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DEVELOPMENT OF AN IPM PACKAGE TO CONTROL GUMMY STEM AND LEAF BLIGHT DISEASES OF BOTTLE GOURD

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ABSTRACT

Three experiments were conducted at the Horticulture Research Centre, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur during Rabi 2019, 2020 and 2021 to develop an integrated pest management (IPM) measure against the gummy stem blight (GSB) disease of bottle gourd. Two IPM packages were tested along with a control treatment, viz. i) IPM-I: soil application of *Tricho*-compost @ 2.5 t/ha + Bordeaux paste at 12 inch from soil level + foliar spray of *Tricho*-leachate @ 0.2% at 15-day intervals, ii) IPM-II: soil application of talc based *Trichoderma* @ 50 kg/ha + mustard oil extract cake @ 600 kg/ha + foliar spray of *Tricho*-leachate @ 0.2% 0.2% at 15-day intervals and iii) control (regular practices) were used. The treatment IPM-II most effectively reduced the GSB disease incidence and increased the yield of bottle gourd followed by IPM-I. The marginal benefit cost ratio too, was the highest with IPM-II, the values being 5.25 and 5.51 in 2020, 2021 followed by 2.36 and 2.37 with IPM-I, respectively.

Keywords: Bottle gourd, Gummy stem blight, IPM

INTRODUCTION

Bottle gourd (*Lagenaria siceraria* L.) is one of the most popular vegetables in Bangladesh. It is enriched with dietary fiber, vitamins such as vitamin B and vitamin C, minerals like sodium, potassium, iron, etc. and other nutritionally beneficial compounds and has low fat and cholesterol contents (Sharma, 2014, Jones, 2009). It is widely cultivated throughout the country during the Rabi (winter) season. Nowadays, it is cultivated during the summer season, too.

In Bangladesh, bottle gourd is affected by many diseases, mainly soil borne diseases such as Gummosis, *Fusarium* wilt, anthracnose and root knot nematode. The gummy stem blight (GSB) disease has been found to be one of the most destructive diseases of bottle gourd caused by the fungal species *Didymella bryoniae* (*Mycosphaerella melonis*) in the country (Anon. 2008). The GSB fungus can be easily transmitted to a new area with infected seeds (Anon. 2010). The disease also attacks other cucurbitaceous vegetables including pointed gourd (Afroz *et al.* 2012). At early stages of plant growth, plants are found more susceptible and in case of severe attack the plant may die (Agrios 1997; Sherf and Macnab 1986). The pathogen is soil-borne and initially infects the plant base at soil level. Producing air-borne ascospores, the pathogen also infects the crop foliage. Gummy

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stem blight results in crown blight, leaf lesions, defoliation and fruit rot. After some time the infected area becomes cracked and gummy substances trickle out from the stem. The disease markedly reduce yield and a severely infected crop may even fail to set fruits.

Strategies to control the diseases of bottle gourd, as reported by researchers from different countries of the world, included utilization of resistant varieties, use of disease free seeds, application of soil amendments with bio-manures or organic manures, seed/stem treatment with fungicide such as benomyl, mancozeb, ipridione and chlorothantranil as early as first appearance of the disease symptom etc. (Charles 1999; Utkhede and Edward 2004). The vine rot of pointed gourd caused by the GSB pathogen was successfully controlled by applying Cupravit @ 0.25%. Effective disease management was also done using various groups of fungicides like Mancozeb (Dithane, Pencozeb); Maneb (Maneb, Manex); Tebuconazole (Folicur) and Thiophynate methyl (Topsin M) in Florida (Afroz *et al.* 2012). In Bangladesh, however, the GSB pathogen has been recently detected, and management measures have not yet been developed. In view of this, the present investigation was conducted to develop a suitable management measure to control this disease of bottle gourd under epiphytotic natural conditions.

MATERIALS AND METHODS

The experiments was conducted in three consecutive crop seasons of 2019-20, 2020-21 and 2021-22 to find out effective control measures against gummy stem blight disease. The experiments were conducted in the experimental field of Bangladesh Agricultural Research Institute (BARI), Gazipur under natural epiphytotic conditions following randomized complete block design with four replications. Three IPM packages such as T₁= IPM package I: Soil application of Tricho-compost @ 2.5 t/ha + Bordeaux paste at 12 inch from soil level + foliar spray of Tricho-leachate @ 0.2% at 15 days interval T₂= IPM package II: Soil application of Talc base *Trichoderma* @ 50 kg/ha + mustard oil cake @ 600 kg/ha + foliar spray of Tricho-leachate @ 0.2% at 15 days interval and T₃= Control (regular practices) were tested. Tricho-leachate @ 0.2% at 15 days interval were applied in the plants three times. Seeds of bottle gourd variety BARI Lau-4 were collected from Olericulture division, HRC, BARI. Bottle gourd was grown following standard methods. Seedlings were grown in polybags. Twenty five days old seedlings were then transplanted in the experimental plots where the unit plot size was 3m X 2.5m raised beds. Cowdung was applied @ 15 ton ha⁻¹. triple super phosphate, urea and muriate of potash were applied @ 175, 175 and 150 kg ha⁻¹ (Anon., 2005). The intercultural operation and irrigation were done in time and duly. Mustard oil cake was applied in the plots 15 days before transplanting the seedlings. After transplanting, Talc base *Trichoderma* was added in the pit.

Data on disease severity of plant growth was recorded at 70 days based on a 0-5 subjective scale, where, 0= without infection on stem or leaf, 1=1-20% stem/leaf area infected, 2=>20-30% stem/ leaf area infected, 3=>30-50% stem/leaf area infected, 4=>50-70% stem/leaf area infected and 5=>70- 100% stem/leaf area infected. Twenty five leaves were randomly selected to record data.

The severity was expressed in percent disease index (PDI). The PDI and percent of disease reduction was computed according to formula described by Krishna Prasad *et al.* (1979) as follows:

$$PDI = \frac{\text{Sum of numerical ratings (Class rating X Class frequency)}}{\text{Total number of stems or leaves counted X maximum rating scale}}$$

$$\text{Reduction diseased area (\%)} = \frac{\text{Diseased area under any treatment} - \text{Diseased area under control}}{\text{Diseased area under control}} \times 100$$

Application of Bordeaux-paste

In the experiment, trellis was used to support the vine growth. Seedlings were treated with Bordeaux paste (CuSO₄: Ca (OH) 2: Water =1:1:10) 25 days after germination by covering the stem base at the ground level and then transplanted in the plots.

Efficacy of IPM-Package to control foliar blight of bottle gourd

Twenty five leaves were randomly selected from each replication to record data on severity of leaf blight based on a 0-5 subjective scale (Mehta and Mandal, 1978) and finally expressed as percent disease index (PDI) according to Krishna Prasad *et al.* (1979) as mentioned earlier. Yield data was also recorded from the treated plots and compared with control.

Marginal Benefit Cost Ratio (MBCR) was computed based on the following standard formula

$$\text{MBCR} = \text{Adjusted net return (t/ha)} / \text{Cost of treatment (Tk/ha)}$$

Statistical analysis

Statistical analysis of the data of the experiment was performed according to Zaman *et al.*, (1982). Data were analyzed after necessary transformation. The recorded data were analyzed statistically to find out the level of significance and the variations using STAR analysis of R software.

RESULTS

Diseased area on stem ranged 6.47-13.56 cm² at 25 days and 2.33-13.44 cm² at 55 days after transplanting under IPM Package I and IPM Package II. At first count 25 days after planting only IPM Package I and IPM Package II caused 44.69 and 52.29% reduction in disease severity over control. At the second count 55 days after transplanting, both the IPM package showed remarkable reduction of gummy stem blight severity compared to control within the range of 72.09-82.66%. IPM Package II gave the highest disease reduction followed by IPM Package I. Interestingly the diseased areas under the control treatment remain unchanged at 25 and 55 days of plant growth in this experiment (Table 1). Bordeaux paste was also found effective in controlling stem bleeding of coconut when the wound was cleaned by chisel and dressed it with Bordeaux paste (Rahman *et al.*, 1989).

Table 1. Effectiveness of IPM packages in controlling gummy stem and leaf blight of bottle gourd seedling, 2019-20

Treatment	Diseased area (cm ²)		Disease reduction over control (%)	
	at 25 Days after transplanting	at 55 Days after transplanting	at 25 Days after transplanting	at 55 Days after transplanting
T ₁	7.50 b	3.75 b	44.69	72.09
T ₂	6.47 c	2.33 c	52.29	82.66
T ₃	13.56 a	13.44 a	-	-

*Values within the same column with a common letter(s) do not differ significantly according to DMRT.

The highest PDI of 68.23, 68.23 and 66.91 was recorded from control plants in 2019-20, 2020-21 and 2021-22 cropping years. Results showed that both of the IPM packages significantly ($p=0.5$) reduced the PDI value over control in all the three years. IPM package II: Soil application of Talc base *Trichoderma* @ 50 kg/ha + mustard oil cake @ 600 kg/ha + foliar spray of Tricho-leachate @ 0.2% at 15 days interval was ranked the best against the diseases of bottle gourd which was significantly different from other treatments in three years. IPM package I: Soil application of Tricho-compost @ 2.5 t/ha + Bordeaux paste at 12 inch from soil level + foliar spray of Tricho-leachate @ 0.2% at 15 days interval was also significantly differed with control (T₃). The IPM packages could reduce Percent Disease Index (PDI) about 40.93 to 46.3%, 41.94 to 46.02% and 38.62 to 45.33% in 2020, 2021 and 2022, respectively over T₃ (Table1).

The yield was significantly increased in T₂ (IPM package II) and T₁ (IPM package I) compare to T₃ (control) (Table2).

Table 2. Effectiveness of IPM packages for disease management of bottle gourd 2019-20, 2020-21 and 2021-22

Treatment	Years								
	2019-20			2020-21			2021-22		
	PDI values	PDI reduction (%)	Yield (t/ha)	PDI values	PDI reduction (%)	Yield (t/ha)	PDI values	PDI reduction (%)	Yield (t/ha)
T ₁	27.30 (22.5) b	40.93	32.08 b	26.29 (21) b	41.94	33.30 b	28.29 (24) b	38.62	32.87 b
T ₂	21.93 (15) c	46.3	46.80 a	22.21 (15.5) c	46.02	48.17 a	21.58 (14.5) c	45.33	48.87 a
T ₃	68.23 (89) a	-	19.20 c	68.23 (89) a	-	20.23 c	66.91 b (87.50) a	-	19.77 c
CV (%)	5.82		10.54	7.61	-	11.17	4.56		10.76

** Values denoted by similar letter in a column do not differ significantly according to DMRT figures in the parenthesis are angular transformed values.

T₁=IPM package I: Soil application of Tricho-compost @ 2.5 t/ha + Bordeaux paste at 12 inch from soil level + foliar spray of Tricho-leachate @ 0.2% at 15 days interval

T₂= IPM package II: Soil application of Talc base *Trichoderma* @ 50 kg/ha + mustard oil cake @ 600 kg/ha + foliar spray of Tricho-leachate @ 0.2% at 15 days interval

T₃= Control (regular practices)

Economics of different treatments are presented in Table 3. The maximum gross return of Tk 963400/ha was obtained from the treatment T₂ followed by T₁ (Tk. 666000/ha) and T₃ (Tk. 404600/ha). Similar result was also observed in case of gross margin. In case of Marginal Benefit Cost Ratio, the highest value was obtained from T₂ (5.25) which was followed by T₁ (2.36).

Table 3. Cost and return analysis of different treatments assigned for the management of Gummy Stem and leaf Blight disease in bottle gourd during 2020-21

Treatment	Gross return (Tk/ha)	Cost of treatment (Tk/ha)	Gross margin (Tk/ha)	Adjusted net return (t/ha)	Marginal Benefit Cost Ratio
	Total				
T ₁	666000	77795	588205	183605	2.36
T ₂	963400	89410	873990	469390	5.25
T ₃	404600	-	404600	-	-

T₁=IPM package I: Soil application of Tricho-compost @ 2.5 t/ha + Bordeaux paste at 12 inch from soil level + foliar spray of Tricho-leachate @ 0.2% at 15 days interval

T₂= IPM package II: Soil application of Talc base *Trichoderma* @ 50 kg/ha + mustard oil cake @ 600 kg/ha + foliar spray of Tricho-leachate @ 0.2% at 15 days interval

T₃= Control (regular practices)

Details of economic analysis

Treatments	Details of calculation	Treatment cost (Tk/ha)
T ₁ =IPM package I: Soil application of Tricho-compost @ 2.5 t/ha + Bordeaux paste at 12 inch from soil level+foliar spray of Tricho-leachate @ 0.2% at 15 days interval	Cost Tricho-compost @ 2.5 t/ha =20*2500 Tk=50,000 Tk Cost of Bordeaux paste =14985 Tk For 1 ha Quick lime (CaO) need 40kg @ 80tk/kg and Copper Sulphate (CuSO ₄) need 40 kg @ 290tk/kg Cost of 1 hectare land for per spray = 12.35 liter X 200 Tk = 2470 Tk (three sprays)=7410 Tk Labor cost for Soil application of Tricho-compost @ 600tk (three times)=1800 Tk Labor cost for application of Bordeaux paste @ 600tk (three times)=1800 Tk Labor cost for Tricho-leachate spray @ 600tk (three sprays)=1800 Tk	77795

Treatments	Details of calculation	Treatment cost (Tk/ha)
T ₂ =IPM package II: Soil application of Talc base <i>Trichoderma</i> @ 50 kg/ha + mustard oil cake @ 600 kg/ha + foliar spray of Tricho-leachate @ 0.2% at 15 days interval	Cost of Talc base <i>Trichoderma</i> @ 50 kg/ha @100 gm 80 Tk=50000*80 Tk=40000 Tk Cost of mustard oil cake @ 600 kg/ha @70 Tk/kg=42000 Tk Cost of 1 hectare land for per spray = 12.35 liter X 200 Tk = 2470 Tk (three sprays) =7410 Tk Labor cost for Soil application of Talc base <i>Trichoderma</i> @ 600tk (three times) =1800 Tk Labor cost for application of mustard oil extract cake @ 600tk (three times)=1800 Tk Labor cost for Tricho-leachate spray @ 600tk (three sprays)=1800 Tk	89410
T ₃ = Control (regular practices)	-	-

Market price of Bottle gourd @ 20Tk/Kg, Spray coverage-600 L/ha, Tricho-compost @ 20 Tk/Kg, Tricho-leachate @ 250 Tk/L, Mustard oil cake@ 70 Tk/Kg, Talc base *Trichoderma* @ Tk/Kg, Chun-80 Tk/Kg, Tute-290 Tk/Kg

In 2021-22 crop season the maximum gross return of Tk 977400/ha was obtained from the treatment T₂ followed by T₁ (Tk. 657400/ha) and T₃ (Tk. 395400/ha). Almost similar trend was also observed in case of gross margin (Table 4). In case of Marginal Benefit Cost Ratio, the highest value was obtained from T₂ (5.51) which was followed by T₁ (2.37).

Table 4. Cost and return analysis of different treatments assigned for the management of Gummy Stem and leaf Blight disease in bottle gourd during 2021-22

Treatment	Gross return (Tk/ha)	Cost of treatment (Tk/ha)	Gross margin (Tk/ha)	Adjusted net return (t/ha)	Marginal Benefit Cost Ratio
	Total				
T ₁	657400	77795	579605	184205	2.37
T ₂	977400	89410	887990	492590	5.51
T ₃	395400	-	395400	-	-

T₁=IPM package I: Soil application of Tricho-compost @ 2.5 t/ha + Bordeaux paste at 12 inch from soil level + foliar spray of Tricho-leachate @ 0.2% at 15 days interval

T₂= IPM package II: Soil application of Talc base *Trichoderma* @ 50 kg/ha + mustard oil cake @ 600 kg/ha + foliar spray of Tricho-leachate @ 0.2% at 15 days interval

T₃= Control (regular practices)

DISCUSSION

In the present investigation, seedlings were treated with Bordeaux paste 25 days after germination by covering the stem base at the ground level and transplanted in the plots. Bordeaux paste protected the seedlings from the gummy stem blight disease. IPM package II: Soil application of Talc base *Trichoderma* @ 50 kg/ha + mustard oil cake @ 600 kg/ha + foliar spray of Tricho-leachate @ 0.2% at 15 days interval was ranked the best against the diseases of bottle gourd which is significantly different from other treatments in three years. Bordeaux paste was also found effective in controlling stem bleeding of coconut when the wound was cleaned by chisel and dressed it with (Nene and Thapliyal; 1979, Rahman *et al.*, 1989). In my previous study, it was found that Bordeaux paste gave the highest disease reduction followed by mustard oilcake and poultry liter. Soil amendment with poultry liter and mustard oilcake and application of Bordeaux paste on infected stem gave respectively 70.14, 77.43 and 91.93% disease reduction (Afroz *et al.*, 2012).

In our study, Bordeaux paste was appeared the most promising treatment in controlling gummosis of jackfruit. The yield was 26.3%-38.8% higher in Bordeaux paste treated plants compared to control (scoped plants). In this study, the effect of gummosis control for tree plants like jack fruit was calculated after one and half years of disease control and cumulative effective on yield supposed to be higher in course of time. The benefit cost ratio (BCR) was found higher in Bordeaux paste treatment compared to other treatments (Rahman and Afroz, 2016). Bordeaux paste/coaltar was more effective to control gummosis disease of citrus and increased the yield (Anon. 2018).

Santosand Café Filho (2005) reported that the gummy stem blight or stem canker of melon, induced by the fungus *D. bryoniae* can be controlled by using Mancozeb. Application of Dithane M-45 (Mancozeb) proved as the best treatment for controlling *D. bryoniae* and increased yield of watermelon (Bharath *et al.*, 2005). The Marginal Benefit Cost Ratio (MBCR) was found 5.25 and 5.51 in IPM package II: Soil application of Talc base *Trichoderma* @ 50 kg/ha + mustard oil cake @ 600 kg/ha + foliar spray of Tricho-leachate @ 0.2% at 15 days interval in both the year which found higher compared to other treatments. The farmers got benefit from sailing bottle gourd as the yield increased.

CONCLUSION

Experiments were conducted to develop an integrated pest management (IPM) measure against the gummy stem blight (GSB) disease of bottle gourd. Two IPM packages were tested along with a control treatment, viz. i) IPM-I: soil application of *Tricho*-compost @ 2.5 t/ha + Bordeaux paste at 12 inch from soil level + foliar spray of Tricho-leachate @ 0.2% at 15-day intervals, ii) IPM-II: soil application of talc based *Trichoderma* @ 50 kg/ha + mustard oil extract cake @ 600 kg/ha + foliar spray of *Tricho*-leachate @ 0.2% at 15-day intervals and iii) control (regular practices) were used. The treatment IPM-II most effectively reduced the GSB disease incidence and increased the yield of bottle gourd, followed by IPM-I. The marginal benefit cost ratio, too, was the highest with IPM-II.

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DETECTION OF *BRUCELLA ABORTUS* IN DAIRY CATTLE OF BANGLADESH BY PCR AND MULTILOCUS PHYLOGENETIC ANALYSES

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ABSTRACT

Brucellosis is a chronic disease caused by the intracellular bacteria *Brucella* spp. Among the *Brucella* species, *Brucella abortus* causes bovine brucellosis and affects the reproductive system which affects fertility and reduces milk and meat production in cattle and buffalo. Brucellosis is endemic in Bangladesh, but still little is known about the prevalent *Brucella* species. At the field level, brucellosis diagnosis is made by the Milk Ring Test (MRT) and the Rose Bengal Plate Test (RBT), but there are chances of false positive diagnoses due to lack of specificity of the tests. For accurate diagnosis of brucellosis molecular techniques like the Polymerase Chain Reaction (PCR) can be used. In this study, sera, milk and cultures tested strongly positive with MRT and RBT were confirmed by PCR. Aborted fetal membranes were used for the isolation of *Brucella* species through cultures which were then genotyped by the Bruce Ladder PCR technique. The sequence of conventional PCR products was used for the multilocus phylogenetic analyses. Only *B. abortus* was detected from fetal membrane, serum and milk samples. This study suggested that the use of the Bruce Ladder PCR technique and molecular phylogeny could lead to a more reliable diagnosis of brucellosis in dairy cows in Bangladesh.

Keywords: Brucellosis, *Brucella abortus*, Bruce ladder PCR, Conventional PCR, Multilocus Phylogenetic Analyses.

