieties cultivated	BARI Sarisa-9, -14, -15, -16, & BAU Sampad	BARI Chinabadam-5, -6, & Dhaka No. 2	BARI Til-3, -4, & BINA Til-1	BARI Soybean-5, & -6
1. Yield (kg ha ⁻¹)	1,641.26	2,398.98	1,458.30	1,598.30
2. Farm gate price (Tk kg ⁻¹)	46.5	59.97	37.0	29.6
3. Gross return (Tk ha ⁻¹)	80,105	146,248	56,796	48,171
Main product	76,319	144,934	54,333	47,475
By-product	3,786	1,314	2,463	696
4. Total variable cost (Tk ha ⁻¹)	23,496	36,028	24,527	26,669
5. Total fixed cost (Tk ha ⁻¹)	27,750	26,020	18,391	17,741
6. Total cost (Tk ha ⁻¹)(4+5)*	51,246	62,048	42,918	44,410
7. Gross margin (Tk ha ⁻¹) (3-4)	56,609	110,220	32,269	21,502
8. Net return (Tk ha ⁻¹) (3-5)	28,859	84,200	13,879	3,761
9. Net return (Tk ha ⁻¹)	3,895	11,363	1,873	508
10. Benefit cost ratio				
Over variable cost (3÷4)	3.41	4.06	2.32	1.8
Over total cost (3÷5)	1.56	2.36	1.32	1.1

Note: 1 acre = 3 bigha = 100 decimal; *total cost includes the rental value of land and cost of family supplied labour

There are ample opportunities to create value addition through promoting mustard oil and various food items based on sesame and groundnut in case of assured markets. There is also a good demand for soya foods in the urban markets. Therefore, promotion of the production and consumption of different types of soybean-based products can create value addition at both producer and national levels.

Groundnut is being used as an important ingredient of *Chanachur* in many food products companies. Mustard oils are marketed in different brand names by different companies in Bangladesh. Soybean is being used by some companies for preparing soya foods. Besides, the cultivation of groundnut requires a higher amount of cash (Tk. 57,332/ha) compared to other oilseed crops. Hence, there is an opportunity for the state authorities to promote contract farming systems. This will enable farmers to link with traders/companies who can provide them with technical knowledge regarding oilseeds production, access to larger buyers, and credit to buy inputs (FGD, 2013).

CONCLUSIONS AND RECOMMENDATIONS

An in-depth investigation has been done to explore the current challenges and opportunities existed in the oilseeds sector of Bangladesh. The study has identified different strengths and opportunities in the oilseeds sector, such as research

capability, good varieties, higher profitability, farmers' interest, existing extension services, availability of potential areas, and private sector involvement. Also at the production and post-harvest processing levels, there are some potential for mechanical interventions that might add value to current oilseed production processes, and allow poor farmers to earn more income from oilseed cultivation. In addition, there are also some challenges in oilseeds sector which are climate variability, high competition with other crops, lack of improved variety, and insect& disease infestation. Overall findings suggest that the opportunities existed in the oilseed sector are outweigh the challenges involved in this sector.

The following recommendations are made to enhance oilseed production for attaining self-sufficiency in Bangladesh.

- Existing improved short-duration rice and oilseed varieties should be disseminated among the farmers.
- The improved quality seeds of oilseeds and rice should be made available at farm level.
- Existing extension services should be strengthened for developing farmer-extension personnel linkage.
- Potential areas should be brought under oilseed cultivation as soon as possible.
- Private sectors should be involved in oilseed production and value addition.
- Oilseed research and development should be strengthened in collaboration with international research institutes for developing improved oilseed varieties.
- Regular training programme should be conducted for farmers and extension personnel.
- Institutional credit facilities may be provided to oilseed farmers.

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Status Paper

**DAIRYING IN SOUTH ASIAN REGION:
OPPORTUNITIES, CHALLENGES AND WAY FORWARD**

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ABSTRACT

South Asian region is blessed with high diversity of dairy animal genetic resources. The role of dairying in livelihood, nutritional and food security of millions of people living in south Asian countries has been well understood. Among livestock, dairy animal assumes much significance since dairying is acknowledged as the major instrument in bringing about socio-economic transformation of rural poor and sustainable rural development. Dairying provides a stable, year-round income, which is an important economic incentive for the small holder farmers. Dairying directly enhance the household income by providing high value output from low value input besides acting as wealth for future investment. This region is home for about 745 Million of Dairy Animal Populations that accounts 21% of global dairy animals. Besides, 25% of world's cattle and buffaloes, 15% of the sheep and goat, and 7% of the camel are inhabitant in the region. South Asia is currently producing about 200 Million tons of milk that accounts around 20% of global production despite low productivity of the dairy animals. This study focused the data related to dairying in different countries of the region and situation analyses of input and delivery system for identifying the points of interventions to boosting dairy production and processing. In gist, this study documented the facts about the current dairying in the south Asia and envisages the priorities to make the dairying sustainable and more productive with the aim to cater the inclusive development of dairying in the region. It is hoped that this will provide a wealth of information to the researchers, planners, entrepreneurs and other stakeholders for upliftment of dairy industry in the region.

Keywords: Dairying, productivity, opportunity, challenges and way forward

INTRODUCTION

Food security exists when all people at all times have access to adequate levels of safe, nutritious food for an active and healthy life (World Food Summit, 1996).

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The livestock sector is important to food security, not only for rural smallholders who rely directly on livestock for food, incomes and services, but also for urban consumers, who get benefit from affordable high-quality animal-based food. Like other livestock the dairy animals play an important role in all four main dimensions of food security: availability, access, stability and utilization. Dairying is acknowledged as the major instrument in bringing about socio-economic transformation of rural poor in developing countries. Dairy industry provides newer avenues for employment, both direct and indirect, and improves the nutritional standards of people. Dairy cattle/buffaloes have an immense contribution for sustainable rural development as unlike crop which is seasonal, dairying provides a stable, year-round income, which is an important economic incentive for the smallholder farmer to take up dairying. Milk plays a major role in reducing poverty and is a source of nutritious food in rural and urban population. For the small-scale producer milk is a key element for household income and food security and is a regular source of income for rural families and their survival. FAO estimates that for every 100 litres of milk produced locally, up to five off farm jobs are created in related industries like collecting, processing and marketing (FAO, 2012). Daily one glass of milk to the children in Asia can contribute tremendously to improving the nutritional levels in the region (Siddiky, 2015). Thus focused attention on dairy development would not only improve the milk production but also enhance the livelihood and food security of this segment of population.

Ensuring food security have to be an issue of great importance for South Asia where a considerable proportion of the population is estimated to be absolutely poor and significant proportion of children malnourished in one way or another. Food security means ensuring a sustainable supply of food at affordable prices that meets existing dietary preferences. Food security is a complex issue with both global and local dimensions that are intimately linked together. The two most important factors that determine the access of a household to food are household income and prevailing prices of essentials. Since milk and milk products are the most preferred food across the region irrespective of religion and socioeconomic status the supply of dairy products has to be addressed as part of any debate on food security (Siddiky, 2015).

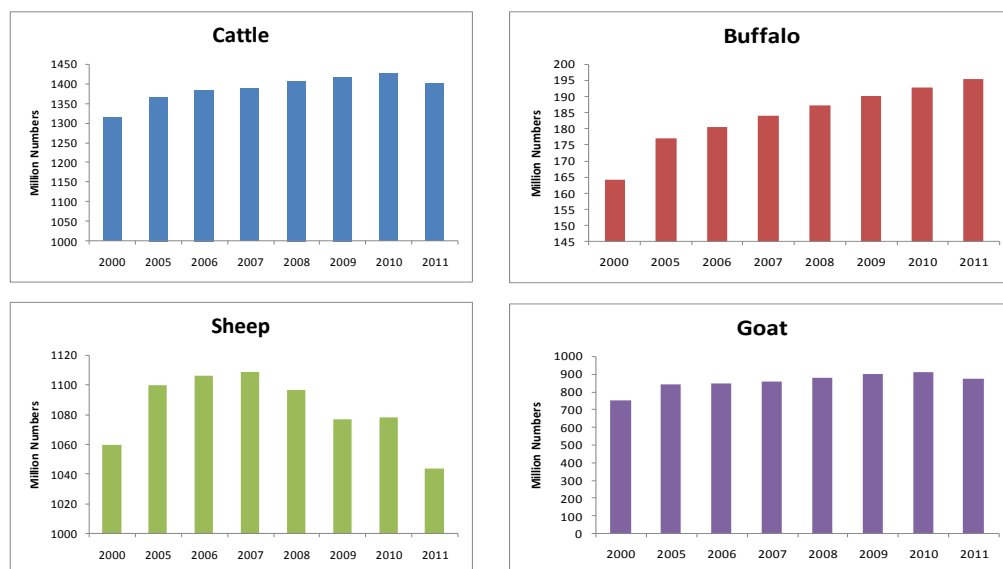
Currently the global milk demand is growing by 15 million tons per year, mostly in developing countries. This increased volume of milk is being produced by small-scale dairy farmers, and millions of jobs per year may be created in primary production. This presents a unique opportunity for building up a sustainable dairy chain that sources milk from smallholder dairy farmers to meet not only the demands of local consumers but also those of the world market. While capitalizing on this opportunity could generate significant wealth in rural areas and provide benefits to all stakeholders involved in the dairy value chain, it calls for a sound dairy development strategy. Since smallholder dairying is considerably affected by factors such as resource access, service delivery, food safety standards as well as national and international subsidies, effective strategies are to be evolved considering all these

factors. If the technological competence of the rural people in dairying is substantially improved, it would not only improve the self employment and also enhance the rural economy and livelihood (Siddiky, 2015).