INTRODUCTION

Brucellosis is one of the major zoonotic disease of humans, livestock and wildlife worldwide. This disease is responsible for significant economic losses to the livestock industry as it causes morbidity and mortality in farm animals (Ali *et al.*, 2015), abortion, infertility, the birth of weak dead offspring, increased calving interval and reduction of milk yield. Brucellosis has been found to be endemic in Bangladesh (Rahman *et al.*, 2014a; Rahman *et al.*, 2019a). The seroprevalence of brucellosis has been reported in different domestic animals in Bangladesh including cattle (0.6%-20.4 %), buffaloes

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(4.0%), sheep (1.2%-7.3%), goats (1%-3.6%), pig (4.8%), horse (1.79%), dogs (4.0%) and humans (2.5 to 18.6%) (Rahman *et al.*, 2012; Islam *et al.*, 2013; Rahman *et al.*, 2013; Millat *et al.*, 2018; Rahman *et al.*, 2019b). *Brucella abortus* DNA was also reported from humans, cattle, buffalo, goats and gayal sera (Rahman *et al.*, 2014a; Rahman *et al.*, 2017). The identification and characterization of *Brucella* spp. from human and animal origin require culture, physical and biochemical tests (Ali *et al.*, 2019). The Milk Ring Test (MRT) and the Rose Bengal Plate Test (RBT) are widely used techniques for screening brucellosis in eradication programmes (Sarker *et al.*, 2017). These tests are easy and less time-consuming to perform on a large scale and require no special equipment and expertise, but false positive results may occur. Samples tested by MRT and RBT can be confirmed by PCR which is more sensitive and specific than those tests. Genotyping of the brucellosis pathogen using PCR helps determine the phylogenetic and geographic association of zoonotic agent as such *B. abortus*. In the present study the Bruce Ladder PCR technique was used for detecting *Brucella abortus*, identifying genetic variations in *B. abortus* isolates collected from cattle and an attempt was made to uncover the association between them.

MATERIALS AND METHODS

Serum and milk testing

A total of 1003 sera samples and 1003 milk samples from dairy cattle-503 from Central Cattle Breeding and Dairy Farm (CCBDF) and 500 from Military Dairy Farm (MDF), Savar, Dhaka were collected with the history of reproductive disturbance. Serum samples were tested by RBT, iELISA and SAT according to the method described previously (Rahman *et al.*, 2019b). Milk samples were tested by Milk Ring Test according to the previously described method (Sarker *et al.*, 20117).

Isolation of *Brucella* spp.

The *Brucella* were isolated from aborted fetal membranes collected from CCBDF & MDF, Savar. Fetal membranes were collected from late aborted cases between the 5th and 8th months of gestation (Megid *et al.*, 2010). *Brucella* agar base (HIMEDIA, M348-500G) was used for the isolation of *Brucella*. Suspected colonies were stained at Livestock and Human Brucellosis Laboratory in the department of Medicine according to standard procedures by modified Ziehl-Neelsen staining and tested biochemically (Alton *et al.*, 1988).

Molecular methods

The conventional PCR and sequencing were performed at the Livestock and Human Brucellosis Laboratory in the Department of Medicine, Bangladesh Agricultural University, Mymensingh, Bangladesh and Bruce Ladder PCR was performed at Federal Research Institute for Animal Health, Reference Laboratory for Brucellosis, Jena, Germany.

The genomic DNA was extracted from positive serum, milk and bacterial isolates using the High Pure PCR Template Preparation Kit (Roche Diagnostics®, Mannheim, Germany) according to the manufacturer's instructions. The conventional PCR was performed using nine pairs of primers the 'gap' (glyceraldehyde 3-phosphate dehydrogenase), 'aroA' (3-phosphoshikimate1-carboxyvinyl transferase), 'glk'

(glucokinase), ‘dnak’ (chaperone protein), ‘gyrB’ (DNA gyrase B subunit), ‘trpE’ (anthranilate synthase), ‘omp’ (25 kDa outer-membrane protein), ‘cob’ (cobyric acid synthase) and ‘int-hyp’ (upstream and extreme 5’ of hypothetical protein) for PCR assay as described by Whatmore *et al.* 2007 under MLSA-9 schemes primers (Table 1). The PCR products were visualized by electrophoresis in a gel documentation system and the PCR product were directly sent to commercial service for purification and sequencing. A total of 483 reference strains for the nine loci were retrieved from ‘*Brucella* Pub MLST Databases’. Multiple alignments of the nucleotide sequences of concatenated data sets of 9 genes of four Bangladeshi isolates were done with respective reference strains using a multiple sequence alignment program MAFFT (Multiple Alignment using Fast Fourier Transform). The maximum-likelihood phylogenetic tree from nucleotide alignment was constructed using RAxML (Stamatakis, 2006). The tree was visualized by Tree Graph2 (Stover and Muller, 2010).

Bruce-ladder PCR

Species-level molecular identification was undertaken by multiplex PCR (Bruce ladder 2.0) which was performed as described (Lopez-Goni *et al.*, 2011) where mit Qiagen Multiplex Mastermix (12.5 μ M) was used. In brief, each reaction contained 6.25 μ M master mix (Qiagen MultiplexPCR kit2x), 4.25 μ M, PrimerMix 18 1 μ M and Tamplate DNA 1 μ M and with the following conditions: Step 1: 95 °C 15 min, Step 2: 94 °C 30 s, Steps 3: 58 °C 90 s, Step 4: 72 °C 3 min, Step 5: 72 °C 10 min. Step 2, 3 and 4 were repeated in 25 cycles. After completion of PCR, the products were separated by electrophoresis in 1.5% agarose gel stained by ethidium bromide and the electrophoresis was carried out at 130V for 60 min. The size of the amplicon (775 BP), forward and reverse primers were selected automatically by the Eppendorf neu Programm: Ladder 2.0 software.

Table 1: **Primers for conventional PCR: *Brucella* MLSA-9 schemes (Whatmore *et al.* 2007, BMC Microbiology 7: 34)**

Locus	Putative function	Primer sequences	Core sequence length
<i>gap</i>	glyceraldehyde 3-phosphate dehydrogenase	5' YGCCAAGCGCGTCATCGT 3' 5' GCGGYTGGAGAAGCCCCA 3'	589 bp
<i>AroA</i>	3-phosphoshikimate 1-carboxyvinyltransferase	5' GACCATCGACGTGCCGGG 3' 5' YCATCAKGCCCATGAATTC 3'	565 bp
<i>glk</i>	glucokinase	5' TATGGAAMAGATCGGCGG 3' 5' GGGCCTTGTCCTCGAAGG 3'	475 bp
<i>dnaK</i>	chaperone protein	5' CGTCTGGTCGAATATCTGG 3' 5' GCGTTTCAATGCCGAGCGA 3'	470 bp
<i>gyrB</i>	DNA gyrase B subunit	5' ATGATTTTCATCCGATCAGGT 3' 5' CTGTGCCGTTGCATTGTC 3'	469 bp
<i>trpE</i>	anthranilate synthase	5' GCGCGCMTGGTATGGCG 3' 5' CKSCCGCCATAGGCTTC 3'	486 bp
<i>omp25</i>	25 kDa outer-membrane protein	5' ATGCGCACTCTTAAGTCTC 3' 5' GCCSAGGATGTTGTCCGT 3'	490 bp
<i>cobQ</i>	cobyric acid synthase	5' GCGGGTTTCAAATGCTTGGA 3' 5' GCGTCAATCATGCCAGC 3'	422 bp
<i>int-hyp</i>	upstream and extreme 5' of hypothetical protein	5' CAACTACTCTGTTGACCCGA 3' 5' GCAGCATCATAGCGACGGA 3'	430 bp

RESULTS AND DISCUSSION

Out of 1003 sera, samples 43 samples (26 from CCBDF and 17 from Military Dairy Farm, Savar) reacted positive in RBT. Out of 1003 milk samples, 14 samples (8 from CCBDF and 6 from Military Dairy Farm, Savar) reacted positive in MRT, and 24 in ELISA & SAT. High grade positive sera, milk and culture were detected strongly as *Brucella abortus*. Seven hundred seventy five base pair band was detected in infected tissue and positive sera and milk tissues through Bruce ladder PCR. It was indicated that positive DNA of *B. abortus* was observed through Bruce ladder PCR (Figure 1).

Brucella abortus which causes bovine brucellosis is an important zoonotic disease with a great economic impact. In Bangladesh, there is limited control or eradication program in place and there is some specialized molecular laboratory in Bangladesh and have facilities to diagnose Brucellosis from *Brucella* isolates and analysis of its phenotype. PCR-based methods can reduce the risk of laboratory infection and are useful tools in epidemiology to identify links. These techniques have also the potential to be implemented in diagnostic laboratories in Bangladesh. Commonly, *B. abortus* is prevalent in case of brucellosis in bovines, *B. melitensis* and *B. suis* have also been identified as the causative agents in regions where bovines have close contact to goats and sheep or pigs, respectively (Liu *et al.*, 2012; Fretin *et al.*, 2013). Under this condition, the use of multiplex PCR is very important for the differentiation of *Brucella* species. A new Bruce-ladder multiplex PCR assay was introduced for diagnosis of *B. abortus* (biovar 1, 2 or 3) and all biovars of *B. melitensis*, *B. suis* and *B. ovis* biovar 1 and to differentiate between vaccine strains S99 and RB51 of *B. abortus* (Lopez-Goni *et al.*, 2011). In the present study, all isolates could be confirmed genetically as *B. abortus* based on Bruce-Ladder PCR, which is in accordance with previous findings from other countries i.e. India and Tanzania (Nagalingam *et al.*, 2012; Mathew *et al.*, 2015).

A total of 483 reference strains for the nine loci were retrieved from 'Brucella Pub MLST Databases'. Multiple alignments of the nucleotide sequences of concatenated data sets of 9 genes of four Bangladeshi isolates were done with respective reference strains using a multiple sequence alignment program MAFFT (Multiple Alignment using Fast Fourier Transform). The approximately-maximum-likelihood phylogenetic tree from nucleotide alignment was constructed using Fast Tree in Geneious v10. The multigene fast tree has been visualized by Tree Graph (Figure 2). The phylogenetic relationship of local isolates of *B. abortus* was investigated successfully using genotyping which is proof of the effectiveness of this genotyping method as a useful tool to trace back infection in the Bangladesh setting. However, in the present study, only one single genotype of *B. abortus* (biovar 3) was identified as the causative agent of brucellosis in cattle pointing to the fact that a single introduction event or single source led to the spread of brucellosis in the study area.

Now a day for the detection of brucellosis veterinary diagnostic laboratories utilize Milk Ring Test (MRT) for the diagnosis of bovine milk samples, which indirectly identifies *Brucella* spp. in the host (Chimana *et al.*, 2010). But MRT positivity does not indicate acute infection. To declare acute infection evidence of *Brucella* organism or detection of *Brucella* DNA in the animal sample is essential (Bricker, 2002; Gupta *et al.*, 2006; Hamdy and Amin, 2002). Acute infection of brucellosis in dairy cattle can be determined

by MRT, RBT and multiplex PCR techniques. This finding will help to cull dairy cattle acutely infected with brucellosis having serious public health hazards.

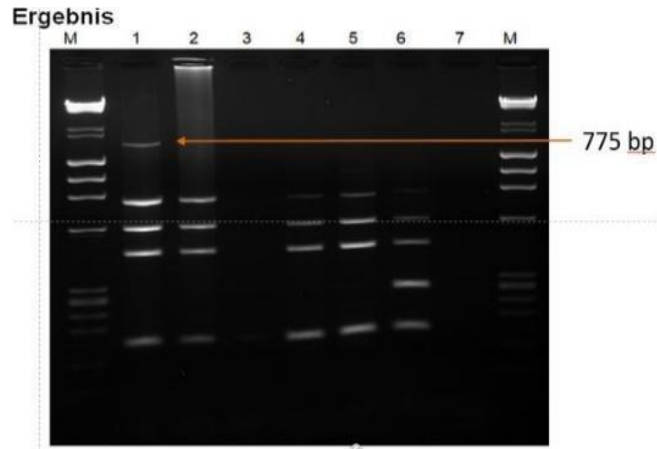


Figure 1: Bruce-Ladder Multiplex 2.0 PCR agarose gel image used to confirm the *Brucella* spp. Lane 1 *Brucella abortus* amplicon size 775 bp. *Brucella* spp. isolate.

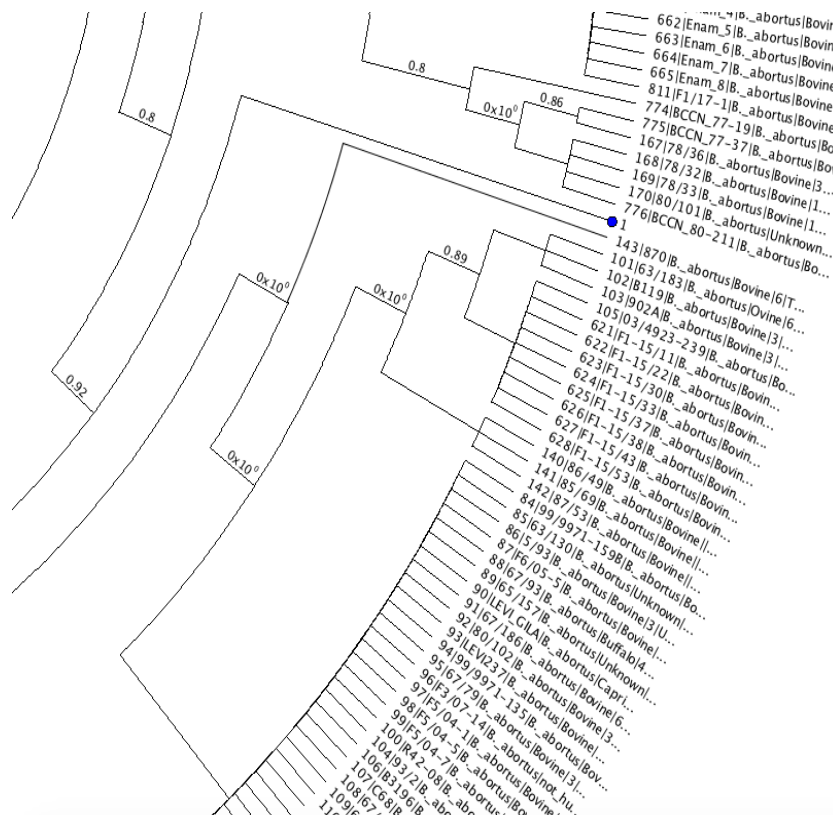


Figure 2: Screenshot of Multigene fast tree of the isolate 1 in *B. abortus* clade with well-supported value.

CONCLUSION

In Bangladesh, brucellosis in cattle is caused by *B. abortus*. Serological, cultural and molecular assays were used in this study to detect *Brucella* infection in both animals and humans. Diagnosis with PCR in association of phylogenetic tree formation was found to be more effective and confirmatory than the serological tests. The use of the Bruce Ladder PCR technique and multilocus molecular phylogeny could lead to a more reliable diagnosis of brucellosis in dairy cows in Bangladesh. The results of this study may be used to control brucellosis in cattle and ensure the quality of animal protein which has an important bearing in food and nutrition security of the people of Bangladesh.

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ECONOMIC IMPACT OF BOVINE TUBERCULOSIS IN CATTLE IN SELECTED DISTRICTS OF BANGLADESH

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ABSTRACT

Bovine tuberculosis (bTB) is considered to be an endemic disease having zoonotic importance in Bangladesh, but no economic analysis describing the burden of this disease has been reported so far. The objective of this study was to calculate the economic losses of bTB in crossbred farming cattle focused mainly on livestock production loss including decreased milk and meat production, neonatal deaths, infertility and other associated losses in two selected cattle-dense districts of Bangladesh. Utilizing the data from a cross-sectional survey on bTB prevalence conducted in 2019 combined with field interview data collection on different financial parameters on livestock goods and published secondary data, estimation of economic loss for a single year in two studied district (Dhaka and Mymensingh) of Bangladesh was done. The study confirmed an annual estimated economic loss due to bTB in farming cattle was BDT 1347.72 million (95% CI = 997.43-1801.65), equivalent to US\$ 16.04 million (95% CI= 11.87-21.45). The study showed that the highest economic loss was due to decreased milk production (BDT 697.25 million) followed by decreased meat production (BDT 531.22 million), other pertinent losses (vet health care, cleaning and disinfection costs, and restocking costs, if required), infertility and neonatal deaths. Due to a high animal-level prevalence of bTB in Dhaka, the study confirmed a higher economic loss in this district (BDT 717.80 million, 95% CI= 617.61-819.72) than that in Mymensingh (BDT 629. 92 million, 95% CI= 379.82-981.93). The study provides an in-depth insight into the impact of bTB on livestock productivity in two cattle rearing districts of Bangladesh. The model developed through this study could be applied to the estimation of the overall bTB burden in Bangladesh.

Keywords: Bovine tuberculosis (bTB), economic impact, farming cattle, Dhaka and Mymensingh districts, Bangladesh

INTRODUCTION

Bovine tuberculosis (bTB) is an important bacterial disease of animals caused by members of the *Mycobacterium tuberculosis* complex (MTC), primarily by *M. bovis*, but also by *M. caprae* and to a lesser extent *M. tuberculosis*, and *M. orygis* (van Ingen *et al.*,

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2012). This infectious disease of cattle also affects other domesticated animals and certain wild species resulting in a general state of illness, pneumonia, weight loss, and finally death. Bovine tuberculosis is still common in less developed countries, and severe economic losses can occur from livestock deaths, chronic diseases and trade restrictions (WOAH, 2018). The disease affects the safety standard of animal-origin food and livelihood of marginalized livestock raisers globally (WHO, 2017).

The economic burden of a livestock disease differs across geographical locations, affected species, rearing systems and the capacity of the national veterinary healthcare system (McDermott et al., 2013). Due to lack of active surveillance in Bangladesh, the actual burden of bTB is still underestimated. However, the country is routinely notifying to the WOAH (founded as OIE) the presence of bTB as *Mycobacterium tuberculosis* complex (MTC) in cattle (OIE, 2020a). During 2017-20, a cross-sectional survey was conducted in two cattle dense districts of Bangladesh with an aim to estimate the animal level prevalence of bTB and associated risk factors. The study generated data on the prevalence of bTB for Dhaka district (12.70%) and Mymensingh district (6.80%) at >4 mm cut-off value (Islam et al., 2020). Nearly 85% of the national cattle population consists of non-descript indigenous and the remaining around 15% crossbred cattle in Bangladesh (Hamid et al., 2017). However 100% crossbred cattle are being kept in urban areas of the Dhaka City Corporation (DCC) due to a high demand of cow's milk and meat from the city dwellers.

Indigenous cattle are less productive and incapable to meet the country's demands of milk and meat. To meet the demands for milk and meat, targets were set to maximize the productivity of indigenous cattle by artificial insemination (AI) with exotic breeds (mostly Holstein Friesian) as early as 1980. Therefore, the numbers of cross-breed animals are increasing gradually in Bangladesh (DLS, 2007). Growing demands for animal-origin food have resulted in an n higher density of cattle population in Bangladesh (145 large ruminants /km² with production clusters in 2010) (WB, 2018). This production intensification increases the likelihood of the burden of zoonosis including bTB especially on crossbred cattle which are more susceptible to bTB than the indigenous breeds (Ameni et al., 2007; O'Reilly & Daborn, 1995).

The bTB disease and its burdens are predominately associated with livestock production losses, with increased mortality and lower meat and milk production along with relevant losses. An evaluation of such losses is of paramount importance in countries where a huge livestock populations are being raised (Azami & Zinsstag, 2018). As a part of the bTB control program, the "test and slaughter" practice has been adopted in the many countries in the world, but due to financial constraints, developing countries like Bangladesh are sparsely following this method (Cousins, 2001). The pivotal elements limiting control of bTB in developing countries may be lack of facilities for culturing and genotyping pathogen isolates, inadequate resources for compensation of culled animals and vaccination, insufficient reporting systems, collaboration, and coordination among public health and veterinary services, low/absence of food safety compliance in both organized and unorganized dairy systems, poor knowledge of risk factors, no national control programs, inadequate government commitment (Zinsstag et al., 2006) etc.

As a large number of MTC susceptible domestic ruminant species are present in Bangladesh including 24 million cattle, 1.5 million buffaloes and 26.0 million goats. Approximately 70% (20% directly and 50% indirectly) of rural people depend on livestock. The contribution of livestock to the national economy was estimated as 1.47% in the year 2018-2019 and this is increasing at the rate of 3.47% annually. The country has achieved self-sufficiency in meat recently and efforts is underway to optimize milk production (DLS, 2019b). Several studies have been conducted in many parts of the world for estimating economic losses (Homem et al., 2016; Tschopp et al., 2012; Gilsdorf et al., 2006), but in formation in this respect is still lacking in Bangladesh. The objective of this study was to evaluate economic losses incurred due to bTB infections of cattle in two important cattle rearing districts, i.e., Dhaka and Mymensingh, of Bangladesh.

MATERIALS AND METHODS

Ethical approval

The protocol PR-17121 was approved by the Ethical Review Committee (ERC) of the International Center for Diarrhoeal Disease and Research (icddr, b), Bangladesh and also permitted by the Animal Welfare and Experimentation Ethical Committee (AWEEC) of Bangladesh Agricultural University (AWEEC/BAU/2019/24). An oral consent was taken from the owner/manager of the cattle farm before collection of data as a substantial proportion of cattle farmers were illiterate and they could not read and write.