Dairy Animal Population in South Asia

The Asian region has emerged as a major player in global dairy production and consumption. Aggregate consumption gains in dairy products in Asia over the past decade have exceeded twice the annual global average (FAO, 2014). The rising demand for milk is producing a shift in the dairy sector from subsistence to a market-oriented with higher input needs. Unlike developed countries where the number of dairy farms is decreasing while the number of heads per farm is increasing but in South Asian region, smallholder farmers owns a majority of dairy animals, with an average of 2-10 cows per household and contributes to a major chunk of milk produced in the region (Siddiky, 2015). It is well known fact that South Asia has large population of milch animals, but in most of the countries the dairy production is far below their national requirement due to low productivity of dairy animals (Pal, S.K. and Siddiky, 2011).

The dairy animal population in the world including cattle, buffalo, sheep, goat and camel was 3534.63 million during 2011. Among the dairy animals, cattle dominated with 1399.9 million heads followed by the sheep which was 1043.7 million heads (FAOSTAT, 2013). While the cattle population more or less was increasing at slow pace, the buffalo population increased steadily from 164.11 million in 2000 to 195.3 million in 2011 (Siddiky, 2015). The dynamics of other dairy animal population across the globe is given in figure 1.



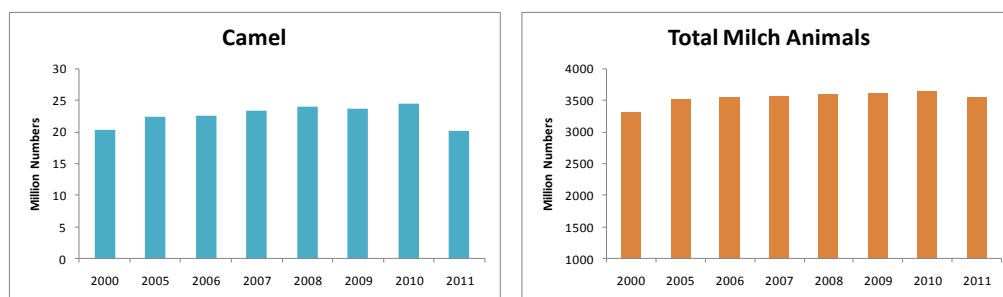
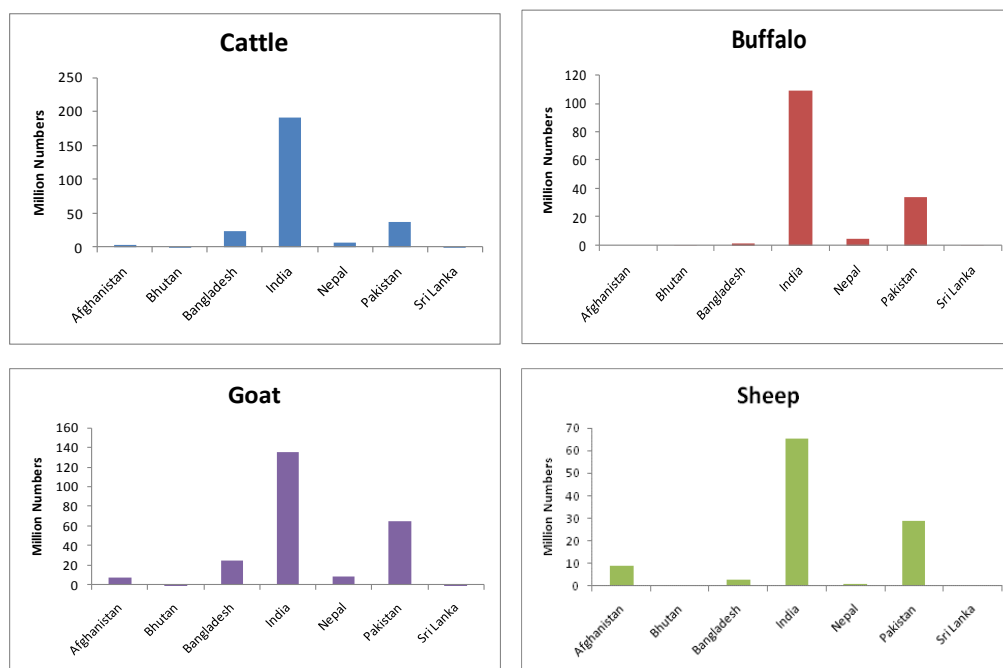


Figure 1. Dynamics of dairy animal population in the world

During 2007, the total dairy animal population in South Asia was 745.11 million that accounts to 21% of the world's dairy animal population. About 25% of world's cattle and buffaloes, 15% of the sheep and goat, and 7% of the camel were present in south Asia (FAOSTAT, 2013; Siddiky, 2015; Abdullah, 2012). Among the south Asian countries, India had huge dairy animal population with 517.08 million heads followed by Pakistan with 138.12 million heads. India accounted for 69.4% of the total dairy animal in the region, while Pakistan accounted for 18.54%. The dairy animal population in Afghanistan, Bangladesh, Bhutan, Nepal and Sri Lanka all accounted for 12.06 % of the total dairy animal population in the SAARC countries (Siddiky, 2015; Pal and Siddiky, 2011). The individual dairy animal populations in south Asia are depicted in figure 2.



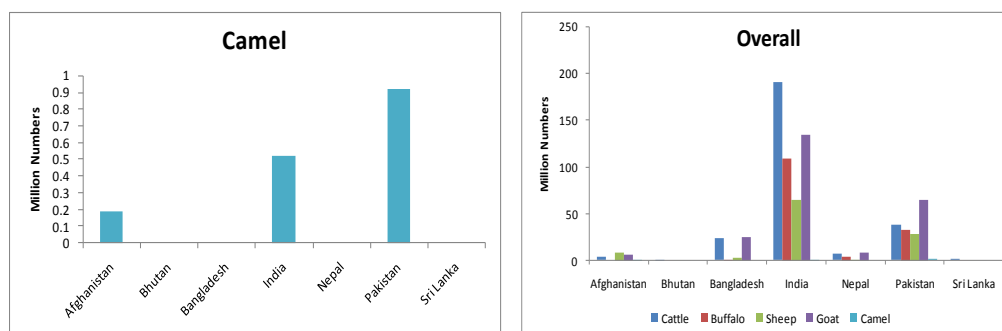


Figure 2. Distribution of individual dairy animal populations in south Asian countries

Growth Rate of Dairy Animal Population in South Asia

For analyzing the Annual Growth Rate (AGR) of dairy animal population, the period was divided into two viz., 1992-2002 and 2003 to afterwards. In Afghanistan, the AGR of cattle was positive during both the periods but the rate was higher during the period 2003-2010. The AGR of goats during both the period were negative while the AGR of sheep was negative during 1996-2002, which turned positive during 2003-2010 (Afghanistan National Livestock Census, 2008). In Bangladesh, the AGR of cattle and buffalo were positive during both the period however the AGR of sheep and goat population was negative during 2002-2010 (Siddiky, and Tareque, 2014). During the period 1992-2002, population of all the dairy animals except sheep showed a positive AGR in Bhutan, however during the period 2003-2010, all dairy animals except goat had negative growth rate (Statistical Year Book of Bhutan, 2012). Both India and Pakistan had positive AGR during both the periods (19th Livestock Census of India, 2012). The AGR of cattle, buffalo and goat was higher during the period 2001-2012 in Pakistan (Pal, S.K. and Siddiky, 2011). In Nepal, except sheep all other dairy animals had a positive growth during both the periods. All the dairy animals had a negative AGR in Sri Lanka during 1992-2002 while the AGR of cattle and buffalo was positive during 2003-2012 (Siddiky, 2015). Over all, the population of dairy animals in the region, during last few decades, showed a positive growth indicating that the population of dairy animal is increasing in a steady pace (Table 1).

Table 1. Annual growth rate of dairy animal population in SAARC Countries

	Afghanistan		Bangladesh		Bhutan		India		Nepal		Pakistan		Sri Lanka	
	1996-2003	2003-2010	1992-2002	2002-2010	1992-2002	2002-2010	1992-2002	2003-2007	1992-2002	2003-2012	1992-2002	2001-2012	1992-2002	2003-2012
Cattle	0.07	3.38	0.20	0.75	0.42	-0.67	0.77	1.50	1.07	0.39	2.62	6.74	-0.02	0.78
Buffalo	--	--	-0.02		9.09	-1.85	1.18	1.52	1.91	2.99	2.85	5.09	-2.38	4.64
Goat	-2.31	-2.53	3.95	-5.78	6.02	7.41	0.69	2.60	2.02	3.62	2.91	4.43	-0.23	-0.73
Sheep	-7.50	4.95	1.96		-2.61	-4.44	1.44	3.28	-0.72	-0.12	-0.87	1.74	-2.87	--

Source: SAARC Dairy Outlook, 2015

Milk Production Scenarios in South Asia

In the last three decades, world milk production has increased by more than 50 percent, from 500 million tons in 1983 to 769 million tons in 2013 (FAO, 2016). Asia is accounted for most of the increase, with output in India, the world's largest milk producing country, by producing 132.4 million tons in 2012-13 (Siddiky and Tareque, 2014). Developing countries are house of two-third of world dairy herd but contribute to one-third of the world milk production (Siddiky, 2015). The most significant milk producers in developed countries are the European Union and the United States. In developing countries India and China rank first and second, respectively in milk production. The growth rate of milk production in India, China, Pakistan, Argentina and Brazil shows future prospects. The world average level of consumption of milk and milk products is 103.6 kg/capita/year and it is expected to increase in both developing and developed countries. Although this region contributes a considerable portion of milk to the world's milk pool, the productivity of animal remains low. In 2011 the world milk production stood around at 730 million tons, of which the south Asian countries contributed 165.4 million tons (22.66%). Of the total milk produced in the region, 42.75% of milk was contributed by cattle while 52.26% was from buffaloes (Siddiky, 2015). Goat and camel contributed to 4.86% and sheep (mainly in Afghanistan) contributed to 0.12% in total milk production (Figure 3). About three-fourth of the milk produced in the region is contributed by India alone. Next major contributor for milk production is Pakistan, which produced about 22.14% of the total milk produced in the region. All the other countries in the region contributed to the remaining part of the total milk produced. The AGR in total milk production in the region between 2006 and 2011 was 4.11%. The milk production was 132.64 million tons, which increased to 165.40 million tons in 2011 with the AGR of 3.89%. The trends in total milk production in south Asian countries are given in figure 4.

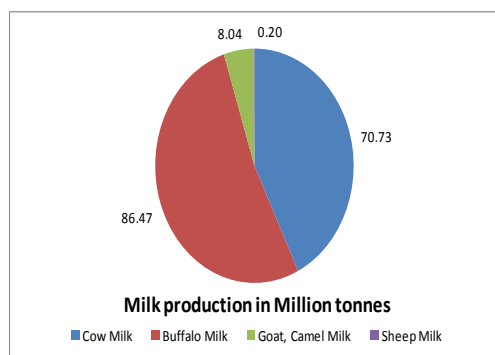


Figure 3. Contribution of different dairy animals in total milk production in the region

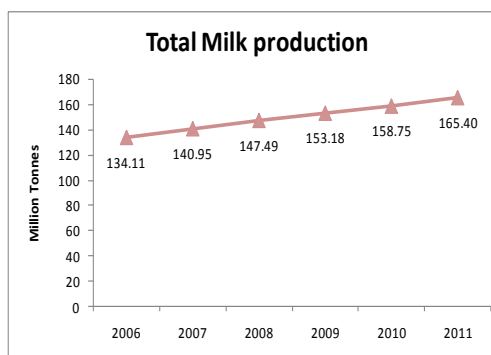


Figure 4. Trend in total milk production in the region

Milk Production Trend in Different South Asian Countries

In 2006, the total milk production in Afghanistan was 1.62 million tons, which increased to 1.72 million tons in 2011. The AGR in milk production between the periods were 1.04%. Bangladesh produced 2.69 million tons of milk during 2006, which increased to 6.97 million in 2015 with the annual growth rate of 17.67% during this period (DLS, 2016). The milk production in Bhutan was 0.042 million tons in 2006 and the total milk production decreased to 0.039 million tons in 2011 with AGR of -1.41%. India witnessed a positive milk production from 2006 to 2011. The milk production increased to 121.8 million tons in 2011 from 97.0 million tons in 2006 (DAHDF, 2012). Nepal also showed a positive trend in milk production. The AGR of milk production during the period from 2006 to 2011 was 3.02%. In Nepal, the total milk production increased from 1.38 million tons in 2006 to 1.63 million tons in 2011 (DLS, 2012). Pakistan produced 31.18 million tons in 2006, which increased to 36.62 million tons in 2011. The AGR in milk production between the periods were 2.91% (MNFSR, 2012). Sri Lanka had a high growth rate in milk production from the period 2006-2011 with the AGR of 5.23%. The total milk production in the country was 0.20 million tons in 2006, which increased to 0.26 million tons in 2011 (DAPH, 2012). All the south Asian countries except Bhutan had a positive growth in milk production (Pal and Siddiky, 2011; Siddiky, 2015).

Productivity of the Dairy Animals in the Region

The data on per cow milk productivity, as indicated by FAO for the year 2011, in component countries is used to analyze and compare the individual animal productivity in South Asia as a whole. The average milk production per cow during 2011 was 627.86 kg/year, while the average milk production per buffalo stood at 1257.96 kg/year. On an average a goat in the region produced 83.45 kg/year in 2011. Among the different south Asian countries, the milk productivity per cow per year was higher in Pakistan followed by India. Lowest milk productivity per cow per year was observed in Bangladesh and Bhutan. The milk productivity per buffalo per year was also higher in Pakistan followed by India. The milk production/cow/year in developed countries like USA, Denmark, Sweden, Finland, The Netherlands etc are above 7500kg whereas in South Asian countries the average milk production/cow/year is less than 1500 kg indicating enough scope to improve the productivity (Siddiky, 2015). There are high variations in per animal milk productivity among the South Asian countries (Figure 5).

Average Milk Production Per Cow

Among the South Asian countries, the average milk production per cow was highest in Pakistan (1229.96 kg/cow/year) followed by India (1191.54 kg/cow/year) in 2011. Sri Lanka stood at third position (683.26 kg/cow/year) among the South Asian countries regarding the average milk production per cow (Siddiky, 2015; Pal and Siddiky, 2011).

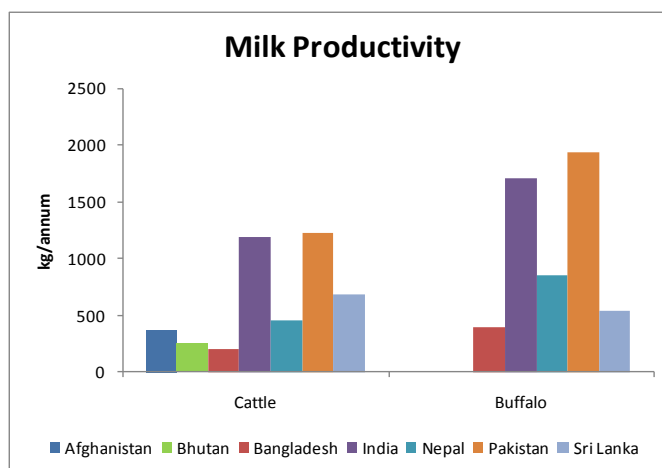


Figure 5: Per animal milk productivity among the South Asian countries

Average Milk Production Per Buffalo

The average milk production per buffalo was highest in Pakistan (1934.96 kg/buffalo/year) followed by India (1700.78 kg/buffalo/year) in 2011. Nepal stood at third position among the South Asian countries regarding the average milk production per buffalo (858.85 kg/buffalo/year).

The average milk production per buffalo was 537.35 kg/buffalo/year in Sri Lanka (Siddiky, 2015; Pal and Siddiky, 2011).

Average Milk Production Per Goat

India stands first regarding the average milk production per goat with the productivity of 150.16 kg/goat/year followed by Pakistan (140.56 kg/goat/year). Bangladesh stood at third position among the SAARC countries regarding the average milk production per goat (80 kg/goat/year). As a whole in the region the average milk production per goat was around 50 kg/goat/year or less (Siddiky, 2015; Pal and Siddiky, 2011).

Dairy Animal Breeds in South Asian Countries

The first report on State of World's Animal Genetic Resources published by the FAO in 2007 indicated that 9% of breeds were extinct and 20% are under risk. Further 36% of the breeds were classified under unknown status. The report indicated that only 35% of world's breeds are enjoying not at risk status, which is an alarming situation for the entire world (FAO, 2007). South Asia harbors a good number of indigenous breeds of dairy animals. These valuable animal genetic resources have been developed over a period of thousands of years through natural selection and human intervention, therefore, well adapted to their respective habitat. However most of the countries, for the genetic improvement of these livestock resources, import

exotic germplasm leading to dilution of local breeds. The adaptation of temperate exotic breeds needs much more sophisticated and scientific management under tropical climates. There has been a change in the utility pattern of these genetic resources which has created a stiffer competition to the local breeds for their survival. Therefore, genetic erosion is a serious concern and a number of local breeds are at the risk of extinction. The local breeds of cattle and buffalo are given in table 2 & 3.