The Study location and design

Based on the dairy farming cattle, the study was conducted in two cattle dense districts (Dhaka and Mymensingh) of Bangladesh (Figure 1) where a bTB prevalence study was conducted during July to December 2019 (Islam et al., 2020). The economic impact analysis was accomplished using the data derived from following sources.

- (1) Initially, the study utilized the data from multiple sources: secondary data/published data from the peer review journals, government document/unpublished data from the government offices (n=14).
- (2) Secondly, a semi-structure questionnaire based face to face interview data collection was done via with different stakeholders (farmers/cattle handlers) (n=79) of five districts of Bangladesh. The questionnaire gathered data on body weight (cow and bull), milk production/cow/day, price of a calf, a cow and a bull, price of milk/liter, treatment cost of a bTB infected animal, restocking cost if needed where a cow/heifer having infertility or abortion. Further, the cost of different parameters of livestock goods (meat and milk), cost of different categories of animals (cow, bull and calf) were validated through discussion with relevant stakeholders [butchers (n=10), milk sellers (n=10) and cattle traders (n=10)]. The overall methodology of this study is presented in Figure 2.

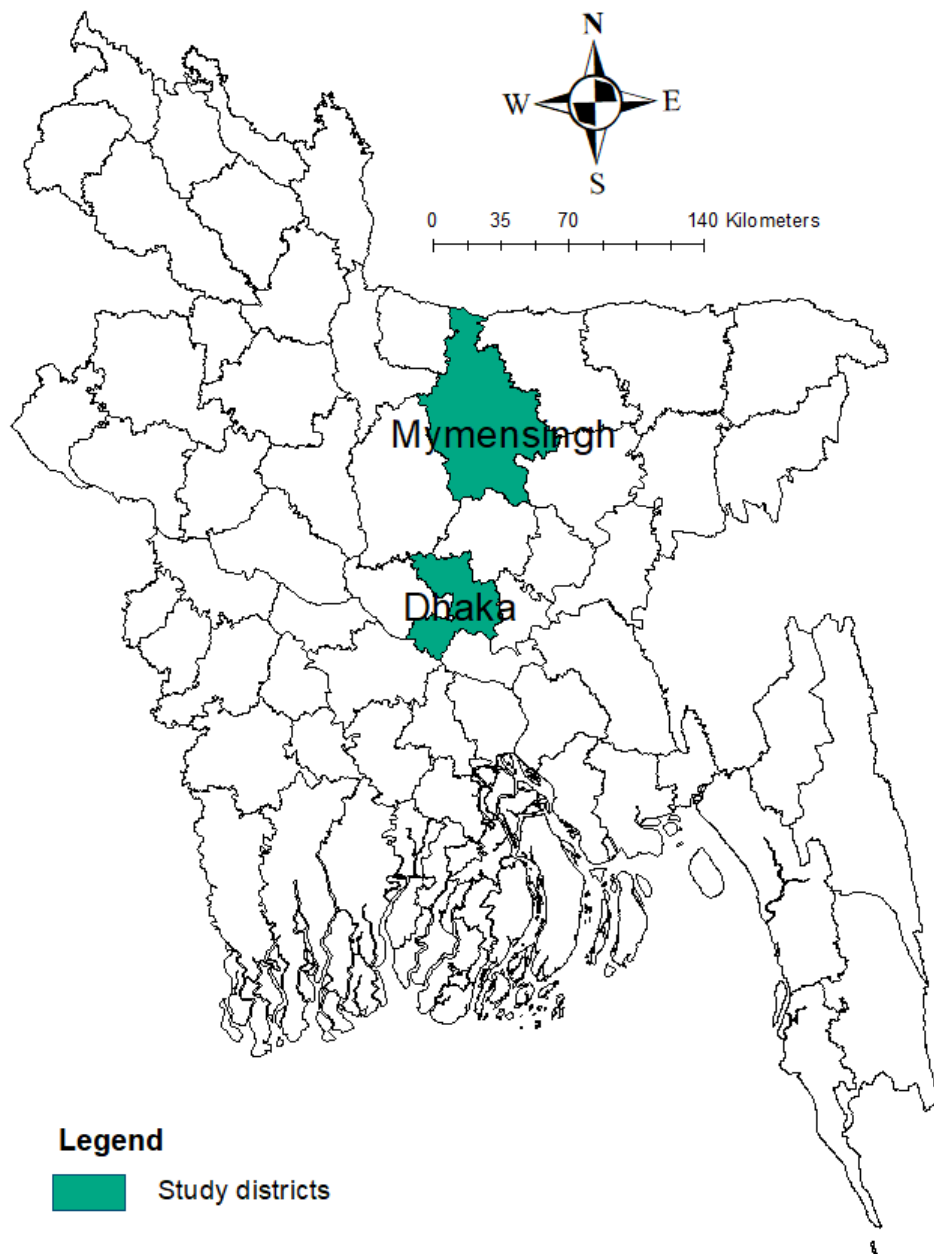


Figure 1: Map showing the study locations of the two districts: Dhaka and Mymensingh where economic impact assessment of bovine tuberculosis conducted.

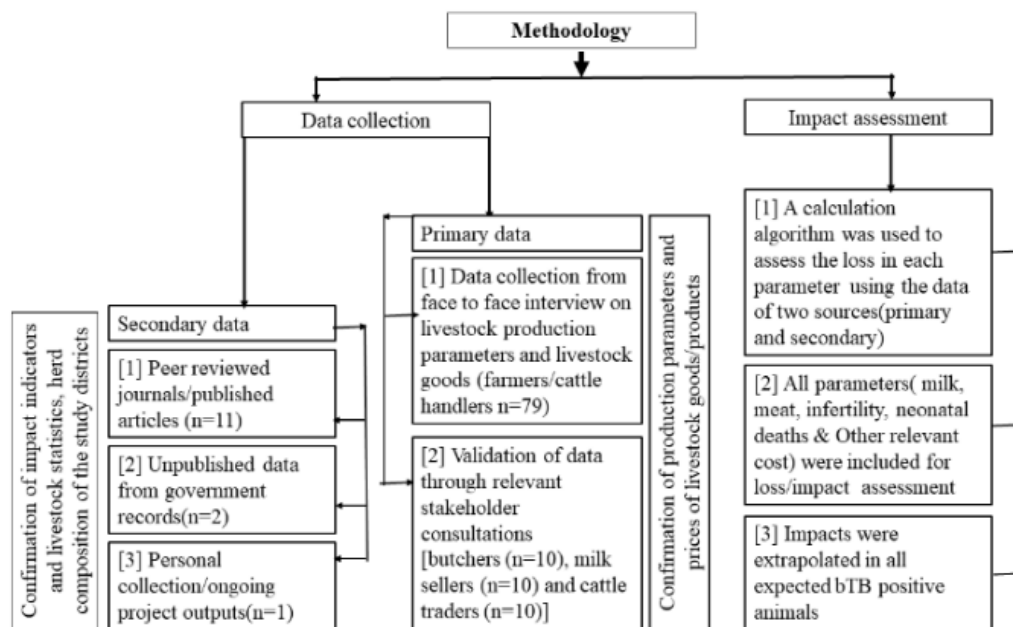


Figure 2: Diagram of financial impact assessment methodology in two cattle dominant districts (Dhaka, Mymensingh) of Bangladesh.

Table 1: Estimation of number of crossbred cattle in study districts (Dhaka and Mymensingh).

District/Sub-district (upazila)/Metropolitan area	Total cattle population (indigenous plus crossbred)	Basis for estimation of cross bred cattle	Crossbred /farming cattle	Reference
District: Dhaka	439,113			DLS. 2020.
Subs district/city corporation area:				
Dhaka metropolitan area (city corporation)	20,590	100%	20,590	
Dhamrai	116,338	15%	17,451	Hamid et al., 2017
Dohar	18,590	15%	2,788	
Keraniganj	73,245	15%	10,987	
Nowabgang	46,850	15%	7,027	
Savar	163,500	15%	24,525	
Total crossbred cattle in Dhaka district			83,368	
District: Mymensingh	917,000			
Subs district:				

District/Sub-district (upazila)/Metropolitan area	Total cattle population (indigenous plus crossbred)	Basis for estimation of cross bred cattle	Crossbred /farming cattle	Reference
Bhaluka	69,538	15%	10,431	Hamid et al., 2017
Dhobaura	62,582	15%	9,387	
Fulbaria	70,538	15%	10,581	
Gaffargaon	74,538	15%	11,181	
Gauripur	69,538	15%	10,430	
Haluaghat	74,570	15%	11,185	
Iswargang	70,538	15%	10,581	
Muktagacha	68,538	15%	10,281	
Sadar	70,544	15%	10,581	
Nandail	77,538	15%	11,631	
Phulpur	73,500	15%	11,025	
Trishal	71,538	15%	10,731	
Tarakanda	63,500	15%	9,525	
Total crossbred cattle in Mymensingh district			137,550	
Grand total in two districts	1,356,113		220,918	

Source: Department of Livestock Services, Krishi Khamar Sarak, Dhaka, Bangladesh

Economic impact assessment model

The livestock contributed 1.47% in national economy in the year 2018-19 with a growth rate 3.47% (DLS, 2019b). The country has 24.2 million cattle (DLS, 2019b), of which 15% crossbred cattle are being kept at farming condition (Hamid et al., 2017). A prevalence study was conducted in 1657 cattle of 66 farms in two districts (Islam et al., 2020). The data collected during the prevalence study has been tailored in this economic model. Of 1657 crossbred farming cattle, of which 86% (n=1419) were female and 14% (n=238) were male cattle included in that prevalence study. Among the two districts 195 positive reactors were identified, of them 177 were in Dhaka district and rest 18 were in Mymensingh district. Of 177 positive reactors in Dhaka district, 169 were female and 8 were male cattle. On the contrary, of 18 positive reactors in Mymensingh district 15 were female and 3 were male cattle. Among the two districts, an animal level prevalence was confirmed as 12.7% in Dhaka district and further confidence interval (95% CI) for prevalence was calculated as 11.0- 14.6% through exact binomial method based on the beta distribution using excel data analysis tool packs (Brown et al., 2001). Similarly, prevalence of bTB was obtained as 6.8% and 95% CI=4.1-10.6% was estimated in Mymensingh district.

In this study, we calculated the losses for the study districts (Dhaka and Mymensingh). The study do not include the indigenous cattle (Zebu) for estimating losses since they are almost resistant to bTB (Ameni et al., 2007) due to different factors in gene profiles are responsible for less susceptibility to indigeneous cattle than other breeds(Vordermeier et al., 2012). Fifteen percent (15%) crossbred animal presented in the national herd (Hamid et al., 2017) was used for estimation of crossbred cattle in two districts. However, one hundred percent crossbred cattle in Dhaka city corporation area was confirmed through discussion with government veterinarians and paraprofessionals and taken under consideration for this study. Finally, 83,368 heads of crossbred cattle in Dhaka district, 137,550 heads of crossbred cattle in Mymensingh district were estimated (Table 1). The proportion of cows and bull, mean age and category animals were taken from a prevalence study conducted by Islam et al., (2020). The prevalence of bTB in two districts with its probability distribution was extrapolated in whole crossbred cattle population, thus, we obtained total number of infected animals in two study districts. The losses reported in published articles in production parameters (like meat, milk, reduction of birth and perinatal deaths, etc.) were used in case of infected animals of study districts. Finally, losses in the livestock goods and relevant costs were then converted in to monetary figures considering the market price of the current year.

The study has assessed economic losses of bovine tuberculosis as per the method described by (Zeng et al., 2019) with minor adjustment stated in the following formula. The areas considered in this study are: decrease in milk and meat production, infertility and neonatal deaths including other costs (treatment, cleaning and sanitation and restocking cost if required).

$$\text{Economic loss}=[Dmi + Dme + Inf + Ned + Oth]$$

Here, ***Dmi***, cost for decreased milk production, where Dmi =number positive adult female X 70% milking cow x average milk production/cow x 250 days (average lactation length) x 18% reduction of milk in infected cow x cost of 1- liter milk).

Dme, cost for decreased meat production, Dme = Number of positive mature cows x average weight of a healthy cow + number positive mature bull x average weight of healthy bull) x 20% reduction of meat x cost for 1 kg of meat

Inf, cost for infertility, Inf = Number of positive adult female x 5% reduction in birth among the infected cows/heifers x cost for 1-month age crossbred calf

Ned=cost neonatal deaths, Ned = Number of positive mature female x 50% pregnant cow x 1% neonatal deaths x cost for 1month old calf.

Oth=other cost, Oth =veterinary health care cost applicable for infected animals+ cleaning/sanitation cost+ restocking cost after adjustment with carcass value (if needed).

Table 2: Parameters utilized in the financial loss assessment extracted from published and unpublished data, Government reports, personal communication from the government offices.

No#	Parameters	Data included in this study	Reference
1	Percentage(%) of crossbred cattle in national herd	15%	Hamid et al., 2017
2	Percentage(%) of crossbred cattle in DCC area	100%	Visualization/ discussion with field vets/paraprofessionals
3	Prevalence of bTB in animal level:		
3.1	Dhaka district	12.70% (95% CI=11-14.60%)	Islam et al., 2020
3.2	Mymensingh district	6.80% (95% CI=4.10-10.60)	
4	Infection status at animal level		
4.1	Cow	92.5%	Islam et al., 2020
4.2	Bull	7.5%	
5	Milking status(milking cow)	70%	
6	Pregnancy status	50%	
7	Average lactation (days) length of a crossbred cow?	250 days	Das et al., 2003, Hamid et al., 2017
8	Type of losses:		
8.1	Reduction of milk production in dairy cows	18%	Barwinek and Taylor, 1996, Ritacco et al., 1991, Rahman and Samad, 2008
8.2	Meat production	20%	Barwinek and Taylor, 1996
8.3	Reduction in births	5%	Barwinek and Taylor, 1996, Bernues et al., 1997
8.4	Perinatal deaths (calves)	1%	Barwinek and Taylor, 1996

Table 3: Parameters utilized in the financial loss assessment through semi-structure interview with cattle farmers/handlers, butcher/meat sellers, government and private veterinarians and paravets

No#	Parameters	Data collection method
1	Body weight a mature cow (average age ≥ 4 years): 60% of live weight	Field interview
2	Body weight a mature bull (average age ≥ 3 years): 60% of the live weight	Field interview
3	Average milk production liter/cow	Field interview
4	Price of a 1 month old cow/bull calf	Field interview
5	Price of a mature cow (≥ 4 years)	Field interview
6	Price of a mature bull (≥ 3 years)	Field interview
7	Price of 1 kg of beef	Field interview
8	Price of 1 liter of milk	Field interview
9	Treatment cost due to bTB infection	Field interview
10	Restocking cost if an animal have infertility or abortion	Field interview

Data management and statistical analysis

Data collected via field interview data collection method, viz., weight of a mature cow (≥ 4 years), weight of a mature bull (≥ 3 years), average milk production (liter/cow/day), price of a 1 month old cow/bull calf, price of a mature cow (≥ 4 years), price a mature bull (≥ 3 years), price of 1 kg of beef, price of a 1 liter of milk, treatment cost of a bTB infected cattle, restocking cost after adjustment of carcass value of a female cattle having infertility or abortion were presented in probability distribution functions (most likely, lowest value, highest value) using ModelRisk (VOSE, 2019) and presented in Table 3.

The data were captured and recorded in Microsoft Excel[®] worksheet. Proportion, percentage, were calculated using an excel data analysis tool pack for analyzing the financial impact in the various parameters like meat, milk, infertility, neonatal deaths along with other pertinent costs.

RESULTS AND DISCUSSION

Related to total number of crossbred farming cattle, the number of mature cows (≥ 3 years old) and bulls (≥ 2 years old) present in the studied districts were calculated as 110,459 (50% of total crossbred cattle population) and 2209 (1% total crossbred cattle population), of which 18,446 (95% CI=13,699-24,746) cows and 1496 (95% CI=1,111-2,006) bulls were predicted to be positive with bTB (Table 4).

Table 4: Expected number of bTB positive animal in study districts (Dhaka and Mymensingh)

District/ animal category	Expected number positive reactors	95% CI		Basis of calculation
		LL	UL	
Dhaka				Table 2 & 3
Cows (≥ 3 years old)	9794	8,483	11,259	
Bull (≥ 2 years old)	794	688	913	
Mymensingh				
Cows (≥ 3 years old)	8652	5217	13487	
Bull (≥ 2 years old)	702	423	1094	
Total cows	18,446	13,699	24,746	
Total Bulls	1,496	1,111	2,006	

CI= Confidence Interval, UL= Upper Limit, LL=Lower limit

The list of the variable inputs captured through field interview data collection: prices of live animals and livestock products used for the estimation economic impact is shown in Table 5.

Table 5: List of the variable inputs captured through field interview data collection: prices of live animals and livestock products used for the estimation economic impact.

Parameters	Pert distribution		
	Most likely	Minimum	Maximum
Weight of a mature cow (≥ 4 years): 60% of live weight (Kg)	240	200	360
Weight of mature bull (≥ 3 years): 60% of the live weight (Kg)	260	160	360
Average milk production (liter/cow)	20	6	32
Price of a 1 month old cow/bull calf (BDT)	40,000	20,000	70,000
Price of a Mature cow (≥ 4 years) in BDT	200,000	120,000	300,000
Price a mature bull (≥ 3 years) in BDT	200,000	70,000	300,000
Price of 1 kg of beef (BDT)	550	500	600
Price of a 1 liter of milk (BDT)	60	45	90
Treatment cost/ infected animal (BDT)	500	200	1,000
Cleaning or disinfection cost/ infected animal	200	500	1,000
Restocking cost of a new animal after adjustment with carcass value if an infected animal having infertility or abortion	68,000	10,000	102,000

The annual economic losses due to decrease milk and meat production, infertility, neonatal deaths along with other relevant costs (veterinary health care, cleaning and disinfection and restocking cost if needed) are presented in Table 6. The total losses from the disease were estimated to be BDT 1347.72 million (95% CI= 997.43-1801.65 million) (Equiv. US\$ 16.04 million, 95% CI= 11.87-21.45 million) in farming cattle of two districts. The largest economic loss was estimated due to decrease milk production (BDT 697.25 million, 95% CI= 517.86 -935.39 million) followed by decreased meat production (BDT 531.22 million, 95% CI= 391.01- 706.27 million). Other relevant costs (veterinary health care, cleaning and disinfection and restocking cost if required) was found to be 3rd position with an annual loss of BDT 71.18 million (95% CI= 53.91-93.87 million). However, infertility and neonatal deaths were also captured as a substantial loss parameter in the study districts (Table 6, Figure 3 & 4).

Table 6: Annual economic losses (in BDT) due to reduced milk and meat production, infertility in female cattle, neonatal deaths along with other losses with 95% CI in the studied districts

Parameters/category	District	Estimated	LL	UL
Milk	Dhaka	370.21	320.66	425.59
	Mymensingh	327.05	197.20	509.81
Total milk		697.26	517.86	935.40
Meat	Dhaka	284.28	242.11	321.34
	Mymensingh	246.95	148.90	384.94
Total meat		531.22	391.01	706.28
Infertility	Dhaka	19.59	16.97	22.52
	Mymensingh	17.30	10.43	26.97
Total infertility		36.89	27.40	49.49
Neonatal deaths	Dhaka	1.96	1.70	2.25
	Mymensingh	1.73	1.04	2.70
Total neonatal deaths		3.69	2.74	4.95
Other cost:				
Vet health care		5.29	4.59	6.09
Cleaning and disinfection		3.18	2.75	3.65
Restocking		33.30	28.84	38.28
Total other cost in Dhaka		41.77	36.18	48.02
Other cost: Mymensingh				
Vet health care		4.68	2.82	72.90
Cleaning and disinfection		2.81	1.69	43.74
Restocking		29.42	17.74	45.86

Parameters/category	District	Estimated	LL	UL
Total other cost in Mymensingh		36.90	22.25	57.52
Total other cost		78.67	539.92	93.87
Total economic loss (1) Dhaka		717.80	617.61	819.72
(2) Mymensingh		629.92	379.83	981.94
Grand total (BDT) in two districts		1347.73	997.44	1801.66
US\$ in million		16.04	11.87	21.45

BDT= Bangladeshi Taka, CI= Confidence Interval, LL=Lower Limit, UP=Upper limit

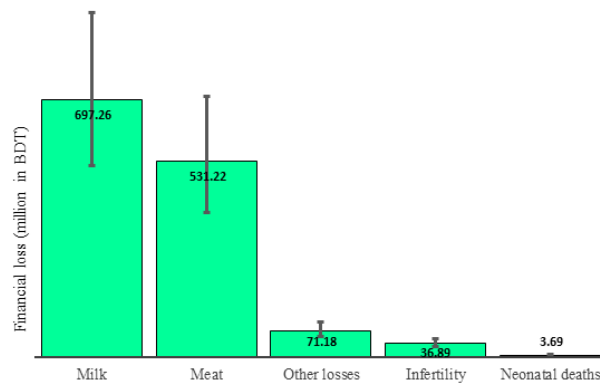


Figure 3: Estimated annual economic losses due to decrease milk, meat production, infertility and neonatal deaths along with other losses (cost for veterinary health care, cleaning and disinfection and restocking cost if applicable) (BDT in million with 95% CI) of bovine tuberculosis (bTB) in the study districts.