Table 2. List of local cattle breeds in the South Asian Countries

Country	Breeds	No. of breeds
Afghanistan	Afghan, Kandahari, Konari, Kunari, Shakhansurri, Sistani, Watani	7
Bangladesh	Red Chittagong, Faridpur, Madaripur, Munshiganj, North Bengal Grey and Pabna	6
Bhutan	Bajo, Jaba, Jatsa, Langu, Nagamee	5
India	Hariana, Hissar, Kangayam, Khillari, Siri, Sunandini, Gir, Ongole, Red Sindhi, Sahiwal, Tharparkar, Alambadi, Amritmahal, Bachur, Bargur, Binjharपुर, Brownsind, Brown Swiss, Burmese Gaur, Cutchi; Dangi, Deoni, Devarakota, Devni, Frieswal, Gangatiri, Gaolao, Gayal, Ghumsuri, Goomsur, Gujamavu, Hallikar, Jellicut, Jersind, Kankrej, Kappiliyan, Karan fries, Karan swiss, Kenkatha, Khamala, Khariar, Khasi, Kherigarh, Krishna valley, Krishnagiri, Kumauni, Ladakhi, Malnad gidda, Malvi, Mampati, Manapari, Mewati, Mhaswad, Motu, Nagami, Nagori, Nakali, Nimari, Ponwar, Pullikulam, Punganur, Purnea, Ramgarhi, Rathi, Red Kandhari, Sanchori, Shahabadi, Son valley, Tarai, Taylor, Thillari, Tho Tho, Umblacherry, Vechur, Zosial, Arunachali and Bengali	78
Nepal	Lulu, Achhami, Khalia, Siri, Yak, Terai and Pahadi	7
Pakistan	Red Sindhi, Sahiwal, Tharparkar, Achai, Bhagnari, Cholistani, Dajjal Desi, Dhanni, Gabarali, Haryana, Hisar, Kankrej, Lohani, Rojhan	
Sri Lanka	Hatton, Kinniya, Sinhala and Thamankaduwa	4

Table 3. List of local buffalo breeds in the South Asian Countries

Country	Breeds	No. of breeds
Bangladesh	Native buffaloes (Non descriptive indigenous type)	
India	Murrah, Mehsana, Nili-Ravi, Surti, Tarai, Assami, Banni, Bhadawari, Chilika, Godavari, Jerangi, Kalahandi, Manda, Marathwada, Nagpuri, Pandharpuri, Sambalpuri, Toda, Jafarabadi	19
Nepal	Lime, Parkote and Gaddi	3
Pakistan	Azi Kheli, Kundhi, Nili, Ravi and Nili Ravi	5
Sri Lanka	Lanka, Mannar and Tamankaduwa	3

Source: Farm Animal Genetic Resources in SAARC Countries, 2014

Dairy Animal Production Systems

Smallholder dairying

The smallholder and landless farmers together constitute over 75 per cent of regional livestock resources. Smallholders obtain nearly half of their income from livestock. Hence a rapid growth in livestock production in the region has the potential to contribute to poverty reduction. Three major milk production systems are rural subsistence, rural market oriented and peri urban plus commercial dairying. Dairy development based on smallholder production system has made an excellent contribution to rural development in both developing and developed countries (Joe Phelan, 2007). In addition to providing employment and income, it has improved the living standards in rural areas. Simulation of increased productivity, better farm financing and improved milk marketing through pro poor dairy development policies have shown that landless rural dairy farmers have the potential to reduce the cost of milk production to the level of large farms and hence theoretically have the potential to maintain a profitable dairy enterprise, especially women. The main risks of dairying by rural landless farmers are not having an animal in milk in any one year, the death of a lactating animal and having to meet the high cost of feed, fodder and poor accessibility to health coverage. Occurrence of any of these events can lead to a reduction of the already low household income by 50% and would probably force the family to abandon the dairy enterprise. Reduction of production risks faced by rural landless dairy farmers requires the availability of improved breeding services, targeted preventive animal health care, better feeding strategies and easy access to formal credit facilities (Torsten et al., 2003).

Periurban Milk Production

Periurban dairying is essentially a market oriented enterprise. Easy access to market prompts smallholders to produce more milk for sale and earn cash income. The number of periurban dairy farms in the region has increased substantially in the recent years due to rapid urbanization and increase in demand for milk. Periurban dairying provides employment and income to the unemployed and low income urban families. Periurban dairying, in general has the technical constraint of limited milk production potential of local breeds, seasonal quantitative and qualitative feed / fodder shortage, poor management and health care. A weak infrastructure base and poor support services have been shown to adversely affect the economic returns of periurban dairy units. Poor roads, unreliable power supply, inefficient cooling and processing capacity can discourage production. Services in terms of credit facility, health coverage, input supply and distribution, technical advisory services are of crucial importance to the successful management of periurban dairy units. In periurban dairying feed accounts for more than 2/3rd of the operational cost as the animals are stall fed with purchased feed and fodder. There is a need to suitably address these issues. In view of the growing contribution of this sector to meet the specialized food needs of growing urban population, periurban livestock production

needs to be recognized as an important component of the national livestock industry. Adequate government investment in infrastructure and incentive to private investment will be the pre requisite to promote this sector.

Dairying through cooperatives

The merit of the cooperative ideology is the coordination and balancing of the fundamental principles of equality, democratic control and equality in institutions, and practices to maximize social welfare. Milk production system in the region is entirely the domain of farmer. Milk production is largely a subsidiary activity to agriculture in rural areas in contrast with organized dairying in western countries. Farmers and landless labourers mostly maintain one to three dairy animals. As a result, small quantities of milk are produced widely spread over the region.

The concept of milk co-operatives has been well structured with one village or a cluster of villages forming the primary cooperative. A group of many primary cooperatives forms a union, which can be a district, region or milk shed area. The third level is the unions joining up to form a Federation at State or National level. The Federation has the power to decide on policies of pricing, human resource, exports/imports, subsidies and credit facility. In South Asian countries the dairy cooperatives are the major means of promoting dairy husbandry and proved to be a strong viable economic institution and a way for improving the living condition of the impoverished rural population. They provide farmers with an organizational support at grass root level in planning, decision-making and scheme implementation. Besides this, the cooperatives also provide services related to animal health, insemination, feed, fodder seeds, fertilizers, credit, training and education. With their vast network and deeper penetration and assured market for the producer they have become a popular sustainable model. There is little success of the cooperative models in the region.

Strengths, Weaknesses, Opportunities and Threats (SWOT) for Dairy Development in South Asia

The SWOT analysis (Siddiky, 2015) of the current dairy scenario of the region would reveal the following:

Strengths

- Constant and sustainable growth (high milk production with high growth rate) despite limited investment from public and private sector
- Mega biodiversity and large bovine population - The vast dairy animal population could prove to be a vital asset for the region. Unlike many other natural resources which may deplete over the years, a sustainable livestock production system will continue to propel the economy.
- Variable agro-climatic conditions and diverse dairy animal production systems (Zero input–low output, low input–moderate output, intensive input–high output)

- Low production cost–Dairy farming in the region thrives largely on crop residues and agricultural byproducts keeping the input costs low. Labor cost is also fairly low making the industry fairly cost competitive. The cost of production of 100 Kg milk is around 20 USD in India compared to 68 USD in Japan and 58 USD in Canada.
- Male are still used for drought agriculture (considerable proportion of agricultural land are cultivated by animals)
- As the milk productivity of dairy animals is low, there is a vast scope for improvement of the milk production and consequently increased marketable surplus of milk for processing.
- Very big domestic market-purchasing power of the consumers is on the upswing with growing economy & continually increasing population of middle class.
- Milk consumption is regular part of the dietary programme irrespective of the region and hence demand is likely to rise continuously.
- Large number of dairy plants in public and cooperative sectors besides several others in the private sector is coming up.
- Vast pool of highly trained and qualified technical manpower is available at least in some countries at all levels to support R&D as well as industry operations.

Weaknesses

- Though cross breeding programmes have significantly improved animal productivity, milk production system in many parts of the region is still largely dominated by low yielding animals.
- Wide gap between availability and requirement of progeny tested proven dairy sires
- Shortage of feed and fodder; continuous reduction in area under fodder production
- Poor condition of roads and erratic power supply remain a major challenge for procurement and supply of good quality raw milk. Furthermore, raw milk collection systems in certain parts of the region remain fairly underdeveloped.
- Maintenance of cold chain is still a major handicap. For organized marketing of milk, the milk produced is required to be transported to nearby processing plant which incurs cold storage and transportation costs which are quite high.
- Majority of producers is unaware about scientific dairy farming, clean milk production and value chain.
- Absence of comprehensive and reliable milk production data, impact assessment studies are almost non-existent, investments in dairy research is also not commensurate with returns and potential.

Opportunities

- Technology driven production enhancement in low producing animals
- Expanding market can create of enormous job and self employment opportunities.
- Economy is growing in the region, consequently, the investment opportunities are also increasing continually.
- Demand for dairy products is income elastic. Continued rise in middle class population will see shift in the consumption pattern in favour of value added products besides the growth in demand for liquid milk.
- Untapped indigenous milk products market - Greatly improved export potential for indigenous as well as western milk products.
- Value addition in raw milk; functional food - Opportunities for utilization of byproducts of the dairy industry for manufacturing value added products.
- Public private partnership

Threats

- Danger of extinction of valuable bio-resources - Excessive grazing pressure on marginal and small community lands has resulted in almost complete degradation of land and Indiscriminate crossbreeding for raising milk productivity could lead to disappearance of valuable indigenous breeds.
- Developed countries are providing huge subsidy & incentive for export of milk and milk products
- Organized dairy industry handles very less percentage of the milk produced. Cost effective technologies, mechanization, and quality control measures are seldom exercised in unorganized sector and remain as key issues.
- Middlemen still control a very large proportion of the milk procurement. Serious efforts need to be taken to eliminate them from the supply chain.

Strategies for Boosting Dairy Production

Now it is well understood that “Animal number driven” dairying may not fulfill the demand for milk and milk products in the region and the dairying need to reorient towards “technology driven mode”. Since dairying is socially and culturally intermingled with farming community and offer livelihood and nutritional security to a major mass of population, this transformation in dairying cannot be made overnight. However, it is high time to develop policies and source the technological options for smooth transition of dairying towards commercial mode while protecting the interests of smallholders. Some of the major issues impeding the dairy animal productivity and technological options to overcome those obstacles are genetic improvement of the dairy animals, conservation and utilization of potential dairy

breeds, genetic improvement of non-descriptive cattle and buffaloes, buy back policy for improving the availability of breeding bulls, enhancing the percolation of artificial breeding facilities, promoting buffaloes as dairy animals, to make availability of feed and fodders of the dairy animals, optimizing reproductive efficiency, milk processing and value addition (Siddiky, 2015; Pal and Siddiky, 2011).

CONCLUSION

The dairy sector in the region is characterized by small-scale, scattered and unorganized dairy animal holders; low productivity; inadequate and inappropriate animal feeding and health care; lack of assured year-round remunerative producer price for milk; inadequate basic infrastructure for provision of production inputs and services; inadequate basic infrastructure for procurement, transportation, processing and marketing of milk; and lack of professional management. Low productivity of dairy animals is a serious constraint to dairy development in the region. The productivity of dairy animals could be increased by crossbreeding the low yielding nondescript cows with high yielding selected indigenous purebreds or suitable exotic breeds in a phased manner. The breeding policy should not only focus on milk yield but should also provide for the production of good quality breeding bulls. Upgrading of nondescript indigenous buffalo through selective breeding with high yielding purebreds, such as Murrah, Nili Ravi, should be given high priority in all areas where buffalo are well adapted to the agro-climatic conditions.

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Short Communication

**INFLUENCE OF RESIDUAL LIME AND PLANT GROWTH
REGULATOR (NAA) ON THE MORPHOLOGICAL AND
PHYSIO-CHEMICAL TRAITS OF AROMATIC RICE CV.
KATARIBHOG**

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Aromatic rice possess high potential to draw attention rice consumer of the world for its aroma, fragrance, grain morphology, quality and other desirable traits. The production of aromatic fine rice is profitable due to its prices up to 10 times more than common rice on international market (Chaudary et al., 2001). In Bangladesh, a number of fine rice cultivars are grown by the farmers specially Kataribhog, Chinisagar, Badshabhog, Kalizira, Tulsimala, Dulabhog, Basmati, Banglamoti (BRRI dhan50), BRRI dhan34, BRRI dhan37 and BRRI dhan38. Liming is a normal agronomic practice to manage acid sulfate soils for crop production. In Malaysia, some areas of acid sulfate soils have been reclaimed for rice cultivation using lime. In the acid sulfate soils of the Muda Agricultural Development Authority (MADA) granary areas in Kedah-Perlis coastal plains (northwest coast of Peninsular Malaysia), for instance, rice yield improved significantly after applying 2.5 tons of ground magnesium limestone (GML) ha⁻¹. However, over liming may reduce crop yields due to lime induced P and micronutrient deficiencies (Fageria, 1984). Plant growth regulators (PGRs) are organic compounds, other than nutrients that modify plant physiological processes and also called biostimulants or bioinhibitors that act inside plant cells to stimulate or inhibit specific enzymes or enzyme systems and thus regulate plant metabolism. Recently, there has been global realization of the important role of PGRs in agriculture for better growth and yield of crop (Prasad and Paudel, 1994). These (PGRs) are being used as an aid to enhance yield. Naphthalene Acetic Acid (NAA) is one of the growth promoting hormones, which may play significant role to change growth characters and yield in BRRI dhan28.

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The PGRs play vital roles in coordination of many growth and behavioral processes in rice, which regulates the amount, type and direction of plant growth (Anjum et al., 2011). The use PGRs, as NAA, GA₃ or their compounds, is becoming popular to ensure efficient production. Remarkable accomplishments of PGRs such as manipulating plant growth and crop yield have been actualized in recent years (Morinaka et al., 2006; Zvi and Eduardo, 2011). The PGRs modify growth and development in various ways under different growth conditions. NAA and GA₃ is responsible for stimulating the production of mRNA molecules in the cells, which in turn improves the chances of fast growth (Olszewki and Gubler, 2002; Emongor, 2007). Recently, the scientists of Bangladesh are being advised to use PGRs to get higher rice production. But the research on examining the combination effect of NAA and lime application in acid piedmont soil for better rice yield is still in initial stage. Yield is the cumulative effect of a variety with its inherent characteristics, management practices and the environment in which it is grown. Variety is one of the most important factors for increasing yield. Thus a study was undertaken with the objectives of identifying the suitable dose of NAA in order to achieve both quantitative and qualitative effect on aromatic rice production. Work on NAA with lime is limited to improve the rice yield in Northwest Bangladesh. Studies in other countries of the world although provides useful information, that cannot be recommended or practiced without trial in our local condition. Therefore, more researches or trials are necessary to investigate the efficacy of NAA on aromatic rice. Thus, the present study was carried out - i) to study the effect of NAA and residual lime effect on growth characteristics of Kataribhog. ii) to know the flag leaf composition of aromatic rice and the yield of Kataribhog.

The study was conducted at agricultural farm, Department of Agricultural Chemistry, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur during February to May, 2011. The rice variety Kataribhog rice was used as a test plant. Lime were applied (see modification in abstract) @ T₁ = 0 t ha⁻¹ (Control), T₂ = 1.0 t ha⁻¹, T₃ = 1.5 t ha⁻¹, T₄ = 2 t ha⁻¹, T₅ = 2.5 t ha⁻¹ used in previous crop at summer mungbean cv. BARImung-7. Treatment @ H₀ = 0 ppm NAA (Control), H₁ = 100 ppm NAA, H₂ = 150 ppm NAA and H₃ = 200 ppm NAA spraying at vegetative and pre-flowering stages while water was used as control (H₀). The experiment was laid out in a randomized complete block design with three replications. Thirty five days old seedlings were transplanted in February, 2012 using three seedlings hill⁻¹. Recommended dose of triple super phosphate (180 kg ha⁻¹), muriate of potash (100 kg ha⁻¹), gypsum (20 kg ha⁻¹) and zinc (7.5 kg ha⁻¹) were incorporated to soil at final land preparation. Urea (215 kg ha⁻¹) was applied in three equal splits at 20, 35 and 55 DAT. Data on yield and yield contributing parameters, plant height, flag leaf length, flag leaf width, leaf numbers plant⁻¹, tiller numbers hill⁻¹ were recorded at maturity, while the panicle numbers hill⁻¹, filled grains panicle⁻¹, unfilled grains panicle⁻¹, 1000-grain weight, grain length, grain width, grain yield ha⁻¹, straw yield ha⁻¹, harvest index were recorded after harvest (synchronized all

parameter). Chemical compositions of flag leaves under different treatments using PGR in the lime tested plots were estimated. The K, Ca, Mg, Zn, Cu and Fe content of flag leaves were estimated as per standard methods. Leaf chlorophyll-a, chlorophyll-b and total carotenoid were also monitored from green flag leaves. The data were statistically analyzed to compare treatment means using the MSTATC computer software developed, (Russell, 1986). If the treatments were significant the differences between pairs of means were compared by LSD followed by Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1984).

Plant height (cm) at maturity stage in table 1 indicated that combined effect of different levels of plant growth regulator (PGR) and residual lime had significant stimulatory effect. Maximum height (138.70 cm) was obtained from the application of both 2 t ha⁻¹ residual lime and 100 ppm NAA while shortest plant (107.00) cm was recorded from T₁ × H₂ and T₂ × H₀ treatments respectively. It seems to be due to intact cells elongation. Watanabe and Saigusa, 2004 stated that plant height was significantly increased by the application of 50 ppm ethephon, 100 ppm GA₃ alone or in combination over that of control. The number of tillers is one of the most important factors for yield. Tiller production especially the number of effective tillers is the potential factor for yield component. Both the number of tillers and effective tillers hill⁻¹ was significantly increased with the concentrations of 100 ppm NAA and 2 t ha⁻¹ residual limes. The highest number of tillers and effective tillers hill⁻¹ were 14.67 and 12.00 respectively while the lowest tiller numbers and effective tillers (10.00) were recorded from 200 ppm NAA and no residual lime application (Table 1). Similar trend was reported through Miyodo application by (Islam, 2007). Leaf numbers, flag leaf length and flag leaf breadth are the most important growth characters of plant on which crop yield depends. The number of leaves was highest at maximum tillering stage of growth and decreased thereafter (Islam, 2008). The decreased in leaf number at later stages of growth might be due to senescence or drying up of leaves. Maximum number of leaves (7.00) was obtained from 100 ppm NAA with 1.5 t ha⁻¹ residual lime application. Both NAA and residual lime markedly increased flag leaf length and breadth (Table 1). Plant hormones play important role in regulating the leaf numbers and leaf area (Liu et al., 2012).