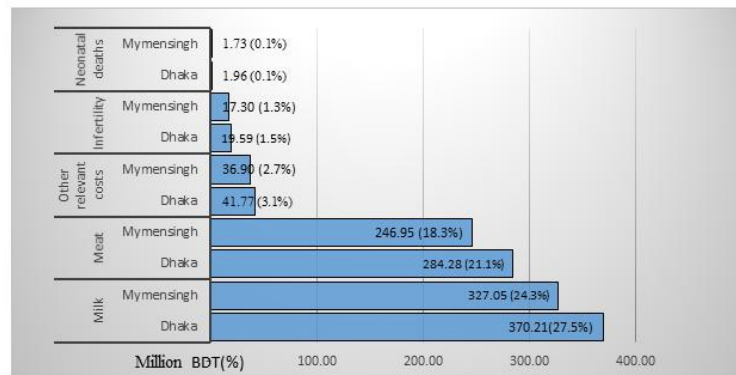


Figure 4: Comparison of economic losses incurred at different loss parameters (milk, meat, infernality, neonatal deaths and other relevant costs in two study districts.

A number of economic studies have been conducted in different countries in the world highlighting the potential losses for the livestock raisers (Homem et al., 2016; Tschopp et al., 2012; Gilsdorf et al., 2006). However, no research has been conducted in Bangladesh. The study has estimated an economic loss of BDT 1347.72 million (95% CI= 997.43-1801.65) in farming cattle of two districts is reasonably found to be higher due to high prevalence of bTB in these study districts. Moreover, the study has accounted a higher economic loss of bTB in Dhaka due to influence of comparatively animal-level higher prevalence among two districts. Due to paradigm shifting of livestock rearing from indigenous to crossbred exotic cattle in urban and periurban settings of Bangladesh since 1980s (Islam et al., 2021) that enables to the burden of bTB (Ameni et al., 2007).

The study revealed that highest bTB losses related to decrease milk production in farming cattle (BDT 697.25 million, 95% CI= 517.86 -935.39 million) followed by decrease meat production (BDT 531.22 million, 95% CI=391.01-706.27 million). This finding is coherent with another research was conducted in Brazil (Homem et al., 2016) and discreetly consistent with another study conducted in Ethiopia (Tschopp et al., 2012). According to the Department of Livestock Services(DLS), Bangladesh, the country's total milk production was 9.40 million tonnes in 2018 against 15.4 million tonnes that implies 63 percent demand was fulfilled through own production (DLS, 2018). However, Bangladesh imported 0.11 million tonnes of powdered milk to lessen the production gap in the same year (BBS, 2018). Bangladesh is now self-reliant in meat production after various measures were taken by the government to boost the country's livestock sector. At present Bangladesh is exporting meat on a small-scale to Middle East countries and the Maldives (DLS, 2018) and is eyeing to export at a wider scale to other countries in near future (LAW, 2019). The impact of bTB will impede on meat and milk production that widens the gap between demand and supply of animal origin food due to production losses, and further it will delay to export meat and meat products as non-compliance of the parameters set by World Organization for Animal Health (WOAH, 2019).

In the past, there was no program to support for mitigating the burden of bTB in Bangladesh, like active surveillance, regular screening program or to devise new techniques for disease diagnosis. Recently, government has taken a several development projects (4.35 billion BDT investment) through World Bank and Government of Bangladesh (GOB) financing will support to the dairy farmers on sustainable and safe livestock production with an aim create market linkage; and to mitigate the burden on zoonotic diseases (anthrax, bTB, brucellosis and rabies) through assist to enhancing veterinary public health service namely enhancing laboratory diagnostic capacities, screening facilities along with provision of compensation payment to the cattle farmers due to loss of zoonotic disease (DLS, 2019a).

Bovine tuberculosis (bTB) is not a disease that obtains the more consideration in developing countries like many other economic important infectious diseases like foot and mouth disease (FMD) and peste des petits ruminants (PPR). Moreover, as the burden of bTB has not yet been evaluated in most of the countries, key stakeholders are not aware of the actual impact of this disease including problem in human health (Juleff et al., 2019).

Dairy production system in low and middle income countries deserves to significant development support as majority of production comes from small scale dairy farms, hence dairy development has contributed to economic growth, nutrition and poverty elimination along with food security. As bTB has documented as declined livestock productivity in terms of meat and milk production and infertility that rationalizes to bTB control program in farming cattle as an utmost priority (Juleff et al., 2019). The absence of convincing policies in dealing with bTB is an important barrier where stakeholders did not cogitate to be a major problem in developing countries (Chauhan et al., 2019).

The study has some limitations: it did not include all potential direct and indirect losses incurred due to bTB in farming cattle and not cover the entire dairy zones of Bangladesh that failure to capture the burden of bTB at the full swath. Additionally, the impact in human health did not address in this study. However, cost of TST did not included in this study for estimating bTB burden at source animal as the country is not practicing this approach in full scale. The study highlights the impact on productivity of crossbred farmed cattle along with the associated losses of bTB in study districts.

To overcome the above burden of of bTB policy framing through inclusion of test and slaughter approach could be adopted to reduce the risk further transmission between animal to animal or animal to human at animal-human interface is very much needed. The sustainability of test and slaughter approach depends of compensation payment for affected farmers (Cosivi et al., 1998). This will be helping for getting farmers support as it has a direct impact on their livelihood. However, the country is practicing this approach on a limited scale.

Considering public health and food safety measures, more than half a dozen milk processors are processing 1 million litres of fresh milk daily through Ultra Heat Treatment) (UHT)/pasteurized milk category for supplying safe food for the consumer (Parvez, 2018). The results recommend the bTB has directed to significant losses in livestock and their products. The impacts they trigger on agriculture, livelihood of marginal communities, and food safety on animal origin food should be promptly to communicate to policy planners and prevention and control measures to be taken accordingly. The model formulated in this study is comparatively simple and can be adapted for estimating the burden of bTB for the whole country.

CONCLUSION

In this study, we attempted to estimate losses incurred by cattle farms due to bovine tuberculosis (bTB) disease of crossbred cattle in two important cattle rearing districts, i.e., Dhaka and Mymensingh, of Bangladesh. The losses, estimated from the bTB prevalence in these two districts were found to be of enormous in terms of livestock productivity. Minimization of the burden of bTB, therefore, has become very essential as intensive cattle farming is being practiced in Bangladesh presently with the possibility of further intensification in the future. Importance should be given to bTB screening in urban and periurban dairy zones where intensive cattle farming has developed, and segregation of reactor animals from the infected herds is required. Milk dealers and the processing industries should collect milk only form bTB free herds, which should inspire livestock farmers to be cautious against the infection and spread of bTB in their

respective herds. The government should ensure free of cost routine screening and subsequent confirmatory tests and provide compensation for the culling of animals tested bTB positive. Initiatives should be taken to raise dairy farmers' awareness regarding bTB and educate them on the impacts of bTB on animal and public health, transmission dynamics in a one health approach.

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EFFECT OF FLOCK SIZE ON THE PRODUCTION PERFORMANCES OF JAMUNA BASIN LAMB IN BANGLADESH

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ABSTRACT

The effect of flock size on the production performances of Jamuna basin lambs from selected areas of two upazilas (sub-districts) of Sherpur district in Bangladesh was studied. Growth performance data from 600 lambs were collected during January 2019 to December 2020. Sixty households (30 from Sherpur Sadar upazila and 30 from Nalitabari upazila) participated in the study. Sex had a significant effect on age (1-12 months) related weight ($p < 0.05$). Body weight and average daily gain (ADG) at different ages of lamb were found to be higher for male than those for female lambs, and these were higher for lambs of the 5-head flock than those of lambs of either the 10-head or 15-head flock. The litter size significantly affected the birth weight ($p < 0.001$) and weight at 3 months ($p < 0.05$) of lambs with different flock sizes. Single born lambs had a higher birth weight and ADG than those for twins and triplets and these weights were higher for lambs of the 5-head flock than those of lambs of either the 10-head or 15-head flock. The ADG was found to be higher in char areas than in hilly areas, and, again, lambs of the 5-head flock outperformed those of either the 10-head or 15-head flock. Likewise, ADG was observed to be higher in the winter season than in the summer season, and the effect of flock size was similar. The prevalence of diseases like diarrhoea, bloat, pneumonia and mortality rate were higher for lambs of the 10-head flock (28.24%) or 15-head flock (33.14%) than that for lambs of the 5-head flock. Thus, maintaining a flock size of 5-10 of Jamuna basin lamb lambs appeared to be an appropriate strategy for rural lamb farmers of for semi-intensive sheep rearing in rural livestock farming conditions of Bangladesh.

Keywords: Average daily gain, flock size, growth traits, Jamuna basin lamb, production performance

INTRODUCTION

Bangladesh has 3.607 million sheep securing 3rd position in number among the ruminant species of the country (DLS, 2020). About 50% of the people are indirectly and 20% directly dependent on livestock rearing in Bangladesh and livestock contributes about 1.47% of the country's GDP (DLS, 2020). Most of the sheep are indigenous, with few crossbreeds (Sun et al., 2020) and are capable of bi-annual lambing and multiple births.

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Lack of public awareness, misconception about sheep meat (lamb/mutton) and inadequate nutrition supplementation for the animals are the limiting factors for sheep farming in Bangladesh (Hossain et al., 2021a). Good nutrition and management play a vital role on sheep and other ruminant production (Hossain et al. 2018a). The Jamuna basin sheep lives well on local grass, tree leaves along with some concentrate feeds (Hashem et al., 2020). It is known that more than 90% of the feed consumed by the ruminants are roughages. In the traditional feeding systems, sheep are raised on harvested or fallow lands, roads and canal sides (Sultana et al., 2010) and they also graze on aquatic weeds and grass in knee-deep water without any feed supplementation. Majority of the sheep in Bangladesh can be easily maintained under rural conditions because of their ability to adapt to harsh environments, poor management and feeding practices, and they can meet human needs for animal protein partially (Hashem et al., 2020; Hossain et al., 2021b)

Sheep not only supply much needed meat for the markets of Bangladesh but also are an important source of livelihood and income for the rural population (Hossain et al., 2018b). Sheep farming requires less capital than other agricultural enterprises. However, this sector is characterized by a lack of technical services, training and production incentives (Hahsem et al., 2020). Despite this the rate of growth of livestock production has been the second highest among the sub-sectors of agriculture in Bangladesh (Sarker et al., 2017; Rahim et al., 2018). To improve lamb productivity selection of good breeds of sheep with optimum growth, development and meat yield (Gizaw, 2013). The effect of ewe breed type on performance indicators like lamb performance (Fogarty et al., 2005), lambing difficulty (Speijers et al., 2010), prolificacy rate (Dawson and Carson, 2002) and health traits (Nieuwhof et al., 2008) have been studied extensively. In terms herd management, flock size is an important parameter for financial success of lamb farming. To the authors' knowledge, no study has been carried out on the effect of flock size on lamb production and profitability in char and upland areas of Bangladesh. This study was undertaken to determine the appropriate flock size of lambs for semi-intensive sheep rearing in rural livestock farming conditions of Bangladesh.

MATERIALS AND METHODS

Study area

The study was conducted in two Upazila (Sherpur Sadar and Nalitabari) of Sherpur district. Sherpur district is bounded on the North by India, on the East by Mymensingh district, on the South and West by Jamalpur district and located about 198 kilometres north of capital Dhaka.

Source and management of data

The on-farm data were collected by trained enumerators from July 2019 to June 2020 for the study. Lambs were identified by permanent plastic ear tags. Records on birth date, birth weight, and postpartum ewe body weight, litter size, sex and dam parity were taken within 24 h from birth. The weights of lambs were taken every 30 days using digital scale.

Animals and Management

Flock size

The farmers received according to flock size such as 5, 10 and 15 sheep constitutes a flock of 5, 10 and 15 flocks having one ram in each group. Sixty households were selected in the study areas divided into equal 20 flock sizes in each group of 30 from Sherpur Sadar and 30 from Nalitabari upazilas.

Flock management

All the farmers reared their lamb in semi-intensive system. All lambs were kept inside the house at night and grazed 6-7 hours in day time. Leaves of tree *viz.*, mango, jack fruit, banana leaves or cutting grass from cultivated land was fed during the rainy season. A few farmers used to concentrate (crushed wheat-68%, soybean meal-30%, DCP-0.5%, vitamin-mineral premix-0.5% and common salt-1% containing 18% CP and 12 MJME/kg DM in the morning and afternoon at the rate of 150g/lamb/d and pure drinking water was supplied *ad libitum* for lambs. Farmers supplied a little bit more grass and tree leaves to their lactating ewes than pregnant ewes while they supplied more amount of concentrates feed to their pregnant ewes than lactating ewes. The sheep flocks were de-wormed against internal and external parasites with ivermectin injection and applied three times per year. They were vaccinated against PPR and after one month again vaccinated booster doses for better results.

Statistical analysis

Tabular technique was applied for the analysis of data using descriptive statistical tools such as frequency, average and percentages, standard deviation etc. using SPSS-v-20 version computer software.

RESULTS AND DISCUSSION

Effect of different flock size and sex of lambs on weight of age from birth to 12 months

The effect of different flock sizes and sex of lambs on weight of age from birth to 12 months is presented in Table 1. Male and female had found some variations in age weight at different flock sizes of lambs. Highly significant ($p < 0.001$) variation was observed at birth weight between male and females of 5, 10 and 15 flock sizes lamb. Higher body weight (7.03, 7.00 kg) observed in 5 flock size lambs compared to 10 (6.73, 6.50 kg) and 15 (6.30, 6.25 kg) flock sizes lamb at the age of three months which had significant ($p < 0.05$) effect. Major findings indicated that male lambs had usually higher weight at birth and grew faster than females (Deribe et al., 2014), while few report showed that there was less general effect of sex on body weights (Legesse, 2008). Loos et al. (2001) reported that the presence of a Y-chromosome and the products of sry gene activation to have sex-specific effects on foetal growth and males appeared to be grown faster than respective females in uterus. The higher weight and faster growth of male over female might be explained by the favour of sexual precocity articulated on body growth. This sexual dimorphism favours body growth in males than females. Hormonal differences between sexes and their resultant effects on growth may also be implicated. The difference in sex hormones and sexual dimorphism affects feed intake, growth rate and

feed efficiency (Mabrouk et al., 2010). In this study the general trend of sex variation was evident at later ages of lambs, which agreed with the findings of Hassen et al. (2002) who reported the importance of sex on older lambs in Ethiopia.

Table 1: Effect of different flock sizes and sex of lambs on weight of age from birth to 12 months (Mean±SE)

Age wt. (month)	5 group		10 group		15 group		Level of sig.
	Male	Female	Male	Female	Male	Female	
At birth	1.44±0.12	1.42±0.15	1.47±0.02	1.45±0.03	1.46±0.01	1.44±0.02	***
3	7.03±0.20	7.00±0.20	6.73±0.17	6.50±0.11	6.30±0.16	6.25±0.16	*
6	10.69±0.41	9.93±0.46	10.23±0.41	9.71±0.40	9.35±0.35	9.35±0.36	NS
9	15.05±0.55	14.75±0.56	14.78±0.45	13.78±0.54	13.81±0.45	12.44±0.43	NS
12	19.19±0.71	16.74±0.72	18.21±0.65	16.68±0.63	17.11±0.54	16.49±0.51	NS

Level of significant, * (p<0.05), *** (p<0.001), NS= non-significant

Effect of different flock sizes and sex of lambs on litter size of age from birth to 12 months

The effect of different flock sizes and sex of lambs on litter size of age from birth to 12 months is presented in Table 2. Litter size is also an important parameter for gaining body weight at different stages of life in three flock sizes. The birth weight of male and female lamb from single lambing than twin and triplet lambing observed significantly ((p<0.001) higher in 5 flock size compared to 10 and 15 flock sizes lambs. There is finite capacity to the maternal uterine space to gestate offspring (Gardner et al., 2007) as litter size increases individual birth weights decline. Higher significant (p<0.05) growth weight of male and female at 3 months aged for 5 flock sizes lambs was found from single lambing than that of twin and triplet lambing compared to 10 and 15 flock sizes in the study areas. There were no statistically significant differences of body weight (p>0.05) from 6 to 12 months of ages in all flock sizes of lamb. Prakash et al. (2017) reported that the effect of litter size of lambs significantly affected the growth performance in Garole sheep. The effect of litter size on growth traits showed consistency as the age of lambs advanced. The higher litter size percentage of multiple births single (47%), twins (48%) and triplets (5%) might be resulted in lower body weights of individual lambs at birth but had improved the overall lamb outputs (Hashem et al., 2020). Generally, single births attained higher body weights up to 12 months of age than twins and triplets in three flock sizes. The higher body weights of single births were due to the fact that they were the sole consumers of their dam's milk while twins and triplets compete for limited milk from their dams (Gemiyo et al., 2014).

Table 2: Effect of different flock sizes and sex of lambs on litter size of age from birth to 12 months (Mean \pm SE)

Group	Age	Single		Twin		Triplet		Level of sig.
		Male	Female	Male	Female	Male	Female	
5	Birth	1.61 \pm 0.11	1.53 \pm 0.16	1.48 \pm 0.17	1.38 \pm 0.19	1.29 \pm 0.38	1.27 \pm 0.48	***
10		1.47 \pm 0.11	1.44 \pm 0.16	1.44 \pm 0.17	1.38 \pm 0.19	1.27 \pm 0.38	1.17 \pm 0.48	
15		1.45 \pm 0.10	1.44 \pm 0.14	1.43 \pm 0.16	1.42 \pm 0.18	1.40 \pm 0.28	1.39 \pm 0.38	
5	3 months	6.87 \pm 0.61	6.06 \pm 0.53	6.36 \pm 0.45	5.86 \pm 0.44	5.70 \pm 0.95	5.15 \pm 1.12	*
10		6.70 \pm 0.33	6.33 \pm 0.02	6.33 \pm 0.42	5.69 \pm 0.39	5.52 \pm 0.96	5.41 \pm 1.04	
15		6.06 \pm 0.34	5.83 \pm 0.37	5.43 \pm 0.44	5.33 \pm 0.39	6.60 \pm 0.12	5.21 \pm 0.80	
5	6 months	11.43 \pm 0.87	10.17 \pm 0.77	10.73 \pm 0.51	10.23 \pm 0.64	9.44 \pm 1.31	9.17 \pm 0.70	NS
10		10.54 \pm 0.43	9.99 \pm 0.58	9.84 \pm 0.56	9.23 \pm 0.62	9.62 \pm 1.00	9.52 \pm 1.05	
15		9.91 \pm 0.49	8.99 \pm 0.47	8.59 \pm 0.52	7.78 \pm 0.61	7.73 \pm 0.70	7.34 \pm 0.93	
5	9 months	16.07 \pm 1.11	13.44 \pm 0.95	14.44 \pm 0.67	13.33 \pm 0.81	14.29 \pm 1.67	14.23 \pm 1.1	NS
10		15.16 \pm 0.56	14.20 \pm 0.72	13.73 \pm 0.87	13.22 \pm 0.78	14.02 \pm 1.67	13.32 \pm 1.63	
15		15.90 \pm 0.61	14.72 \pm 0.57	13.52 \pm 0.65	13.22 \pm 0.77	12.8 \pm 0.92	12.40 \pm 1.11	
5	12 months	19.19 \pm 0.71	17.27 \pm 1.15	18.71 \pm 0.78	16.03 \pm 0.97	18.26 \pm 1.96	15.98 \pm 1.12	NS
10		18.81 \pm 0.88	16.58 \pm 1.23	18.08 \pm 1.06	16.63 \pm 1.90	17.20 \pm 1.85	15.20 \pm 1.79	
15		19.19 \pm 0.76	16.69 \pm 1.23	16.92 \pm 0.74	15.04 \pm 1.94	16.32 \pm 0.52	14.92 \pm 1.15	

Level of significant, * ($p < 0.05$); *** ($p < 0.001$), NS= non-significant,

Effect of different flock of litter sizes on average daily gain (ADG) (g/d) from 1 to 12 months of age

The effect of different flock of litter sizes on average daily gain (ADG) (g/d) from 1 to 12 months of age is presented in Table 3. The ADG of litter sizes showed almost similar in all flock sizes lambs having no significant effect ($p > 0.05$) at the age of one month. The higher significant ($p < 0.05$) ADG observed from three months aged in single lambs compared to twin and triplet lambs in 10 flock sizes lamb. The higher significant ($p < 0.01$) ADG observed from nine months aged in single lambs compared to twin and triplet lambs in 5 flock size lamb in the study areas. The flock size and ewe breed type had a significant impact on key flock performances variables (Bohan et al., 2017). The flock size had been shown to affect the labour efficiency of farms across both sheep flock and dairy herds (Austin et al., 2008) with a lower labour requirement per lamb required on larger flock and herds. Previous international sheep researches on flock size had focused on individual flock management and behaviour variables such as lamb grazing time, social foraging (Michelena et al., 2009). Generally, single births attained higher body weights and ADG than twin and triplets in 5 flock sizes than 10 and 15 flock sizes lamb.