The panicle length and number of filled grains panicle⁻¹ as well as number of unfilled grains panicle⁻¹ are very important parameters to evaluate the yield performance of a variety in response to a specific treatment. Table 2 showed that the highest values of panicle length, filled grains panicle⁻¹, unfilled grains panicle⁻¹, grain yield and straw yield (28.00 cm, 139.30, 39.67, 1.63 t ha⁻¹ and 7.00 t ha⁻¹ respectively) where as the lowest values of panicle length, filled grains panicle⁻¹, unfilled grains panicle⁻¹, grain yield and straw yield (18.00 cm, 105.7, 18.00, 0.80 t ha⁻¹ and 4.25 t ha⁻¹, respectively). Panicle length, filled grains and grain yield differed significantly and increased by the application of both PGR and lime (Table 2). Maximum grain yield was observed due to application of 100 ppm NAA and 2 t ha⁻¹ residual effect of lime

Table 1. Effect of PGR and residual lime on morphological and growth characters of Kataribhog rice

Treatments	Plant height (cm)	Tiller numbers	Effective tiller numbers	Leaf numbers	Flag leaf length (cm)	Flag leaf width (cm)
T ₁ ×H ₀	130.70d	11.33ef	9.00de	5.33d	30.17cd	1.06a
T ₁ ×H ₁	131.70c	12.67cd	10.67bc	6.67ab	29.80cde	1.03a
T ₁ ×H ₂	107.00n	10.33hi	7.67g	6.00c	35.67a	0.96a
T ₁ ×H ₃	107.70m	10.00i	6.67i	6.00c	27.83f	1.00a
T ₂ ×H ₀	107.00n	10.67gh	7.67g	6.67ab	27.33f	0.93a
T ₂ ×H ₁	135.00b	12.67cd	9.00de	6.33bc	30.00cde	1.10a
T ₂ ×H ₂	120.30i	11.33ef	7.67g	6.33bc	30.27c	1.16a
T ₂ ×H ₃	113.00j	12.33d	9.00de	6.67ab	23.33h	0.83a
T ₃ ×H ₀	110.70k	10.33hi	7.67g	6.67ab	29.67cde	1.16a
T ₃ ×H ₁	129.30e	13.67b	10.33c	7.00a	33.33b	1.10a
T ₃ ×H ₂	129.00e	13.00c	11.67a	6.33bc	32.57b	1.16a
T ₃ ×H ₃	121.70h	12.33d	9.00de	6.33bc	29.17de	1.03a
T ₄ ×H ₀	126.30g	11.00fg	7.67g	6.67ab	27.33f	1.00a
T ₄ ×H ₁	138.70a	14.67a	12.00a	6.67ab	29.83cde	1.13a
T ₄ ×H ₂	131.90c	14.00b	11.00b	6.33bc	26.93f	1.06a
T ₄ ×H ₃	131.70c	11.67e	8.33f	6.67ab	30.33c	0.90a
T ₅ ×H ₀	107.70m	11.67e	7.00hi	6.00c	30.27c	1.16a
T ₅ ×H ₁	127.70f	11.67e	7.33gh	6.33bc	30.00cde	1.00a
T ₅ ×H ₂	109.30l	12.67cd	8.67ef	6.67ab	25.23g	1.03a
T ₅ ×H ₃	128.00f	12.33d	9.33d	6.67ab	29.00e	1.13a
LSD (5%)	0.5	.35	.33	0.3	0.20	0.17

Mean followed by same letter do not differ significantly at 5% level.

Here, T₁ = 0 t ha⁻¹ (Control), T₂ = 1.0 t ha⁻¹, T₃ = 1.5 t ha⁻¹, T₄ = 2 t ha⁻¹, T₅ = 2.5 t ha⁻¹ and H₀ = 0 ppm NAA (Control), H₁ = 100 ppm NAA, H₂ = 150 ppm NAA, H₃ = 200 ppm NAA

application. (Gurmani et al., 2006), they stated that ABA, BA and CCC plant growth regulators increased grain yield of rice. The finding is also similar with Pandey et al., 2001, whom reported that IAA @ 50 ppm produced significantly maximum grain yield plant⁻¹, 1000-grain weight and yield kg ha⁻¹. The present result also agrees with the findings of previous studies (Andreevska et al., 2004; Islam et al., 2008). Harvest index is the ratio of economic yield and biological yield, and the ultimate partitioning of dry matter between grain and vegetative parts is indicated by

HI, the economic yield of rice is its grain, biological yield of a crop is the TDM at final harvest (Daval and Hamblin, 1976). Table 2 showed that, the highest percentage of Harvest Index (HI) was found at $T_3 \times H_1$ (21.22%) and lowest was at $T_2 \times H_2$ (13.07%). This result confirmed the results of previous studies (Islam, 2007; Islam et al., 2008).

Table 2. Effect of PGR and residual lime on yield attributes and yields of Kataribhog rice

Treatments	Panicle length (cm)	Filled grain panicle ⁻¹	Unfilled grain panicle ⁻¹	Grain yield (t ha ⁻¹)	Straw yield (t ha ⁻¹)	Harvest index (%)
$T_1 \times H_0$	23.33ghi	129.00f	24.33f	0.89cd	7.17a	18.06e
$T_1 \times H_1$	24.30ef	134.30d	35.00b	1.46abc	5.87bc	19.92bc
$T_1 \times H_2$	19.00l	118.00k	21.67i	1.38abcd	6.92a	16.63g
$T_1 \times H_3$	18.00m	121.00j	25.67e	0.94bcd	4.25f	18.11de
$T_2 \times H_0$	22.00k	116.70l	25.33e	0.80d	4.92e	13.99i
$T_2 \times H_1$	27.00b	138.30b	21.33ij	1.43abc	6.25b	18.62d
$T_2 \times H_2$	25.67c	122.00i	32.00c	0.94bcd	6.25b	13.07j
$T_2 \times H_3$	23.00hij	137.70b	20.67j	1.38abcd	5.68bc	19.55bc
$T_3 \times H_0$	22.67ijk	125.00h	23.33gh	0.88cd	5.68bc	13.41j
$T_3 \times H_1$	26.00c	130.30e	24.33f	1.53ab	5.68bc	21.22a
$T_3 \times H_2$	25.33cd	138.00b	30.33d	1.44abc	6.92a	17.22f
$T_3 \times H_3$	23.33ghi	128.00g	23.00h	1.40abcd	5.68bc	19.77bc
$T_4 \times H_0$	27.67ab	102.30o	18.00l	1.38abcd	5.50cd	20.06b
$T_4 \times H_1$	27.00b	139.30a	18.33kl	1.63a	6.15b	20.95a
$T_4 \times H_2$	28.00a	136.70c	21.67i	1.13abcd	5.68bc	16.59g
$T_4 \times H_3$	25.33cd	122.00i	39.67a	1.40abcd	5.68bc	19.77bc
$T_5 \times H_0$	24.67de	121.30ij	19.00k	1.37abcd	6.25b	19.35c
$T_5 \times H_1$	22.33jk	110.30m	22.67h	1.08abcd	5.00de	17.76e
$T_5 \times H_2$	23.67fgh	105.70n	24.00fg	1.13abcd	5.68bc	16.59g
$T_5 \times H_3$	24.00efg	128.30fg	32.33c	1.29abcd	7.00a	15.56h
LSD (5%)	0.90	1.30	2.67	0.30	0.60	0.52

Mean followed by same letter do not differ significantly at 5% level

Chlorophylls are the most widely distributed plant pigments responsible for the characteristic green color of fruit and vegetables (Almela, 2000). A significant variation was found in chlorophyll-a, chlorophyll-b, total chlorophyll and total carotenoid content through combined effect of PGR and lime (Table 3). The highest amount of chlorophyll-a content of flag leaf was observed in $T_4 \times H_1$ (20.60 mg g⁻¹ FW) followed by $T_1 \times H_1$ (20.32 mg g⁻¹ FW) and $T_4 \times H_3$ (20.13 mg g⁻¹ FW)

combination while the lowest chlorophyll-a content of flag leaf was observed in $T_4 \times H_2$ (16.49 mg g⁻¹ FW). The highest value of chlorophyll-b content were found in both $T_2 \times H_2$ and $T_4 \times H_1$ (11.78 mg g⁻¹ FW) and while the lowest chlorophyll-b content was found in $T_5 \times H_1$ (5.69 mg g⁻¹ FW). The highest total chlorophyll was obtained in $T_4 \times H_1$ (32.38 mg g⁻¹ FW) followed by $T_1 \times H_1$ (31.40 mg g⁻¹ FW) while the lowest total chlorophyll content was happen in $T_5 \times H_1$ (24.12 mg g⁻¹ FW) treatment combination (Table 3). The highest mean value of total carotenoid content was found in $T_4 \times H_0$ (2.15 mg g⁻¹ FW) while the lowest carotenoid content was obtained in $T_5 \times H_2$ (0.76 mg g⁻¹ FW).