Table 3: Effect of different flock of litter sizes lambs on average daily gain (ADG) (g/d) from 1 to 12 months of age (Mean \pm SE)

Age month	5 groups			10 groups			15 groups			Level of significance
	Single	Twin	Triplet	Single	Twin	Triplet	Single	Twin	Triplet	
1	122 \pm 0.40	113 \pm 0.15	93 \pm 0.66	122 \pm 0.4	112 \pm 0.28	93 \pm 0.66	122 \pm 0.40	113 \pm 0.28	94 \pm 0.62	NS
3	71 \pm 0.57	68 \pm 0.45	60 \pm 1.04	73 \pm 0.38	66 \pm 0.42	61 \pm 1.00	66 \pm 0.36	60 \pm 0.39	58 \pm 0.75	*
6	60 \pm 0.82	58 \pm 0.58	52 \pm 1.01	57 \pm 0.57	53 \pm 0.59	53 \pm 1.03	53 \pm 0.48	45 \pm 0.57	42 \pm 0.82	NS
9	55 \pm 1.03	51 \pm 0.74	53 \pm 1.39	54 \pm 0.64	50 \pm 0.83	51 \pm 1.65	50 \pm 0.71	49 \pm 0.59	47 \pm 1.02	**
12	52 \pm 1.32	48 \pm 0.88	48 \pm 0.88	49 \pm 1.06	48 \pm 1.48	45 \pm 1.80	46 \pm 1.00	44 \pm 1.34	43 \pm 0.84	NS

Level of significant, *($p < 0.05$), ** ($p < 0.01$); NS- non-significant

Effect of different flock sizes of lambs on ADG at different locations from one to 12 months of age

The effect of different flock sizes of lambs on ADG at different locations from one to 12 months of age is presented in Table 4. A significant higher ($p < 0.01$) ADG (120g/d) observed from 5 flock size of char area compared to hilly area from one month than that of 10 and 15 flock sizes. The variation in growth traits among locations also concurred with previous results that had compared performances of sheep in different locations (Mukasa-Mugerwal et al., 2000). They showed significant differences between Horro and Menz lambs in birth weight (2.4 vs. 2.1kg). In the present study the lambs born in grazing land available area (char area) showed higher growth rate indicating that feed was available for lambs and lactating ewes. Lambs in char area from 10 and 15 flock sizes weight were heavier than hill area at the age of 1 to 12 months except 5 grouped lamb sizes at six month of age. The variation in growth performance between these areas might be an indication that lambs in char area had better management of flock sizes lamb. The difference in growth performance between areas might also be a positive feature to improve the management practices.

This variation was recorded in growth performance between char and high land areas could be an asset and gave a pin picture to improve the genetic potentials of local sheep with a long-term selection process. Hassan and Talukder (2011) observed differences birth and body weight gain to the lambs of three ecological zone of Bangladesh which was closer to the present study. Lamb born in char area had higher daily weight gain than hilly area. These values were agreed with observation of Gbangboche et al. (2006). Both locations were not significant ($p > 0.05$) effect in all stages of ages of flock sizes under the study, except ADG of one month of age. Male lambs were superior over their female contemporaries but sex effect had not significant in the study in all flock sizes. Deribe et al. (2014) reported the higher ADG of male than female which was similar to the present study.

Table 4: Effect of different flock sizes of lambs on ADG at different locations from one to 12 months of age (Mean \pm SE)

Age (Month)	Locations						Level of significance
	Char			Hilly			
	5 group	10 group	15 group	5 group	10 group	15 group	
1	120 \pm 1.90	109 \pm 1.80	106 \pm 2.06	108 \pm 1.78	104 \pm 1.79	102 \pm 1.77	**
3	73 \pm 2.55	73 \pm 2.56	67 \pm 2.46	69 \pm 2.49	69 \pm 2.48	60 \pm 2.33	NS
6	52 \pm 3.05	57 \pm 3.19	52 \pm 3.06	56 \pm 3.26	52 \pm 3.26	47 \pm 2.91	NS
9	52 \pm 3.76	57 \pm 3.91	51 \pm 3.72	53 \pm 3.77	51 \pm 3.80	47 \pm 3.56	NS
12	50 \pm 4.25	52 \pm 4.32	46 \pm 4.07	47 \pm 4.10	46 \pm 4.01	45 \pm 4.01	NS

Level of significant, ** ($p < 0.01$), NS= Non-significant

Effect of different flock sizes of lambs on ADG at different seasons from one to 12 months of age

The effect of different flock sizes of lambs on ADG at different seasons from one to 12 months of age is presented in Table 5. The ADG of winter season in three month of age compared to summer season observed almost similar in three flock sizes lamb which had significant effect ($p < 0.05$). Same trend of ADG of winter season compared to summer season was found in 9 months of age which had statistically significant ($p < 0.01$). No statistical significant effect ($p > 0.05$) was found from both seasons of one, six and 12 months of age in three flock sizes. Generally, higher birth weight and subsequent weights were found better during the winter season in all flock sizes of lambs. This could be associated with seasonal nutrient fluctuations and feed selection behavior of sheep (Deribe et al., 2014). The influence of season on birth weight, weight at various ages and ADG had been well documented at station (Teklebrhan et al., 2014) and under smallholder management systems (Hassen et al., 2002). They reported higher body weights and ADG of lambs during the dry season and part of light rainy seasons was consistent with other reports (Teklebrhan et al., 2014). The higher body weight and faster gain during early winter (dry season) at some ages was partly due to better body reserves fat of dams at the end of summer season, and the associated higher milk yield. It had been noted that during late winter lambs born with higher weight and usually grew faster due to feed flushing of the dams during early winter season (Deribe et al., 2014). Yilmaz et al. (2007) reported seasonal differences on weight of lambs due to differences in ambient temperature and maternal prenatal effects during gestation. According to Gbangboche et al. (2006) effects of heat stress affects feed intake and consequently body weights among indigenous lambs in sub-humid ecological zones. The quality of feed and variations in feed composition as well as milk yield of dams was a factor that had frequently reported to influence lamb weight during early growth period (Yilmaz et al., 2007) which was in accordance with the findings of Zapasnikiene (2002). The ADG of winter born lambs for 5 flock size compared to 10 and 15 flock sizes was better than summer born lambs. Suitable environment, plenty of feed were available in the winter season under the study areas. Similar observation was noted by Deribe et al. (2014). In summer (June to

September), both area were affected by flood. This causes the scarcity of feed that leads to the lower ADG than winter season in all flock sizes of lambs.

Table 5: Effect of different flock sizes of lambs on ADG at different seasons from one to 12 months of age (Mean \pm SE)

Age (Month)	Seasons						Level of significance
	Winter			Summer			
	5 group	10 group	15 group	5 group	10 group	15 group	
1	113 \pm 0.18	108 \pm 0.11	107 \pm 0.07	111 \pm 0.05	107 \pm 0.10	106 \pm 0.05	NS
3	71 \pm 0.23	63 \pm 0.19	57 \pm 0.21	70 \pm 0.12	63 \pm 0.16	57 \pm 0.20	*
6	56 \pm 0.28	55 \pm 0.33	49 \pm 0.36	54 \pm 0.29	55 \pm 0.22	49 \pm 0.33	NS
9	50 \pm 0.44	50 \pm 0.44	45 \pm 0.45	48 \pm 0.24	49 \pm 0.40	45 \pm 0.44	**
12	48 \pm 0.63	46 \pm 0.62	43 \pm 0.48	47 \pm 0.22	47 \pm 0.60	42 \pm 0.44	NS

Level of significant, *($p < 0.05$), ** ($p < 0.01$), NS= Non-significant

Mortality of different flock sizes of lambs in the study area

The mortality of different flock sizes of lambs in the study area is presented in Table 6. Average mortality rate was found 24.93%. The highest mortality was 17.50% at the age of one month and no mortality observed at 12 months of age. Mortality rate of winter season (15.30%) was higher than summer (7.52%). The highest mortality rate (12.2%) was found during spring season following (5.5%) in summer season (Lateef and Bashir, 2014). This findings did not agree with the present study. The seasonal differences in mortality might be the result of severe drought conditions leading to fodder shortage in the study areas. Triplet mortality was higher (33.33%) than single (12.50%) and twin lambing (15.50%) due to lacking of mother's milk and faulty management. This result was agreed with the result of Mustafa et al. (2014). Fifteen flock size lambs mortality rate was higher (33.13%) than 05 (13.44%) and 10 flock sizes (28.24%) due to lacking of sufficient floor space, adequate nutrition and weak management. Total mortality was found 21% at neonatal stage. The neonatal period was the most vulnerable time in the life of a lamb, with almost half of all pre-weaning mortalities occurring on the day of birth (Nowak and Poindron, 2006). The causes of neonatal death was poor management, poor mothering of ewes, pneumonia and other infectious diseases. The number of lambs born per ewe was certainly an economically important trait in a commercial sheep enterprise. Hassan and Talukder (2011) found average lamb mortality (12.4%) in native sheep which was lower than present study. Similar results observed from Nimbkar et al. (2002) where they found 13.8% mortality in Garole sheep of India which was not agreed to the present study. Better management practices before lambing and care of lambs from birth to three months of age could play an important role in reducing mortality due to the adaptation of survivability. Mortality rate was higher (12%) in hilly area than char area (10%) due to lack of green grass, lack of better management and geographical locations. The overall lamb mortality rate of 5 flock sizes was less than 10 and 15 flock sizes lamb in the study areas.

Table 6. Mortality of different flock sizes of lambs in the study area

Parameter	Number of lamb died	Per cent	Mean±SEM
Age:			
1 month	6	17.50	2.20±0.81
3 month	4	12.25	2.15±1.00
6 month	4	6.15	1.21±0
9 month	2	3.10	1.15±00
12 month	0	0	0
Location:			
Char area	26	10.00	3.75±0.83
Hilly area	34	12.00	4.88±0.02
Season:			
Summer	18	7.52	3.62±0.05
Winter	43	15.30	6.16±0.11
Flock size:			
5 animals	21	13.44	4.25±1.77
10 animals	61	28.24	10.18±1.10
15 animals	56	33.13	7.02±0.45
Litter size:			
Single	68	12.50	8.50±4.34
Twin	84	15.50	12.00±1.31
Triplet	8	33.33	2.66±0.07

SEM= Standard error of mean

Causes of death of different flock sizes of lambs on different diseases

Based on the data obtained from flock monitoring, the death% of the lamb was calculated (Table 7). Death of diarrhoea, bloat and pneumonia was found 4 to 7 % in 5 flock sizes than that of 10 and 15 flock sizes lamb during study period. Hasan and Talukder (2011) reported that death was 23 and 15.4%, respectively due to diarrhoea and pneumonia of Jamuna basin sheep which was not agreed with the present study. The causes of death were more difficult to diagnose without a full necropsy of lambs. Lamb mortality could be greatly reduced by slight modifications of lambing management. Lower birth weight and nutritional deficiency were observed lower in 5 flock sizes than 10 and 15 flock sizes. Metabolic disorders and diseases are certainly the prevalent causes of death. Mortality rate was higher in diarrhoea for 10 and 15 flock sizes compared to 5 flock size lambs. Pneumonia and gastroenteritis were the major diseases which caused the highest mortality rate in lambs (Mustafa et al., 2014) which was not similar to the present study. Proper feeding of the pregnant ewes minimizes the chance of lamb mortality.

More than 3-4% mortality had its causes in faulty or inadequate management of ewes and lambs. Simple improvements providing skill development training in the overall

management of the flock before, during and after lambing would be greatly reduced the number of deaths. Reducing lamb mortality to an acceptable level should be kept from 5 to 10 % for any lamb producers of flock sizes lamb performances in Bangladesh.

Table 7: Causes of death of different flock sizes of lambs on various diseases

Disease	Flock size	No. of lamb died	%
Diarrhoea	5	2	7
	10	4	20
	15	8	40
Bloat	5	2	4
	10	3	15
	15	5	25
Pneumonia	5	1	5
	10	2	10
	15	3	15
Lower body weight	5	3	3
	10	5	25
	15	7	35
Nutritional deficiency	5	0	0
	10	3	15
	15	5	25
Biting (dog/fox)	5	1	5
	10	2	10
	15	3	15
PPR	5	1	5
	10	3	15
	15	4	20
Nitrate Poisoning	5	0	0
	10	0	0
	15	3	15
Dead unknown	5	1	3
	10	1	5
	15	2	10

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CONCLUSIONS

This study revealed that flock size may influence the birth weight, average daily growth, other growth traits and disease prevalence and mortality rate of the Jamuna basin lamb in different areas like chars and hills and different seasons in Bangladesh. . Considering these parameters, maintaining a flock size of 5-10 Jamuna basin lambs appeared to be an appropriate strategy for rural lamb farmers for semi-intensive sheep rearing in rural livestock farming conditions of Bangladesh.

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EFFECT OF SUPPLEMENTARY FEEDING ON THE PRODUCTION AND REPRODUCTION TRAITS, CARCASS AND MEAT QUALITY OF BARIND SHEEP IN BANGLADESH

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ABSTRACT

The study was conducted to evaluate the effect of different feeding regimes on the growth, productive and reproductive performances of the Barind sheep in two upazilas (sub-districts), Paba and Godagari, of Rajshahi district of Bangladesh during February 2018 to September, 2020. Four hundred (360 female and forty male) sheep from the Barind region were selected which were distributed among 40 farmers (20 farmers from each upazila). The farmers received 10 sheep each with an average live weight of 17.48 ± 0.11 kg for the female and 18.27 ± 0.25 kg for the male. The sheep were reared in a semi-intensive condition for up to 1 year and 6 months. The sheep were provided feed concentrate mixtures at the rates of 1%, 1.5% and 2% on the basis of their live weight. The animals of all four groups were allowed to graze for 8 hours every day. The concentrate supplementation had a positive effect on the birth weight of lambs. The highest birth weight (1.43 ± 0.06 kg) was obtained with 2% concentrate supplementation the lowest (1.28 ± 0.04 kg) with 0% concentrate supplementation. Concentrate supplementation positively influenced the litter size, 1.5% and 2% concentrate supplementation producing 1.82 ± 0.07 and 1.83 ± 0.10 kids, respectively, per birth compared with 1.70 ± 0.06 with 0% concentrate supplementation. The highest milk yield (259.64 ± 2.08 ml/day) was obtained with 2% concentrate and the lowest (244.55 ± 1.36 ml/day) with zero concentrate. The highest average daily weight gains (ADG) for up to 6, 9 and 12 months of age were 55.50 ± 1.67 , 48.50 ± 0.30 and 40.00 ± 0.00 g/d with 2% concentrate which, with zero concentrate, were 40.91 ± 0.84 , 31.91 ± 0.97 and 28.88 ± 1.47 g/d, respectively. The sheep with non-supplementing feeding had taken the longest period (206 ± 93 days) to attain maturity (age at maturity) while the shortest period (167.75 ± 1.16 days) was required with 2% concentrate. The gestation length was shorter (146.80 ± 0.24 days) with 2% concentrate than that (150.63 ± 0.33 days) with zero concentrate. Thus, it was concluded that feeding with a relatively level of concentrate supplementation of 2% would beneficially impact important productive and reproductive parameters in lamb production.

Keywords: Feeding, productive, reproductive, sheep and traits.

INTRODUCTION

Lamb production contributes significantly to Bangladesh's agricultural economy. Sheep are sparingly scattered across Bangladesh concentrated in three ecological zones: the

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coastal region of Noakhali and Cox's Bazar, the Jamuna river basin of Tangail, Jamalpur and Sherpur districts including char areas and the Barind region of Rajshahi division (DLS, 2010). Sheep in the Barind region are normally reared in rural areas with no feed supplements and are fed primarily on range land grasses. Many factors influence the reproductive and productive performances, and hence, profitability of sheep of which genotype, the environment (Kafi *et al.*, 2004), management and particularly the nutritional status are important (Al-Ghalban *et al.*, 2004). Low productivity of sheep in the Barind areas are mainly due to poor nutrition and seasonal fluctuations in feed supply both quantitatively and qualitatively. The productive and reproductive potentiality of sheep is deteriorating in the country due to inadequate nutrient supplementation. Supplementary feeding may have a beneficial effect on the growth and development of weaned lambs. The effect of nutrition, particularly underfeeding and flush feeding on female fertility in sheep has been extensively studied by Fernández-Foren *et al.* (2011) which revealed the importance of concentrate supplementation on growth and productivity of sheep. Grazing alone may not be sufficient for optimizing the live weight gain and fertility of sheep (Kochapakdee *et al.*, 1994). The nutritive value and availability of pasture and range land grass in the Barind region are greatly affected by seasonal fluctuations in rainfall and, thus, grazing alone may not be sufficient for optimizing the growth and weight live weight gain of sheep. Commonly available feed resources in Barind regions are crop residues, mixed sward hays and natural pastures on road sides and steep slopes that usually do not meet the nutrient requirements of sheep. Such feed resources are usually low in CP (crude protein) content (less than 7%) which is inadequate to meet the minimum requirement for growth of rumen microorganisms (Van Soest, 1994). There is no scientific feeding programme, and as a result, while the number of sheep increases there is no significant improvement in productive and reproductive traits. This is aggravating economic losses. The potential of Barind sheep for meat production has not been explored in details. The present experiment was designed to study the effects of feed concentrate supplementation on the growth, productive and reproductive performances of sheep raised under grazing conditions.

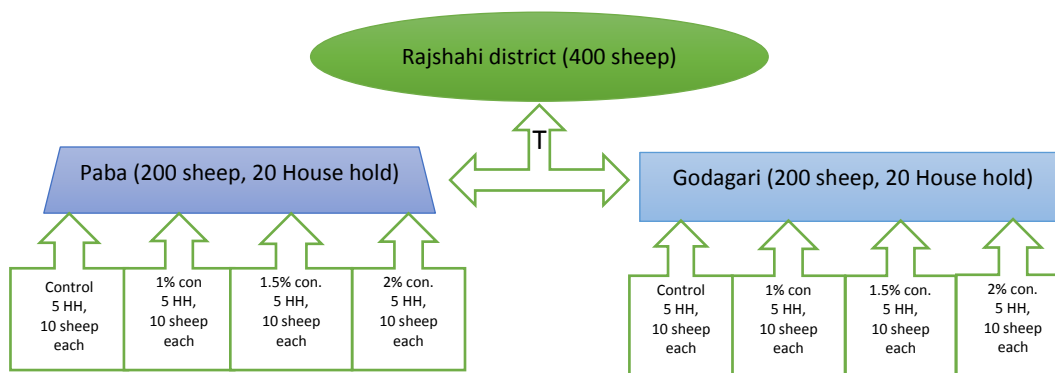
MATERIAL AND METHODS

The study was conducted to observe the effect of different level of supplementation of concentrate along with grazing. The study was carried out in Barind region at two upazilas; Paba and Godagari of Rajshahi district in Bangladesh during from February, 2018 to September, 2020.

Animals and their management:

Four hundred (Three hundred sixty female and forty male) Barind region's sheep (females were early pregnant/mature non pregnant but capable to conceive) were taken under experiment. Sheep were distributed among 40 farmers (Twenty farmers from each Upazila) of 10 sheep each having average live weight 17.48 ± 0.11 kg for female and 18.27 ± 0.25 kg for male. Fifty sheep were allocated to each of the four treatments in a completely randomized design in every Upazila. Sheep were reared under semi-intensive condition up to one year six month's. Same concentrate mixture having 18% crude protein (CP) and 12 MJ energy on dry matter (DM) basis excluding control group was

supplied to other three groups of sheep. The sheep were provided concentrate mixture at the rate of 1%, 1.5% and 2% on the basis of their live weight. The animals of all four groups were allowed to graze for 8 hours every day. The feeding regimes were as follow below the flow chart.



They were housed in well ventilated slated floor. Four sides of the tin roofed house were encircled with wire made net. All sheep were marked by ear-tagging. Before the start of the experiment, the sheep were dewormed against internal and external parasites with the injection of ivermectin as prescribed by the manufacturer and it was continued till to the end of the experiment giving 2 month's interval. All sheep were vaccinated against PPR disease. Sheep were allowed for experimental concentrate from the beginning of the experiment. Body weight changes were monitored fortnightly for 12 months and other parameter were monitored regularly. The initial and final weights were taken twice on two consecutive days and the average of the two was taken as initial and final weights, respectively. After taking weight fortnightly required amount of concentrate calculated and offered to the animal for next 15 days.

Experimental feed and treatments

Animals were divided into four groups each of ten sheep with five replications and fed natural pasture for eight hours daily. After birth kids were offered @ 10 and 20 gram feed respectively at 2nd and 3rd week of age. Then the first group (T_1) was offered zero concentrate (control), the second group (T_2) was offered 1.0% concentrate, third group (T_3) was offered 1.5% concentrate and the fourth group (T_4) was offered 2.0% concentrate of their live weight. Same concentrate mixture (18% CP, 12 MJ energy on DM basis) excluding control group was supplied to experimental sheep. Proportion of ingredients and nutrient composition of concentrate was as follows: Crushed maize 40%, soybean meal 26%, wheat bran 22%, rice polish 10%, vitamin mineral premix 0.5%, DCP 0.5%, salt 1%, CP 18% and ME (MJ/kg DM) 12%. The concentrate mixture was offered twice a day at 7:00-8:00 am and 4:00-6:00 pm into two equal halves before and after grazing respectively. Experimental sheep had free access to clean water.

Data collection:

Following data of growth performance, productive and reproductive parameter were recorded:

- Live weight at different ages on different feeding regime (kg);
- Birth weight (kg);
- Live weight at 6 months (kg);
- Live weight at 9 months (kg);
- Live weight at 12 months (kg);
- Litter size (no);
- Weaning weight (kg);
- Milk Production (ml/day);
- Average daily gain (ADG);
- Average daily gain (ADG) up to six months (g);
- ADG up to nine months (g);
- ADG up to twelve months (g);
- Age at puberty (days);
- Service per conception (no.);
- Conception rate (%);
- Gestation length (days) and
- Age at first lambing (days).

Statistical analyses

Data on productive and reproductive parameters and body weight change were coded after ending of data collection and then compiled, tabulated analyzed using SPSS version 23 for windows. Parameters were given as mean, standard error, p-value and significance level. Data were presented as Mean \pm SE and $p < 0.05$ was considered as significant. DMRT was performed to observe significant differences of productive and reproductive traits and income in different concentrate levels.

RESULTS AND DISCUSSION

Effect of different feeding regime on productive and reproductive traits are shown in Table 1. The highest birth (1.43 \pm 0.06) was obtained on 2% concentrate supplementation and the lowest (1.28 \pm 0.04) on control (0% concentrate supplementation) feeding method. Kochapakdee *et al.* (1994) reported that, better supplementary feeding did not always play potential role in attainment of birth weight or post weaning rate of gain of lambs. The present study insignificantly ($p > 0.05$) higher birth weight was obtained on 2% supplementation than control this result supported the findings of Salim *et al.* (2002). Sarder *et al.* (2015) observed 0.96 \pm 0.32 kg birth weight on grazing. Concentrate supplementation had imposed positive impact on litter size and it was higher on 1.5% and 2% supplementation (1.818 \pm .58 and 1.833 \pm .59 respectively) than control (1.70 \pm 0.06).

Supplementation of 1.5% (T₂) and 2% (T₃) concentrate had given more results (1.82 \pm 0.07 and 1.83 \pm 0.10 respectively) to produce number of kids per birth. Muwalla *et al.* (1995) who showed that inclusion of poultry litter did not influence the performance of lambing rate and number of weaned lambs of ewes. Sarder *et al.* (2015) were observed slightly large litter size (1.85 \pm 0.02) than present findings. Weaning weight on T₀, T₁ and T₂ was 5.18 \pm 0.13, 4.26 \pm 0.28 and 5.54 \pm 0.25 kg respectively. Milk yield obtained from T₀, T₁, T₂ and T₃ was 244.55 \pm 1.36, 247.25 \pm 1.07, 247.60 \pm 1.14 and 259.64 \pm 2.08 ml/day, respectively. Muwalla *et al.* (1995) was observed that ewes fed the control diet (without poultry litter) weaned heavier lambs than those fed the experimental diet due to numeric increase in their milk production. Pulina *et al.* (2006) showed that sheep milk composition is strongly influenced by ewe nutrition, especially in highly productive animals. Average daily gain (ADG) is shown in the Table 1. The highest result (55.50 \pm 1.67 g/d) was observed on T₃ and it was gradually decreased to minimization of

concentrate in ration. Gradual increase in ADG was observed on advanced inoculation level of concentrate giving insignificant difference among treatments. The ADG obtained on T₀, T₁ and T₂ was 31.91±0.97, 36.11±1.06, and 37.63±1.33g, respectively. Though gradual increased result attributed with the advanced inoculation, the variation of result among 4 treatments was not significant and these were 28.88±1.47g, 34.16±1.53 g and 34.28±1.75 g for T₀, T₁ and T₂ respectively. Galal and Kebede (1979) observed that the final live weights of lambs increased ($p<0.0001$) with increased concentrate level and the ADG of lambs was highest ($p<0.0001$) at the highest concentrate offered. It was reported that rams fed on urea treated straw supplemented with wheat bran achieved ADG of 65.6 g. Dessie *et al.* (2010) observed that sheep supplemented with the high level of CM (concentrate mixture) had higher final BW ($P<0.01$) as well as BW change and daily BW gain ($P<0.001$) than sheep in the control treatment and those supplemented with the low level of CM. Solomon Melaku and Betsha (2008) showed the differences in BW ($P>0.05$) gain between the non-supplemented and supplemented sheep and the absence of such differences between the supplemented sheep agrees with the results reported by Ngwa and Tawah (2002) and their report also consistent to the present findings. Salo *et al.* (2016) observed that the supplemented sheep had significantly ($P < 0.05$) higher final weight and daily live weight gain (ADG) compared with the sheep on the control treatment. The sheep had taken long period (206±.93 days) to attain maturity on concentrate less feed (T₀). Average service required for each successful conception was 1.8±0.04. Less service for a successful pregnancy was on T₃ (2% concentrate supplementation). Average conception rate showed 87.88±0.30 given highest result (93±0.53) on 2% concentrate supplementation (T₃) and lowest conception rate on control feeding (T₀). Length of post-partum heat period gradually increased with the decreasing of inoculation level of concentrate showing insignificant ($p>0.05$) effect. Gestation length recorded on T₁ and T₂ was 148.57±0.34 days and 146.80±0.24 days, respectively. Shorter (335.25±1.16 days) period for first time lambing was obtained on treatment T₃ then it was gradually longer with reducing concentrate supplementation to ration. Jha *et al.* (2021) mentioned in the first study concerning the reproductive performance of native rams in Bangladesh that rams supplemented with concentrate gained weight faster ($p<0.05$) than controls, and body weight was higher ($p<0.05$) at the end of experiment in this group. The average gestation length recorded by Salim *et al.* (2002) for goats was 143.5 days. Mukasa-Mugerwa and Ezaz (1992) stated that the level of nutrition had effect ($p<0.05$) on body growth rate (48.5 ± 1.6 g/d) from weaning to puberty in Menz ram lambs. Sultana *et al.* (2011) measured average milk yield was not significantly ($p>0.05$) higher (281.0g/d) in intensive system than semi-intensive feeding system (264.50g/d). Sultana *et al.* (2011) observed higher (98.0%) conception rate in semi-intensive system compare to intensive system (83.0%). On grazing feeding Sarder *et al.* (2015) observed 162±2.43 days lambing interval in ewes of Rajshahi areas.

Table 1: Effect of different feeding regimes on productive and reproductive traits (Mean±SE)

Productive and reproductive parameters	Feeding regimes					Significant level
	Grazing (T ₀)	1% (T ₁)	1.5% (T ₂)	2% (T ₃)	Overall	
Birth weight (kg) n=498	1.28±0.04 n=221	1.29±0.03 n=106	1.42±0.04 n=111	1.43±0.06 n=60	1.39±0.02 N=498	NS
Litter size (no) n=285	1.70±0.06 n=130	1.73±0.08 n=61	1.82±0.07 n=61	1.83±0.10 n=33	1.75±0.04 N=285	NS
Weaning weight (kg) n=213	5.18±0.13 n=99	4.26±0.28 n=42	5.54±0.25 n=44	6.17±0.25 n=28	4.27±0.10 N=213	NS
Milk (ml/d) n=229	244.55±1.36 ^b n=82	247.25±1.07 ^b 60	247.60±1.14 ^{ab} 56	259.64±2.08 ^a n=31	249.87±0.82 N=229	**
AdG up to 6 month (g/d) n=426	40.91±0.84 ^b n=182	41.83±0.88 ^b n=96	46.94±1.67 ^b n=90	55.50±1.67 ^a n=58	42.64±0.53 N=426	**
AdG up to 9 month (g/d) n=255	31.91±0.97 ^b n=96	36.11±1.02 ^b n=60	37.63±1.33 ^{ab} n=51	48.50±0.30 ^a n=48	35.95±0.54 N=255	*
AdG up to 12 month (g/d) n=85	28.88±1.47 ^b n=28	34.16±1.53 ^b n=18	34.28±1.75 ^b n=20	40.00±0.00 ^a n=19	33.51±0.76 N=85	*
Age at puberty (days) n=90	206.04±2.90 ^b n=24	181.50±1.51 ^b n=28	181.83±1.32 ^b n=20	167.75±1.16 ^a n=18	195.02±1.96 N=90	***
Service per conception (no) n=363	2.0±0.08 n=116	1.5±0.06 n=91	1.5±0.06 n=84	1.40±0.06 n=72	1.8±0.04 N=363	NS
Conception rate (%) n=454	85.2±0.42 n=128	90.00±1.34 n=112	90.0±1.12 n=116	93.00±0.53 n=98	87.88±0.30 N=454	NS
Postpartum heat (days) n=154	21.5±0.84 n=40	19.61±0.81 n=37	17.14±0.51 n=42	15.62±0.52 n=39	18.38±0.04 n=154	NS
Gestation length (days) n=239	150.63±0.33 ^b n=72	148.57±0.34 ^b n=84	146.80±0.24 ^{ab} n=56	146.00±0.27 ^a n=27	149.26±0.20 N=239	**
Age at first lambing (days) n=239	368.41±3.88 n=72	351.40±0.77 n=84	343.71±4.43 n=56	335.25±1.16 n=27	358.13±2.01 N=239	NS

^{abc} values having different superscript differed significantly. Parentheses indicate that total number of observation, SE= Standard error of mean, NS= Non-significant, *p= Significant at 5% level, **p= Significant at 1% level, ***p= Significant at 0.1% level. T₀= only grazing, T₁= 1% concentrate, T₂= 1.50% concentrate, T₃= 2% concentrate. n= no. of observation.

Effect of concentrate supplementation on birth weight of lambs of different litter is given in the Table 2. Irrespective of feed heavier (1.69±0.03) kids were obtained in single and lighter (0.99±0.04) in triplet birth. On the other hand, Inoculation level of reproduction had a positive effect on birth weight for all births. Birth weight of lambs on treatments varied significantly (p<0.05) but the variations were highly significant (p<0.001) to the litter size.

Table-2. Birth weight of kid of different litter on different feeding regime

Parameter	Litter size	Feeding regimes				Overall	Significant
		T ₀	T ₁	T ₂	T ₃		
Birth weight	Single n=206	1.53±0.06 ^b n=63	1.71±0.04 ^a n=58	1.72±0.07 ^a n=45	1.75±0.10 ^a n=40	1.69±0.03 N=206	NS
	Twin n=232	1.15±0.05 ^b n=62	1.28±0.03 ^a n=58	1.30±0.04 ^a n=64	1.31±0.05 ^a n=48	1.27±0.02 N=232	***
	Triplet n=36	0.63±0.02 ^b n=6	0.96±0.05 ^a n=9	0.96±0.05 ^a n=12	1.01±0.10 ^a n=9	0.99±0.04 N=36	**

The values are Mean±SE, ^{ab} values having different superscript differed significantly. Parentheses indicate that total number of observation, SE= Standard error of mean, NS= Non-significant, **p= Significant at 1% level, ***p= Significant at 0.1% level. T₀= only grazing, T₁= 1% concentrate, T₂= 1.50% concentrate, T₃= 2% concentrate, n= no. of observation, N= no. of total observation.

Effect of concentrate supplementation of live weight at various age group of lambs of different litter is observed in the Table 3. Live weight at 1 month of lambs on treatments varied significantly ($p < 0.01$) but the variations were highly significant ($p < 0.001$) to the litter size. Live weight at 3 month of lambs on treatments varied insignificantly ($p > 0.05$) but the variations were highly significant ($p < 0.001$) to the litter size. Irrespective of feed heavier (10.15 ± 0.28) lambs were obtained in single and lighter (8.43 ± 0.14 kg) in twin birth. Birth weight of lambs on treatments varied significantly ($p < 0.01$) but the variations were highly significant ($p < 0.001$) to the litter size. Live weight at 9 month of lambs on treatments varied insignificantly ($p < 0.01$) but the variations were significant ($p < 0.01$) to the litter size. Live weight at 12 month of lambs on treatments varied insignificantly ($p > 0.05$) but the variations were significant ($p < 0.01$) to the litter size.

Effect of concentrate supplementation on average daily gain (ADG) of different litter size of lamb up to 6, 9 and 12th month's of age is shown in the Table-4. The highest result (56.47 ± 3.17 g) was observed on T₃ and it was gradually decreased to minimization of concentrate in ration. Gradual increase in ADG was observed on advanced inoculation level of concentrate giving insignificant ($p > 0.05$) difference among treatments. It was positively related to the amount of concentrate supply. Average ADG observed up to 12 month's of age was 45.58 ± 3.41 g and best result obtained on treatment T₃ (36.60 ± 3.08 g). The ADG obtained on T₀, T₁ and T₂ was 31.36 ± 2.60 , 34.30 ± 1.80 , and 35.18 ± 2.05 g respectively. Average ADG observed up to 12 month's of age was 33.51 ± 7.04 g and best result obtained on treatment T₃ (40.10 ± 1.16 g). Awuk and Tamir (2007) obtained concentrate supplementation promoted daily live weight gain while feeding hay only resulted in body weight loss. The high mean daily body weight gain of the goats on concentrate-supplements was in agreement with the findings of Galal and Kassahun (1981) in local highland goats supplemented with concentrates. Jha *et al.* (2021) mentioned in the first study concerning the reproductive performance of native rams in Bangladesh that rams supplemented with concentrate gained weight faster ($p < 0.05$) than controls, and body weight was higher ($p < 0.05$) at the end of experiment in this group. Salim *et al.* (2002), Tufarelli *et al.* (2011) obtained positive effect on growth rate. These

were consistent with the present findings. Fahmy (1990) observed ADG of lambs fed fish meal (226 g) and corn gluten- blood meal (217 g) were higher ($P < 0.01$) than those of lambs fed the soybean meal (189 g) and those fed the control diet (186 g). Shinde *et al.* (1998) reported lambs grow faster than kids under stall feeding while kids grow faster than lambs under semi- intensive system (grazing and concentrate supplementation). Karim and Verma (2001) reported that the average daily gain of 95 ± 4.4 g in grazing with supplementation group and 79 ± 4.4 g in intensively feeding group. Kochewad *et al.* (2018) conducted a study on performance of Deccani sheep lambs (3-12 month's age) in intensive farming system, semi intensive farming system and extensive farming system and reported ADG (43.53, 38.51, 34.70 g), average body weight gain (11.75, 10.40, 9.36 kg) and feed conversion efficiency (14.43, 14.87, 15.12%). Javanthi *et al.* (2003) and Agnihotri *et al.* (2006) reported average daily gain of 79 g in intensive system and 35g in semi-intensive system because of adequate nutrition management under intensive system.

Table 3: Weight change of kids at different stages of life on different feeding regimes

Age	Litter size	Feeding regimes				Overall	Significant
		T ₀	T ₁	T ₂	T ₃		
1 month	Single	3.22±0.12 ^b n=47	3.50±0.18 ^b n=32	3.78±0.21 ^b n=22	4.11±0.12 ^a n=25	3.64±0.09 N=126	**
	Twin	2.42±0.06 ^b n=62	2.52±0.08 ^b n=64	2.87±0.10 ^b n=56	2.91±0.09 ^a n=58	2.74±0.04 N=240	**
3 month's	Single	6.34±0.34 ^b n=28	6.41±0.31 ^b n=21	7.62±0.64 ^b n=16	7.97±0.26 ^a n=18	6.63±0.19 N=83	**
	Twin	4.87±0.14 ^c n=52	4.91±0.15 ^b n=53	4.98±0.16 ^{ab} n=44	4.20±0.11 ^a n=46	4.97±0.08 N=195	**
6 month's	Single	9.51±0.52 n=20	10.09±0.44 n=15	11.45±0.86 n=12	12.80±0.45 n=13	10.15±0.28 N=60	NS
	Twin	8.34±0.27 ^b n=42	8.40±0.25 ^{ab} n=33	8.58±0.43 ^{ab} n=34	9.33±0.29 ^a n=36	8.43±0.14 N=145	*
9 month's	Single	11.29±0.82 n=16	12.46±0.52 n=14	12.78±1.29 n=10	15.00±1.29 n=11	12.32±0.36 N=51	NS
	Twin	9.26±0.22 n=32	10.43±0.41 n=26	10.63±0.28 n=26	13.90±0.06 n=31	10.42±0.36 N=115	NS
12 month's	Single	12.29±1.29 n=10	14.77±0.57 n=12	15.00±1.42 n=9	16.78±0.10 n=11	14.46±0.83 N=42	NS
	Twin	11.72±0.38 n=31	12.30±0.36 n=28	13.03±0.51 n=16	14.04±0.08 n=13	12.88±0.22 N=88	NS

The values are Mean±SE, ^{abc} values having different superscript differed significantly. Parentheses indicate that total number of observation, SE= Standard error of mean, NS= Non-significant, *p= Significant at 5% level, **p= Significant at 1% level. T₀= only grazing, T₁= 1% concentrate, T₂= 1.50% concentrate, T₃= 2% concentrate. n= no. of observation, N= no. of total observation.

Table 4: Average daily gain (ADG) of kid of different litter on different feeding regimes (Mean±SE)

Age	Litter size	Feeding regimes				Overall	Sig.
		T ₀	T ₁	T ₂	T ₃		
AGG upto 6 month's of age	Single	43.54±2.69 n=19	45.68±2.37 n=14	46.46±2.03 n=14	56.47±3.17 n=10	47.05±1.58 n=57	NS
	Twin	36.68±1.32 n=24	38.86±1.45 n=40	41.25±2.40 n=22	43.49±1.70 n=13	39.47±0.90 n=99	NS
AGG upto 9 month's of age	Single	36.68±1.28 n=16	37.56±0.90 n=14	39.58±3.37 n=10	52.48±2.46 n=11	40.90±1.28 n=51	NS
	Twin	31.36±2.60 n=21	34.30±1.80 n=33	35.18±2.05 n=17	38.12±3.96 n=12	34.29±0.79 n=83	NS
AGG upto 12 month's of age	Single	32.47±0.84 n=10	33.30±1.03 n=12	36.60±3.08 n=9	45.58±3.41 n=11	37.02±1.15 n=42	NS
	Twin	30.60±1.67 n=20	30.62±2.03 n=28	33.38±1.61 n=16	36.74±2.80 n=11	32.10±0.66 n=75	NS

NS= Non-significant, T₀= only grazing, T₁= 1% concentrate, T₂= 1.50% concentrate, T₃= 2% concentrate. n= no. of observation.

CONCLUSIONS

Supplementary feeding had a remarkable effect on the production and reproduction traits, carcass and meat quality of Barind sheep in two upazilas of Rajshahi district of Bangladesh. The supplementation feeds (1.5% and 2%) substantially increased the litter size and birth weight of the sheep under study. The highest birth weight and average daily gain (ADG) in weight for up to 6, 9 and 12 months, the lowest kid mortality and the highest milk yield were achieved with 2% concentrate feed supplementation. Also, increasing feed supplementation was positively correlated with shorter gestation and maturation periods. Thus, it was concluded that feeding with a relatively level of concentrate supplementation of 2% would beneficially impact important productive and reproductive parameters in lamb production.

Finally, it concluded that the increased level of concentrate (2%) had imposed positive effect on important productive and reproductive traits of lamb production.