Table 3. Effect of PGR and residual lime on Chlorophyll content (mg g⁻¹) in flag leaf of Kataribhog rice

Treatments	Chlorophyll-a	Chlorophyll-b	Total Chlorophyll	Total carotenoid
$T_1 \times H_0$	17.45f	9.26gh	26.71j	1.89abc
$T_1 \times H_1$	20.32a	11.08bc	31.40b	1.41bcde
$T_1 \times H_2$	19.34bc	11.54ab	30.88bcd	1.18def
$T_1 \times H_3$	17.42fg	9.92ef	27.34i	1.17def
$T_2 \times H_0$	20.58a	10.69cd	31.27bc	1.48bcde
$T_2 \times H_1$	19.39bc	10.61cd	30.00e	1.50bcde
$T_2 \times H_2$	17.99ef	11.78a	29.77e	1.10ef
$T_2 \times H_3$	16.86h	9.08h	25.94k	1.28def
$T_3 \times H_0$	19.06bc	9.72efg	28.78f	1.49bcde
$T_3 \times H_1$	19.52b	11.32ab	30.84bcd	1.73abcd
$T_3 \times H_2$	17.50f	7.30i	24.80l	2.01ab
$T_3 \times H_3$	19.39bc	11.27ab	30.66d	0.96ef
$T_4 \times H_0$	18.82cd	7.10i	25.92k	2.15a
$T_4 \times H_1$	20.60a	11.78a	32.00a	0.91ef
$T_4 \times H_2$	16.49h	9.56fgh	26.05k	0.96ef
$T_4 \times H_3$	20.13a	10.61cd	30.74cd	1.27def
$T_5 \times H_0$	18.50de	9.07h	27.57hi	1.50bcde
$T_5 \times H_1$	18.43de	5.69j	24.12m	0.98ef
$T_5 \times H_2$	16.90 gh	11.36ab	28.26fg	0.76f
$T_5 \times H_3$	17.80f	10.15de	27.95gh	1.29cdef
LSD (5%)	0.52	0.41	0.21	0.30

Mean followed by same letter do not differ significantly at 5% level.

Table 4 showed there is no significant variation in K content in flag leaves. The highest K content were found in T₂×H₃ and T₄×H₃ (0.59 mg l⁻¹) while the lowest was obtained in T₁×H₃ (0.47 mg l⁻¹). The residual effect of 2 ton ha⁻¹ lime along with 200 ppm NAA helps to uptake highest rate of K in flag leaves. The status of K improved the rice straw quality. The variation of K content in different varieties was variable (Sarwar et al., 2010). A significant variation was found in flag leaves Ca content in relation to application of various levels of residual lime and NAA (Table 4). Maximum Ca content was found in T₃×H₁ (131 mg l⁻¹) while the lowest was found in T₁ × H₃ (100 mg l⁻¹). This result revealed that 100 ppm NAA may have positive impact on Ca content in rice leaf. Secondary nutrient plays important roles in

Table 4. Effect of PGR and residual lime on leaf nutrient composition (mg g⁻¹) in flag leaf of Kataribhog rice

Treatments	K	Ca	Mg	Zn	Cu	Fe
T ₁ ×H ₀	0.50	111g	22.20k	0.15	0.18	2.56a
T ₁ ×H ₁	0.57	113f	29.20de	0.14	0.24	2.04abc
T ₁ ×H ₂	0.57	106j	29.00e	0.09	0.26	2.02abc
T ₁ ×H ₃	0.47	100n	20.70l	0.02	0.17	1.08e
T ₂ ×H ₀	0.48	108h	21.00l	0.16	0.17	1.97abc
T ₂ ×H ₁	0.56	115d	22.50k	0.14	0.19	1.87bc
T ₂ ×H ₂	0.53	102m	25.60h	0.09	0.24	1.69bcd
T ₂ ×H ₃	0.59	100n	25.60h	0.05	0.15	1.97abc
T ₃ ×H ₀	0.46	106j	25.50h	0.05	0.21	1.71bcd
T ₃ ×H ₁	0.58	131a	33.60a	0.27	0.26	2.49a
T ₃ ×H ₂	0.57	124b	31.10b	0.18	0.34	2.29ab
T ₃ ×H ₃	0.51	120c	29.60d	0.14	0.31	2.54a
T ₄ ×H ₀	0.56	106j	29.55d	0.03	0.23	1.83bc
T ₄ ×H ₁	0.57	114e	31.50b	0.14	0.20	1.88bc
T ₄ ×H ₂	0.53	107i	28.10f	0.32	0.12	1.47cde
T ₄ ×H ₃	0.59	103l	23.40j	0.02	0.16	1.21de
T ₅ ×H ₀	0.52	104k	24.70 i	0.16	0.07	1.07e
T ₅ ×H ₁	0.55	103l	26.50g	0.15	0.13	1.44cde
T ₅ ×H ₂	0.57	108h	26.80g	0.03	0.12	1.79bcd
T ₅ ×H ₃	0.50	115d	30.50c	0.21	0.14	2.22ab
LSD (5%)	ns	2.00	0.30	ns	ns	0.38

Mean followed by same letter do not differ significantly at 5% level.

plant life. Calcium is an essential part of cell structure and plays its role in cell division (Tandon, 2000). The total Mg uptake differed significantly with respect to application of different levels of lime and PGR applied in combination (Table 4). The highest Mg content was recorded in T₃×H₁ (33.60 mg l⁻¹) followed by T₄×H₁ (31.1 mg l⁻¹). On the other hand, the lowest was recorded in T₁×H₃ (20.70mg l⁻¹). Magnesium is an integral part of chlorophyll and thus, linked with photosynthesis. It plays an important role in energy transfer processes in plants (Tandon, 2000).

There is no significant variation in Zn and iron content in flag leaves (Table 4). Similar result was obtained by (Tomasevei and Anicic, 2010). The iron content of the flag leaf of Kataribhog rice cultivar also showed a significant variation in relation to NAA and residual effect of lime (Table 2). The highest amount of flag leaf iron content was found in T₁×H₀ (2.56 mg l⁻¹) followed by T₃×H₃ (2.54 mg l⁻¹) treatments. On the other hand, the lowest was found in T₃×H₀ (1.07 mg l⁻¹).

From the above findings, it can be concluded that Naphthalene Acetic Acid and residual effect of lime significantly affected the yield as well as yield contributing parameters of Kataribhog rice, especially at 100 ppm NAA and 2 t ha⁻¹ of lime application.

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screened by the managing editor/ associate editor solely or with the help of members of editorial board. If the article is found to be in order as per the 'Guidelines' and there is adequate original information, it is sent to an expert for peer review. On the contrary, the articles that contain inadequate information or are not conform to 'Guidelines' are rejected as such or returned to the author for revision. After the article has been examined by the reviewer or followed by over viewer, it is also suitably edited by a relevant member of Editorial Board. Both reviewer/ over viewer and editorial board comments along with the manuscript are passed on to an author, who resubmits it after incorporating suggestions and comments. The thoroughly revised article will be checked by the editorial board and managing editor in the light of review/ over viewer comments whether it would be worth for publishing or not. The articles accepted for publication by editorial team are checked by a professional editor for English language, uniformity and any other ambiguity before sending to the press for composing. Proofs are mailed to the corresponding authors if needed and are also gone through by the managing editor/ associate editor/ member of editorial board.

Article Processing Charges

Articles are published in the SAARC Journal of Agriculture (SJA) free of charge.

Title and Authorship Information

The following information should be included

- Paper title
- Full author names
- Full mailing addresses
- Email addresses

Abstract

The manuscript should contain an abstract. The abstract should be self-contained and citation-free, single paragraph and should not exceed 200/250 words. An ideal abstract may contain background, rationale, objectives, materials & methods, results and conclusions.

Keywords

Up to 10, in alphabetical order and separated by comma.

Introduction

This section should be succinct, with no subheadings.

Materials and Methods

This part should contain sufficient details so that all procedures can be reflected. It can be divided into subsections if several methods are described. It should include year and place of study.

Results

The content of this section should permit full comprehension of the data reported in figures and tables.

Discussion

A comprehensive discussion section is required to justify the results. Normally a comparison between your results and results from previous works should be given in the Discussion. The Results and Discussion could be merged in a single section if necessary.

Conclusions

This should clearly explain the main findings of the work highlighting its importance and relevance.