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INFLUENCE OF LOCATION AND GENOTYPE ON GROWTH, YIELD AND QUALITY OF STRAWBERRY IN THE SUBTROPICAL REGION OF BANGLADESH

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ABSTRACT

Location effects on the yield and quality of five strawberry genotypes were investigated in this study. The plants were grown at three different locations, i.e., Chattogram, Gazipur and Rajshahi of in Bangladesh varying in soil and environmental conditions. Data on plant growth attributes such as days to first flowering, first harvesting and fruiting duration and fruit quality parameters like total soluble solids (TSS) and ascorbic acid (AA) concentration were recorded. Environment significantly affected plant growth and development and almost all fruit quality attributes. Irrespective of genotype the strawberry plants performed the best, in terms of almost all the plant growth and fruit quality parameters studied, at Rajshahi among the locations. The longest fruiting duration and the highest fruit yield plant⁻¹, TSS and ascorbic acid contents in fruit were recorded strawberry grown at Rajshahi while the shortest fruiting duration and the lowest fruit yield plant⁻¹, TSS content and AA content were recorded from the strawberry plants grown at Chattogram. At Rajshahi, the highest yield 19.87 t ha⁻¹ was recorded for the variety Camarosa which was statistically similar to 18.74 and 17.71 t ha⁻¹ from Sweet Charlie at Rajshahi and Gazipur; the lowest yield of 9.29 t ha⁻¹ was recorded for the genotype FA 008 at Chattogram and also at the other two locations.

Keyword: Strawberry; Location; Genotypes; Sub-tropical; Growth; Yield

1. INTRODUCTION

The major cultivated strawberry species, *Fragaria x ananassa*, is a hybrid of *F. chiloensis* and *F. virginiana* (Hancock *et al.*, 2008). The fruits are rich in vitamin C and are commonly eaten fresh as a dessert fruit, are used as a pastry or pie filling, and may also be preserved in many ways. It is photo-sensitive as well as thermo-sensitive. It has been adapted to different environmental conditions and is being cultivated across the world. Strawberry is a temperate zone crop but nowadays, its commercial cultivation has been started in Bangladesh (Rahman, 2016) gaining popularity as a popular fruit crop due to its quick growing nature, good taste and fine aroma. The cultivars grown in Bangladesh have short-day plants. Environmental conditions prevailing during October to March, i.e., in the Rabi season (winter) are suitable for the growth and development of strawberry in Bangladesh. Strawberry has a great significance as it is

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grown in the winter season in Bangladesh when native fruits are rare. Tangail, Rajshahi, Joypurhat, Bogura, Cumilla, Satkhira, Khagrachari, and Cox's Bazar are the key strawberry producing districts of Bangladesh, although different cultivars respond differently to the prevailing environmental conditions in those districts (Rahman *et al.*, 2016). Understanding the relationship between genotype and environmental conditions at the locations of cultivation is crucial to optimizing the growth, yield and fruit quality of strawberry (Hossan *et al.*, 2013; Islam *et al.*, 2013; Rahman *et al.*, 2013; Ahmed *et al.*, 2019). In view of this, the present study was undertaken to evaluate the performance of five strawberry genotypes under environmental conditions obtaining in Rajshahi, Gazipur and Chattogram districts of Bangladesh.

2. MATERIALS AND METHODS

2.1. Experimental sites

The experiment was conducted by the Pomology Division, Horticulture Research Center (HRC) of the Bangladesh Agricultural Research Institute (BARI), Gazipur during September 2019 to May 2020. The experimental locations were (a) research farm of the Pomology Division, HRC, BARI, Gazipur (site-1), (b) Fruit Research Station, HRC, BARI, Binodpur, Rajshahi (site-2) and (c) Regional Agricultural Research Station (RARS) of BARI, Hathazari, Chattogram (site-3). Site-1, site-2 and site-3 represent the sSouth-central zone, western zone and south-eastern zone of Bangladesh, respectively. The geographical locations, climatic conditions and characteristics of soils at the three experimental sites have been given in Fig. 1 and Table 1.

Table 1. Soil and climatic conditions of different experimental sites (August 2019 to May 2020)

Factor	Site-1	Site-2	Site-3
District	Gazipur	Rajshahi	Chattogram
Geographic profile			
Altitude	14.33 m	21.64 m	16.46 m
Latitude	23 ⁰ 59' N	24 ⁰ 22' N	22 ⁰ 21' N
Longitude	90 ⁰ 24' E	88 ⁰ 39' E	91 ⁰ 48' E
Climatic condition			
Cumulative rainfall	108 mm	69.4 mm	98 mm
Relative humidity	79.4 %	76.3 %	72.6 %
Temperature (Max)	26.89 °C	20.43 °C	31.63 °C
Temperature (Min)	17.05 °C	14.38 °C	21.54 °C
Soil characteristics			
Agro-ecological zone (AEZ)	28 Madhupur Tract	11 High Ganges River Floodplain	23 Chattogram Coastal Plain
Land type	High	Medium	High

Factor	Site-1	Site-2	Site-3
Texture	Clay loam	Sandy loam	Silt loam
pH	4.8-5.5	6.1-7.9	5.5-6.2
Organic matter	Low	Low	Very low
Nitrogen	Very low	Low	Low
Phosphorus	Very low	Low	Low
Potassium	Low	Medium	Medium

2.2. Experimental design and layout

The experiment was conducted in a RCBD (factorial with location and genotype as the two factors) design with three dispersed replications. The unit plot size was 1 m x 6 m and the plants were spaced 50 x 40 cm on beds raised 30 cm above the ground with a 50 cm drain in between two beds. Each bed had double rows accommodating 30 plants. Strawberry saplings were planted on October 01, 2019 at each location.

2.3. Treatments

The experiment consisted of two factors:

1. Factor A (three locations)
 - i. A₁: Gazipur
 - ii. A₂: Rajshahi
 - iii. A₃: Chattogram
2. Factor B (5 genotypes)
 - i. B₁: Sweet Charlie
 - ii. B₂: Festival
 - iii. B₃: Camarosa
 - iv. B₄: FA 008
 - v. B₅: BARI Strawberry-1

2.4. Intercultural operations

Runners were removed once every 3 to 4 days in order to enable the crown to initiate flowering. Straw mulch was applied around the plants as a normal practice in order to conserve soil moisture, control weeds and provide healthy conditions for the fruits. Hand weeding was done whenever necessary. The plants were irrigated as and when necessary to maintain soil moisture at around the field capacity. All other necessary cultural practices and plant protection measures were followed uniformly for all the plots and treatments during the entire period of experimentation.

2.5 Harvesting

The fruits were harvested at commercial maturity when >80% of the fruit surface showed red coloration. Immediately after harvest, the strawberries were sorted to eliminate damaged fruits and to select for uniform size and color.

2.6 Ascorbic acid determination and total soluble solids (TSS)

Ascorbic acid content was determined by the Merckquant Ascorbic Acid Test (Reflectoquant, Merck) using test strips dipped in strawberry juice (Camin *et al.* 2011). Results were expressed in mg 100g⁻¹ fresh weight (FW).

2.6 Data recording

Data were collected from inner plants from each row to avoid the border effect. In each unit plot 20 plants were selected randomly for recording data on different morphological, vegetative, reproductive, yield contributing and chemical attributes. The data were recorded on plant height (cm), leaves plant⁻¹, days to flowering, days to harvest, fruiting duration, fruit weight (g), number of fruits plant⁻¹, yield plant⁻¹ (g), yield (t ha⁻¹), total soluble solids (TSS%) and ascorbic acid content of fruit (mg 100 g⁻¹).

2.7. Data analysis

The collected data were combined and analyzed after Zaman *et al.* (1982) by using the MSTAT-C computer software. DMRT (Duncan's Multiple Range Test) was used to compare the means.

3. RESULTS AND DISCUSSION

3.1. Growth and phenological responses of strawberry

In general, genotypes at Rajshahi produced the tallest plants and those at Chattogram were the shortest (Table 2). Irrespective of location, Festival and Camarosa produced the tallest plants while BARI Strawberry-1 and FA 008 produced the shortest. The number of leaves plant⁻¹ was the maximum at Gazipur closely followed by Rajshahi and the lowest was at Chattogram (Table 2). The genotypes flowered early at Chattogram and Gazipur while flowering was delayed at Rajshahi (Table 2). Vegetative development of strawberry was highly sensitive to climatic variables which is supported by findings of Riyaphan *et al.* (2005). No location x genotype variety interaction effect was noticed in respect of days to flowering (Table 3). A more favorable day-night temperature regime at Rajshahi (Table 1) may have resulted in better vegetative growth and flower development of strawberry than at the other two locations. Riyaphan *et al.* (2005) found a negative relationship between a higher growing temperature and vegetative growth and flower development, the present findings are strongly in consonance with this. Early workers also reported that seasonal changes, day and night temperature, daylight intensity significantly affected plant growth and flower induction of strawberry (Avigdor-Avidov, 1986; Sønsteby and Heide, 2009).

Table 2. Single effect of location and genotype on plant height, leaves plant⁻¹, days to flowering, days to harvest and fruiting duration of strawberry

Treatment	Plant height (cm)	Leaves plant ⁻¹	Days to flowering	Days to harvest	Fruiting duration (days)
Effect of location					
Gazipur	25.45 ab	40.65 a	72.85 b	23.70 b	86.05 b
Rajshahi	26.85 a	40.40 a	76.70 a	31.65 a	91.50 a
Chattogram	22.15 b	23.30 b	70.30 b	23.00 b	82.35 b
Level of significance	*	**	**	**	**
Effect of genotypes					
Sweet Charlie	26.08 b	37.17 c	73.92 b	25.67 b	87.67 b
Festival	27.75 a	42.08 b	76.42 b	28.58 a	90.08 a
Camarosa	28.58 a	45.42 a	80.33 a	29.33 a	90.75 a
FA 008	21.08 c	24.42 d	67.75 c	23.33 c	81.25 c
BARI Strawberry-1	20.58 c	24.83 d	68.00 c	23.67 c	83.42 c
Level of significance	**	**	**	**	**

Figures followed by the same letter(s) in a column do not differ significantly by DMRT

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

Days to first harvest depended on both genotype and location, ranging from 21.25 to 27.00 days at Gazipur, 28.50 to 36.00 days at Rajshahi and 21.00 to 25.00 days at Chattogram. Fruit harvesting from FA 008 was earliest at Gazipur while that for Camarosa at Rajshahi was the latest. This was in line with the finding of Rahman *et al.* (2016).

Like days to harvest, fruiting duration also varied with genotype and location (Table 2). Fruiting duration ranged from 79.8 to 90.8, 84.5 to 95.3 and 79.5 to 85.8 days at Gazipur, Rajshahi and Chattogram, respectively. Fruiting duration was the shortest in genotype FA 008 at Chattogram and the longest at Rajshahi (Table 3). Temperature influenced the flowering time, high temperature causing a relatively quick bloom compared with the blooming time in lower temperature regions. On the other hand, in low temperature areas (Rajshahi), flowering and fruiting were delayed although the harvest duration was found to be longer. In previous studies, Rahman *et al.* (2016) found that low temperatures were conducive to good plant growth and development, and the plants grown at low temperatures had delayed flowering, fruit set and a relatively long harvest duration. The observed variations in this study might be due to the effect of environmental factors prevailing at the locations. The locations of this study differed considerably with respect to geographic location and elevation (latitude, longitude, altitude), day length, daily mean temperature band soil characteristics (Table-1). Variations in in photoperiod and temperature are the functions of latitude, longitude

and/or altitude (Ledesma *et al.*, 2008). Palmer and Dixon (1990) reported good correlations of climatic conditions with vegetative growth, flowering, fruiting, harvesting and fruit quality of strawberry. Erika *et al.*, (2022) stated that photoperiod, temperature and latitude are the primary factors controlling the transition from vegetative to reproductive growth in strawberry, and genotype, cultivars, on the other hand, is the key determinant of plant response to climatic conditions especially the response regarding floral initiation. Results of the present study corroborate these observations of earlier researchers.

Table 3. Interaction effect of location and genotype on plant height, leaves plant⁻¹, days to flowering, days to harvest and fruiting duration in strawberry

Treatment	Plant height (cm)	Leaves plant ⁻¹	Days to flowering	Days to harvest	Fruiting duration (days)
Gazipur x Sweet Charlie	27.00 bc	45.25 d	73.75	24.25 ef	87.50 c-e
Gazipur x Festival	28.00 bc	48.75 c	76.50	26.00 de	89.30 b-d
Gazipur x Camarosa	28.50 b	54.25 ab	80.50	27.00 cd	90.80 bc
Gazipur x FA 008	22.00 ef	28.50 e	66.50	20.00 g	79.80 b-d
Gazipur x BARI Strawberry-1	21.75 ef	26.50 ef	67.00	21.25 g	83.00 gh
Rajshahi x Sweet Charlie	28.50 b	42.75 d	77.00	30.50 b	92.30 b
Rajshahi x Festival	30.25 a	52.00 b	79.00	33.75 a	95.30 a
Rajshahi x Camarosa	30.75 a	56.25 a	84.25	36.00 a	98.30 a
Rajshahi x FA 008	23.25 e	25.25 fg	71.50	29.50 b	84.50 e-g
Rajshahi x BARI Strawberry-1	21.50 f	25.75 ef	71.75	28.50 bc	87.30 c-f
Chattogram x Sweet Charlie	22.75 e f	23.50 fg	71.00	22.25 fg	83.30 f-h
Chattogram x Festival	25.00 d	25.50 ef	73.75	26.00 de	85.80 d-g
Chattogram x Camarosa	26.50 cd	25.75 ef	76.25	25.00 de	83.30 f-h
Chattogram x FA 008	18.00 g	19.50 h	65.25	20.50 g	79.50 h
Chattogram x BARI Strawberry-1	18.50 g	22.25 gh	65.25	21.25 g	80.00 h
Level of significance	*	**	ns	*	**
CV (%)	3.62	4.63	4.34	4.61	2.51

Figures followed by the same letter(s) in a column do not differ significantly by DMRT

**Significant at 1 % level; *Significant and 5 % level; ns = Not significant

3.2. Yield and quality attributes

The numbers of fruits plant⁻¹ at Gazipur and Rajshahi were statistically similar but significantly higher than that at Chattogram (Table 4). Sweet Charlie was found to be the most productive genotype, the numbers of fruits plant⁻¹ in this genotype at Gazipur and Rajshahi were similar (Table 5). The number of fruits plant⁻¹ was found to be the

minimum for in BARI Strawberry-1 as well as in FA 008 at Chattogram and similar results were obtained at the other locations. Seasonal changes and critical photoperiods and temperatures of the locations might have influenced flowering and fruiting of the genotypes (Opstad, 2011). The coefficient of variation (CV) of fruits plant⁻¹ showed a very stable character at 1% level of significance across locations (Table 5).

Table 4. Single effect of location and genotypes on yield, yield contributing characters and fruit quality attributes in strawberry

Treatment	Fruits Plant ⁻¹	Fruit weight (g)	Yield plant ⁻¹ (g)	Yield (t ha ⁻¹)	TSS ¹ (%)	AA ² (mg 100g ⁻¹)
Location						
Gazipur	37.20 a	15.60 a	580.53 a	14.11 a	7.70 a	73.00 b
Rajshahi	36.55 a	16.89 a	620.88 a	16.55 b	7.88 a	77.30 a
Chattogram	33.50 b	15.28 b	517.60 b	13.27 c	7.23 b	74.45 ab
Level of significance	**	**	**	**	**	*
Genotypes						
Sweet Charlie	39.58 a	17.04 b	676.33 a	17.06 a	7.67 b	70.17 d
Festival	35.75 b	18.13 a	647.25 a	16.00 b	7.94 ab	79.58 a
Camarosa	36.67 b	17.85 ab	655.20 a	16.43 b	8.23 a	78.50 a
FA 008	33.58 c	12.67 d	425.54 b	11.29 d	7.04 c	74.00 b
BARI Strawberry-1	33.17 c	13.92 c	460.67 b	12.03 c	7.13 c	72.33 c
Level of significance	**	**	**	**	**	**

¹ Total dissolved solids; ² Ascorbic acid

Figures followed by the same letter(s) in a column do not differ significantly by DMRT

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

Table 5. Interaction effect of location and genotypes on yield, yield contributing characters and fruit quality attributes in strawberry

Treatment	Fruits Plant ⁻¹	Fruit weight (g)	Yield plant ⁻¹ (g)	Yield (t ha ⁻¹)	TSS (%)	AA (mg 100g ⁻¹)
Gazipur x Sweet Charlie	42.25 a	17.13 ab	725.10 ab	17.71 ab	7.53 de	79.00 bc
Gazipur x Festival	35.25 cd	18.25 a	642.80 bc	14.71 bc	8.10 ab	77.80 bc
Gazipur x Camarosa	35.00 cd	17.38 ab	607.50 c	14.21 c	8.45 a	70.30 g
Gazipur x FA 008	36.00 cd	12.00 e	430.50 d-f	10.92 d-f	7.28 ef	71.30 fg
Gazipur x BARI Strawberry-1	37.50 bc	13.25 de	496.80 d	12.64 d	7.15 ef	66.80 h
Rajshahi x Sweet Charlie	40.25 ab	18.00 a	723.90 ab	18.74 ab	8.05 a-c	82.00 a
Rajshahi x Festival	35.25 cd	18.63 a	656.00 bc	17.08 bc	8.18 ab	80.50 ab

Treatment	Fruits Plant ⁻¹	Fruit weight (g)	Yield plant ⁻¹ (g)	Yield (t ha ⁻¹)	TSS (%)	AA (mg 100g ⁻¹)
Rajshahi x Camarosa	40.25 ab	14.25 d	746.50 a	19.87 a	8.43 a	77.30 cd
Rajshahi x FA 008	34.75 cd	13.50 de	494.30 d	13.74 d	7.30 d-f	74.00 ef
Rajshahi x BARI Strawberry-1	32.25 de	15.00 cd	483.80 de	13.02 de	7.43 de	72.80 e-g
Chattogram x Sweet Charlie	36.25 cd	16.00 bc	580.00 c	14.72 c	7.43 de	77.80 bc
Chattogram x Festival	36.75 bc	17.50 ab	643.00 bc	16.22 bc	7.55 c-e	77.30 cd
Chattogram x Camarosa	34.75 cd	17.63 ab	611.60 c	15.43 c	7.80 b-d	74.50 de
Chattogram x FA 008	30.00 e	11.75 e	351.90 f	9.29 f	6.55 g	71.80 e-g
Chattogram x BARI Strawberry-1	29.75 e	13.50 de	401.50 ef	10.42 ef	6.80 fg	71.00 g
Level of significance	**	*	**	**	*	**
CV (%)	5.93	4.78	7.17	8.02	3.44	11.58

Figures followed by the same letter(s) in a column do not differ significantly by DMRT

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

The maximum individual fruit weight was found at Rajshahi which was statistically similar to that at Gazipur. The individual fruit weight was minimum at Chattogram (Table 4). The effect of location and genotype x location interaction on fruit weight differed significantly (Table 5). The fruits of Festival and Sweet Charlie were the largest in size at Rajshahi. At Gazipur and Chattogram also the largest fruits were produced by Festival. Camarosa also gave large fruits at Chattogram. The smallest individual fruit was found in FA 008 at Chattogram, Gazipur and Rajshahi and in BARI Strawberry-1 at Chattogram. The prevailing environmental conditions of the locations might have influenced fruit weight as also observed by Wang and Camp (2000).

Both fruit yield plant⁻¹ and yield hectare⁻¹ were the highest at Rajshahi, and yield plant⁻¹ was statistically similar at Gazipur (Table 4). Both parameters were significantly influenced by location and genotype (Table 5). At Rajshahi the highest yield hectare⁻¹ was recorded for Camarosa. Sweet Charlie yielded similarly at Rajshahi and Gazipur. The lowest yield hectare⁻¹ was recorded for FA 008 at Chattogram followed by Gazipur. BARI Strawberry-1 yielded similarly at Chattogram, and the results were consistent across the locations (Table 5). These results compared well with the findings of Wang and Camp, 2000. The CV% of 8.2 at the 1% level of significance (Table 5) indicated a very stable yield across the locations.

The highest TSS content of 7.88% was recorded in fruits at Rajshahi, the TSS value for fruits from Gazipur (7.70) compared well while TSS was the lowest (7.23%) in fruits from Chattogram (Table 4). The pre-harvest temperature of a given location may influenced TSS content of strawberry fruits (Darnell, 2003; and Ledesma *et al.*, 2008). With regard to genotype, the highest TSS was found in Camarosa at Gazipur and Rajshahi. TSS values in Festival at Rajshahi and Gazipur and in Sweet Charlie at Rajshahi were also high. It is noteworthy that Camarosa had the highest average TSS

value 8.23% and the lowest CV% of 3.44. The lowest TSS values of 6.55 and 6.80% for FA 008 and BARI Strawberry-1 were recorded at Chattogram (Table 5). A relatively high pre-harvested temperature at Chattogram may have been the cause of the low TSS content of fruits there. Shaw (1990) and Wang and Camp (2000) observed that high temperature considerably reduced TSS content of strawberry.