Acknowledgments

Acknowledgments of people, grants, funds, etc. should be placed in a separate section before the reference list. The names of funding organizations should be written in full. Please check this section carefully before publication, as amendments or corrections are not allowed after publication

SHORT COMMUNICATIONS

Short Communications typically describe research techniques, apparatus, and observations which were not confirmed normally by repetition (preliminary findings based on a single experiment). These articles are usually shorter than research papers and there are no individual abstract, introduction, materials and methods, results and discussion. Instead, they are written in continuous form without any sub headings.

References

1. In general, not more than 10-15 references would be required. Recent and relevant not more than 20 years old references are encouraged.
2. There is no need to give references for standard procedures of soil and plant analysis, as well as for routine statistical analysis; only the methodology may be indicated.
3. All references quoted in the text must appear at the end of the article and vice-versa. The spellings author's names and dates or years at the two places should be carefully checked.
4. The references should include names of all authors, years (not within brackets), full title of the article, full name of the journal (in italics) (no abbreviations), volume number, issue number, and pages. For book or monograph, the name of the publisher should also be given as well as its volume, edition and relevant pages.
5. The references cited together in the text should be arranged chronologically. The list of references should be arranged alphabetically on author's names, and

chronologically per author.

6. References from standard scientific journals should be preferred, while those concerning unpublished data are generally to be avoided or mentioned as 'Personal communications' in the text. These need not be given in the reference list. A few examples for correct citation of references in the list are given below:

Journal Article

- Buhler, D.D. and Mester, T.C. 1991. Effect of tillage systems on the emergence depth of giant and green foxtail. *Weed Science*, 39: 200-203
- Mowla, G.M., Mondal, M.K., Islam, M.N. and Islam, M.T. 1992. Farm level water utilization in an irrigation project. *Bangladesh Rice Journal*, 3 (1&2): 51-56
- Rahman, M.M. 1990. Infestation and yield loss in chickpea due to pod borer in Bangladesh. *Bangladesh Journal of Agricultural Research*, 15(2): 16-23

Book/Bulletin/Reports/Series

- Bhuiyan, S.I. 1982. Irrigation system management research and selected methodological issues. *IRRI research paper series no 81*. Los Banos, Manila
- De Datta, S. K. 1981. Principles and practices of rice production. Los Banos, Manila
- International Rice Research Institute. 2000. International rice trade: a review of 1999 and prospects for 2000. *International Rice Commission Newsletter*, IRRI, Manila
- Steel, R.G.D. and Torrie, J.H. 1980. Principles and procedures of statistics: A biometrical approach. 2nd ed. McGraw-Hill, New York
- Westerman, R.L. (ed.) 1990. Soil testing and plant analysis. 3rd ed. SSSA Book Ser. 3. SSSA, Madison, WI

Chapter in a Book

- David, H. and Easwaramoorthy. 1988. Physical resistance mechanisms in insect plant interactions. p. 45-70. In T.N. Ananthakrishnan and A. Rahman (ed.), *Dynamics of insect plant interactions: Recent advances and future trends*. Oxford and IBH Publication, New Delhi
- Johnson, D. W. and D. E. Todd. 1998. Effects of harvesting intensity on forest productivity and soil carbon storage. p. 351-363. In R. Lal et al. (ed.) *Management of carbon sequestration in soils*. Advances in Soil Science. CRC Press, Boca Raton, FL

Conference/Symposium/ Proceedings

- Joshi, B.K. 2004. Crossing frequency and ancestors used in developing Nepalese mid and high hill rice cultivars: Possible criteria for yield improvement and rice genes conservation. p. 502-523. In Proc. *National Conference on Science and Technology*, 4th, Vol. 1. 23-26 Mar., 2004. NAST, Kathmandu, Nepal.
- Ramanujam, S. (ed.) 1979. *Proceedings of International Wheat Genet Symposia*, 5th, New Delhi, India. 23-28 Feb. 1978. Indian Soc. Genet. Plant Breeding, Indian Agric. Res. Inst., New Delhi.

Dissertation

Singh, A.A. 2005. Weed management approaches and modeling crop weed interaction in soybean. M. Sc. (Ag.) thesis. Tamil Nadu Agricultural Univ., Coimbatore.

Software and Software Documentation

Minitab. 1998. MINITAB 12. Minitab, State College, PA.

Online publication

Venugopal, D. 2000. Nilgiri tea in crisis: Causes consequences and possible solutions. Retrieved October 11, 2000 from <http://www.badaga.org>.

Online journal article

Doerge, T.A. 2002. Variable-rate nitrogen management creates opportunities and challenges for corn producers. *Crop Manage.* doi:10.1094/cm-2002-0905-01-RS.

Tables

1. Each table must be typed on a separate sheet (not to be included in the text) and numbered consecutively in the same order as they mentioned in text.
2. The title should fully describe the contents of the table and explain any symbol or abbreviation used in it as a footnote, using asterisks or small letters viz. a, b, etc.
3. Tables should be self-explanatory, not very large (< 10 columns in portrait and <14 columns in landscape formats respectively) and may cover space up to 20-25% of the text.
4. Maximum size of table acceptable is that can be conveniently composed within one full printed page of the journal. The large sized tables should be suitably split into two or more small tables.
5. Standard abbreviations of units of different parameters should be added between parentheses.
6. The data in the tables should be corrected to minimum place of decimal so as to make it more meaningful.
7. Vertical lines should not be used to separate columns. Similarly, horizontal lines should be used only where these are necessary, not in the body of the article.
8. All tables should be tagged with the main body of the text i.e. after references.

Figures

1. Figures may be given in place of tables where a large number of values are presented that can be interpreted through figures. In no case the same data should be presented in both tables and figures.

2. Originals of the figures should be no larger than twice the final size, of good quality and printed clearly in black on plain white paper or in color. The figures may be sized to fit within the columns of the journal (8 cm width for single column or 17 cm for columns i.e. full page).
3. Lines should be bold enough to allow the figure to be reduced to either single or double column width in the journal.
4. Black and white photographs are also accepted if these are necessary to improve the presentation and quality of the article.

Some useful hints

1. All scientific or technical names as well as all data and facts must be rechecked carefully before submitting the manuscript.
2. Dates and years may be mentioned as 28 May 2007, 28 May to 7 June, and 28-30 May instead of May 28, 2007, 28 May-7 June, and 28 to 30 May, respectively.
3. Avoid numerals and abbreviations at the beginning of a sentence; spell out or change the word order if necessary.
4. A comma may be used for data in thousands or more such as 10,000 or 2,30,000 etc. Alternatively, these data can also be presented as 10.0 or 230.0 if a common expression such as 'x10³' is used in tables or figures. Avoid expressing data in 'lakhs', instead use 'thousand' or 'million'.
5. Only standard abbreviations should be used and these should invariably be explained at first mention. Avoid use of self-made abbreviations such as Rhizo., Azo., buta, isop. etc. for *Rhizobium*, *Azotobacter*, butachlor, isoproturon, respectively.
6. For names of plant protection chemicals, the first letter of the name need not be capitalized for scientific names but should be capitalized for trade names. All the names should be checked very carefully.
7. Use of unnecessary abbreviations and treatment symbols such as T1, T2 etc. under Materials and Methods or tables without actually using these under Results and Discussion should be avoided.
8. All weights and measurements must be in SI or metric units. Use kg ha⁻¹, or t ha⁻¹ but not q ha⁻¹. Do not follow the style g/ha, mg/kg, mg/l, mg/g, ml/l or g per ha, etc
9. Use % after numbers, not per-cent, e.g. 7%. In a series or range of measurements, mention the units only at end, e.g. use 30, 100, 170 and 300C; 20 or 30% more instead of 30C, 100C, 170C and 300 °C; 20% or 30% more.
10. Numeral should be used whenever it is followed by a unit measure or its abbreviations e.g. 1 g, 3 m, 5 h, 6 months etc. Otherwise, words should be used

for numbers one to nine and numerals for larger ones except in a series of numbers when numerals should be used for all in the series.

11. For the composition of fertilizers, manures, crops or soil, the elemental forms (K, P, Mg etc.) should be used and not the oxides.
12. Statistical analysis of data in the standard experimental design should be sound and complete in itself with both \pm SE (Standard Error of means) and CD (Critical Difference) or LSD (Least Significant Difference) ($P=0.05$) values given for comparison of treatment means in tables and figures.
13. Use multiplication sign (\times) not a alphabet x for indicating multiplication, crossing, etc

SAARC Agriculture Centre (SAC) is the first SAARC Regional Centre established in 1988 at Dhaka, Bangladesh with an overall objective of promotion of agricultural research and development as well as technology dissemination initiatives for sustainable agricultural development and poverty alleviation in the region.

SAARC Journal of Agriculture (SJA), a half yearly publication from the Centre, is envisaged to serve as a platform for exchange of latest knowledge on breakthrough topics that are of current concern for researchers, extensionists, policy makers and students. It aims to capture the first-hand knowledge on research achievements in the field of agriculture, fisheries, livestock, forestry and allied subjects from the SAARC member countries. SAARC Agriculture Centre welcomes your feedback and suggestions for improving the quality of the journal.