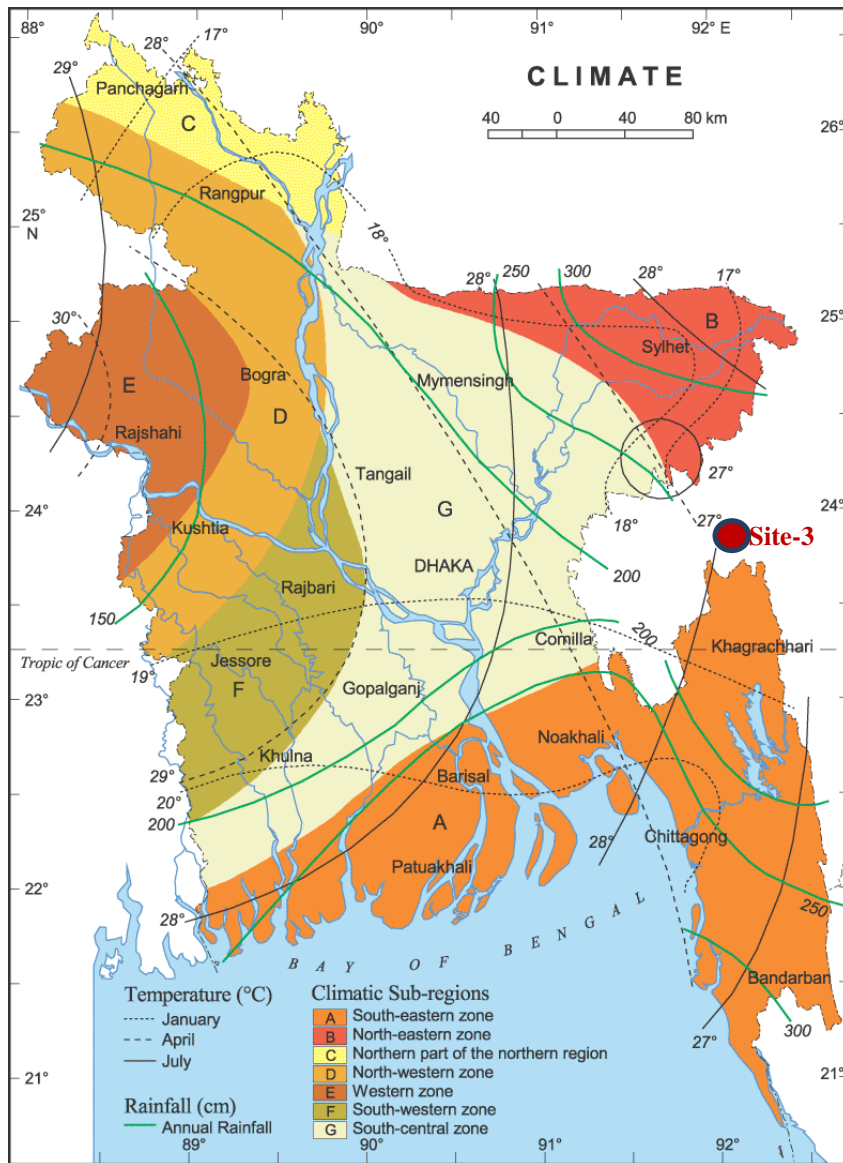


Fig. 1. Climatic map of Bangladesh (Source: Rashid, 2019)

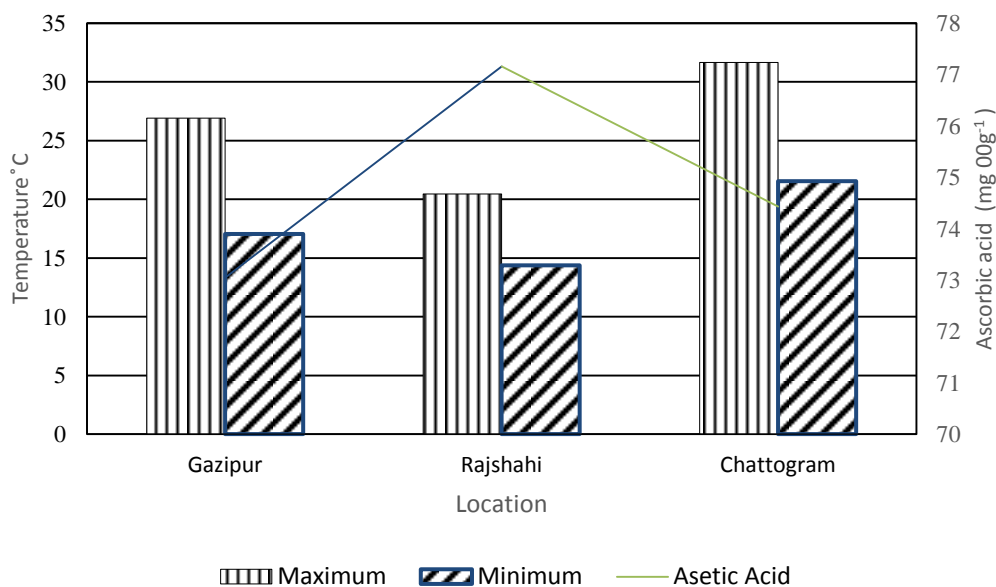


Fig. 2. Effect of location on ascorbic acid content of strawberry

Temperature significantly influenced the ascorbic acid (AA) content of the different strawberry genotypes (Fig. 2). The AA content of fruits was higher in the cooler region (Crespo *et al.* 2010). The highest AA content was recorded in fruits at Rajshahi and the lowest at Gazipur (Table 4). Location and genotypes significantly affected the fruit AA content (Table 5) which ranged from 66.8 to 82.0 mg 100g⁻¹ FW, the highest being for Sweet Charlie at Rajshahi followed by Festival at the same location and the lowest for BARI Strawberry-1 at Gazipur plant. Regardless of genotype, fruits from Rajshahi had a higher AA content (Table 5) which could be due to the low air temperature at this location (Fig 2, Table 1). Crespo *et al.*, (2010) also reported similar findings. Josuttis *et al.* (2012) observed an influence of latitude on the concentrations of bioactive compound in strawberry fruits.

4. CONCLUSION

Five strawberry genotypes were evaluated at three geographically varying locations of Bangladesh in terms of their growth, fruiting duration and yield and quality of fruits. The results indicated that strawberry can be successfully grown all over Bangladesh, but the Rajshahi region offers relatively suitable environmental conditions for strawberry to grow and yield well and produce good quality fruits. Considering growth, yield and fruit quality, the genotypes Camarosa and Sweet Charlie in Rajshahi, Sweet Charlie in Gazipur and Rajshahi and Festival in Chattogram are expected to perform well. Trials with more genotypes in the future in diverse agro-ecological conditions across the country may provide more detailed information on the genotype-wise and region-wise suitability of growing strawberry in the Rabi season.

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EFFECT OF HARVESTING DATE ON PHYTOCHEMICALS ACCUMULATION IN POTATO CULTIVARS

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ABSTRACT

Glycoalkaloids (α -solanine and α -chaconine) are naturally occurring toxic compounds in potato (*Solanum tuberosum* L.) tuber that cause acute intoxication in humans after their consumption. Phenolics provide valuable health promoting antioxidants, whereas glycoalkaloid concentrations exceeding the upper safety limit of 20 mg 100g⁻¹ fresh weight (Fwt) are potential neurotoxins. The aim of this study was to determine changes in the concentrations of glycoalkaloids and phenolic compounds in tubers of three selected early potato varieties during different growth stages. The total glycoalkaloids (TGA) and phenolics were determined by UV spectrophotometry. The tuber TGA content ranged from 7.47 to 9.23 mg 100g⁻¹ Fwt in potato varieties BARI Alu-41 and BARI Alu-13, respectively, and were below the upper safety limit. The total phenolics concentration in the tubers varied from 94.98 to 194.98 mg g⁻¹ Fwt. Glycoalkaloid and phenolic production was significantly reduced from the time of initiation to maturity at 55 and 90 days, after planting (DAP), respectively. Tuber phytochemicals were strongly influenced by variety and stage of maturity of the crop. For nutritional safety and to maintain quality, harvesting of mature potato tubers after 90 DAP is recommended.

Keywords: Glycoalkaloids, Harvesting time, Phenolic compound, *Solanum tuberosum*.

INTRODUCTION

Potato is the third most important crop in Bangladesh next to rice and wheat. It is one of the promising crops for the country due to its high productivity, short duration and wide adaptability. Besides its nutritional value, potato plants produce a variety of secondary metabolites during growth and post-harvest storage. These secondary compounds include glycoalkaloids, phenolic acids, protease inhibitors and lectins (Friedman, 2006). The glycoalkaloids are nitrogen containing steroidal glycosides, derivatives of aglycone solanidine having major constituents known as α -solanine and α -chaconine (Friedman and McDonald, 1997).

The normal season for potato cultivation in Bangladesh starts from mid-November after harvesting of Aman rice (monsoon rice). However, early potato can be harvested in only 60 days, and farmers can grow traditional potato varieties on the same piece of land with

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double benefits. However, the quality, such as, flavour, taste, flesh consistency, mellowness, moisture, texture and chemical composition, etc. of early harvested edible potato tuber may deteriorate with time and its suitability for human consumption may be compromised. This Moreover, potato plants produce a variety of secondary phytochemicals during growth and post-harvest storage such as glycoalkaloids (α -chaconine & α -solanine), phenolic acids, protease inhibitors and lectins. Among these phytochemicals, glycoalkaloids have been widely studied because of their toxicity to humans (Rytel, 2012). Glycoalkaloids concentrations exceeding the upper safety limit of 20 mg/100g fresh weight are potential neurotoxins (Carlson-Nilsson et al., 2000). Early cultivars have often been connected with glycoalkaloid levels above the recommended safety limit (Zolnowski et al., 2002). High levels of glycoalkaloids are reported to inhibit cholinesterase and disrupt cell membranes with clinical symptoms of poisoning that includes abdominal colic pain, diarrhea and vomiting. However, phenolics provide valuable health promoting antioxidants (McGehee et al., 2000). Potato contains several other phytochemicals such as flavonoids, polyamines and carotenoids which are highly desirable in diet because of their beneficial effects on human health. The concentration and stability of these constituents are affected by several factors such as genotype, agronomic factors, postharvest storage, cooking and processing conditions. In spite of this, no information is available on patterns of change in glycoalkaloids and phenolic concentrations in potato tubers during growth and maturity of early potato cultivars in Bangladesh. In this study, changes in the concentrations of different phytochemicals like glycoalkaloids and phenolic compounds in tubers of five popular potato varieties harvested at varying times in Bangladesh.

MATERIALS AND METHODS

Plant materials

Certified seeds of three commercial potato varieties selected for their varied resistance to late blight and suitability for chips, crisps and table-stock use were obtained from Breeder Seed Production Center (BSPC), Bangladesh Agricultural Research Institute (BARI), Debigonj, Bangladesh (Table 1).

Table 1: Commercial potato varieties used for the study

Name	Source	Year of release	Duration to maturity (days)	Optimal production (t ha ⁻¹)	Late blight resistance	Special attributes
BARI Alu-13	Netherlands	1994	90-95	20-30	Tolerant	Popular early variety, good taste and storage quality
BARI Alu-29	Netherlands	2008	85-90	20-26	Fairly tolerant	Early and processing quality variety
BARI Alu-41	Bangladesh (Carlita × TPS-67)	2012	90-95	38-44	Fairly tolerant	Early and processing quality variety

Source: Agro Technology Hand Book- Ninth Edition, BARI

Experimental design

The selected potato varieties were grown under field conditions in rabi season (Nov-Feb) of 2020-21 at Plant Physiology Division, BARI, Joydebpur, Gazipur. The experiment was laid out in randomized complete block design with three replications. The treatments included three potato varieties viz, BARI Alu 13, BARI Alu 29 and BARI Alu 41 with four harvesting times i.e., 55 DAP (days after planting), 65 DAP, 75 DAP and 90 DAP. The unit plot size was 4m × 3m. Whole tubers were planted with a spacing of 60 cm × 25 cm on 23 November, 2020. Well decomposed cowdung was applied @ 10 t ha⁻¹ before land preparation. Fertilizers were applied @ 150-45-125-20 kg ha⁻¹ N, P, K, S in the form of urea, triple super phosphate (TSP), muriate of potash (MOP) and gypsum, respectively. Full amount of TSP, MOP, gypsum and 50% of urea were applied as basal during final land preparation and the remaining amount of area was side dressed at 35 days after planting. Weeding, irrigation, earthing-up and other intercultural operations were done as and when necessary.

Tuber sampling and yield

Sampling and harvesting were performed four times i.e., 55, 65, 75 and 90 DAP as follows treatment. Data on total tuber yield t ha⁻¹ from 1.2 m × 3.5 m area at harvest as the sum of marketable and non-marketable tuber yields. The plants in the outer row were excluded during sampling. Three plants from each variety in each replicate were randomly selected and six tubers each weighing 17-22 g were collected, washed with cold water to remove extraneous materials and thoroughly dried. The unpeeled samples were cut into small pieces using a kitchen chopper. The samples were then thoroughly mixed and sub-samples of 300 g were freeze-dried. The dry samples were ground in a Wiley mill® to pass through a 40-mesh screen and stored at 4 °C until used for extraction and analysis of glycoalkaloid and phenolic components.

Extraction and quantification of glycoalkaloids

Extraction of glycoalkaloids was conducted employing the method adapted from Cataldi et al., 2005. Two and a half g of tuber sample was dissolved with 35 ml of 2% acetic acid for 2 hours. The extract was recovered by vacuum filtration, the residue washed with 15 ml of 2% acetic acid and the combined filtrate centrifuged for 30 minutes at 6000 rpm. The supernatant was heated gently to 75 °C, allowed to cool and subsequently 15 ml of 58% aqueous NH₄OH added to raise the pH to >10, alkaline condition needed to precipitate alkaloids. The alkaloids were rapidly precipitated in an ice-water bath for 1 hour. The precipitate was collected by centrifugation at 6000 rpm for 30 minutes at 1 °C and the pellet washed twice with 1% NH₄OH prior to drying. The final pellet was placed in an oven at 60 °C overnight to evaporate the ammonia and solubilized prior to UV spectrophotometric glycoalkaloid analysis.

Spectrophotometric measurements were carried out using a double-beam UV-1800 UV-VIS spectrophotometer (Shimadzu Scientific Instruments Inc., Kyoto, Japan). All potato tuber extracts were diluted appropriately before analysis to bring the sample absorbance to within the detection range of the UV-Vis spectrophotometer. Spectrophotometric determination of glycoalkaloids was conducted on dry pellets that were reconstituted in 3

ml of a mixture of 50% ethanol and sulphuric acid (1:2; v/v). One ml of 1% formaldehyde was added dropwise to the solution while the flask was stirred vigorously in an ice-bath. The flask was then transferred to a water bath maintained at 23-25 °C for 90 mins and the absorbance of the resulting purple red colour measured at 562 nm using the UV-Vis spectrophotometer.

Extraction and Quantification of Total Phenolics

The concentration of total phenolics in the tuber extracts was determined by Folin-Ciocalteu (Chirinos et al., 2005). Fifty microlitres (50 µl) of each potato extract was transferred into a 15 ml glass tube, diluted with 3.95 ml of distilled water followed by addition of 250 µl of 10% FC reagent (phosphomolybdate & phosphotungstate) and vortexed thoroughly. After 5 minutes, 750 µl of 7% Na₂CO₃ was added and the solution was incubated at room temperature for 2 hrs. Upon reduction of the FC reagent, the absorbance of 100 µl of the resulting blue complex was measured at 765 nm using a UV-Vis spectrophotometer against the reagent blank. The concentration of total phenolics was calculated from gallic acid standard curve and results of total phenolic content were expressed as mg GA equivalents per 100 g fresh weight (mg GA equ/100 g Fwt) of tuber tissue.

Statistical analysis

All data were presented as mean values for each treatment. An analysis of variance was conducted between different treatments. The significance of the differences between control and research treatment were evaluated by LSD tests ($P < 0.05$) using the SAS 9.2 (SAS Institute Inc., Cary, NC, USA) statistical software. Origin Pro version 8.0 (Origin lab corporation, Wellesley Hills, Wellesley, MA, USA) was used to prepare graphs.

RESULTS AND DISCUSSION

Glycoalkaloid content of potato varieties at different stages of maturity

The total glycoalkaloid (mg/100g Fwt) content of tubers from three commercial potato varieties at different stages of maturity as determined by UV spectrophotometry are shown in Fig 1. The results indicate that the influence of variety and stage of maturation on the concentration of TGA in potato tubers were significant. The tuber total glycoalkaloid (TGA) contents ranged from 8.79 to 10.87 mg 100g⁻¹, 7.88 to 9.14 mg 100g⁻¹, 7.03 to 9.12 mg 100g⁻¹ and 6.20 to 7.8 mg 100g⁻¹ at 55, 65, 75 and 90 days after planting (DAP), respectively. The tubers from BARI Alu-41 and BARI Alu-13 varieties had the lowest (7.47 mg) and highest (9.23 mg/100g) concentration of mean TGA, respectively. The data clearly shows that there was a significant ($P < 0.05$). TGA reduction from the period between tuber initiation and maturity.

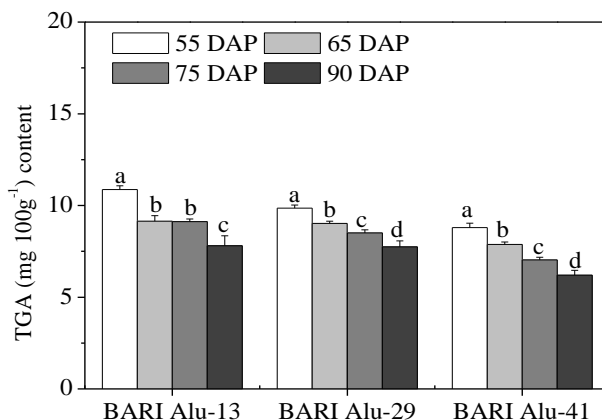


Figure 1: Total glycoalkaloid (TGA) (mg 100g⁻¹) content of potato tubers at different stages of maturity as determined by UV spectrophotometry. Error bars represent SD values (n=3). Different letters indicate significant differences ($P < 0.05$) among the treatments within each genotype.

Total phenolic content of potato tubers at different stages of maturity

The phenolic content of tubers from three commercial potato varieties determined by UV-spectrophotometry is shown in Fig. 2. The mean TP values for potato tubers ranged from 94.98 to 194.96 mg 100g⁻¹. The results indicate that variety and stage of maturity affect the concentration of tuber TP significantly. The corresponding total phenolic (TP) content varied from 77.8 to 228.79 mg 100g⁻¹ Fwt with the highest and lowest concentration measured in vars. BARI Alu-41 and BARI Alu-13, respectively. The observed variations of TP among potato varieties evaluated are within the acceptable limits reported in previous investigations (Im et al., 2008; Reyes et al., 2005) in which tuber TP contents ranged from 1.0-181 mg g⁻¹ Fwt. This study has also established that vars. BARI Alu-41 have great potentials as a source of TP which can be positive in terms of the antioxidant intake but at the same time could speed potato browning reactions that lower their quality.

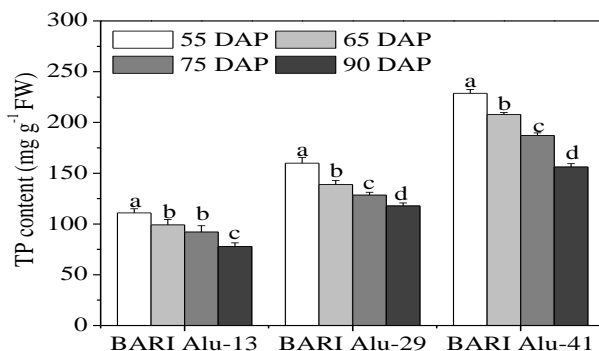


Figure 2: Total phenolic (TP) content of potato tubers at different stages of maturity. Error bars represent SD values (n=3). Different letters indicate significant differences ($P < 0.05$) among the treatments within each genotype.

The concentration of total phenolic content decreased significantly from the period between tuber bulking at 55 days after planting (DAP) and the period when mature tubers were ready for harvest at 90 DAP. This demonstrates that the stage of maturity influences the level of phenolic compounds in potato tubers, an observation which is consistent with results of Reyes et al., 2004 who reported decreased anthocyanins and total phenolics during tuber growth and development. The observed changes suggest that phenolic profiles can be useful to potato breeders and growers in selecting the optimum time for harvesting to increase antioxidant properties in food.

Tuber yield

The mean tuber yield ranged from 9.25 to 12.40 t ha⁻¹, 11.83 to 16.57 t ha⁻¹, 14.07 to 19.44 t ha⁻¹ and 18.39 to 23.06 t ha⁻¹ at 55, 65, 75 and 90 DAP, respectively (Fig. 3). There was a gradual increased tuber at 55 DAP to at 90 DAP. The highest average (55 DAP to 90 DAP) tuber yield was obtained from BARI Alu-41 (18.30 t ha⁻¹) followed by BARI Alu-29 (15.30 t ha⁻¹) and BARI Alu-13 (13.38 t ha⁻¹).

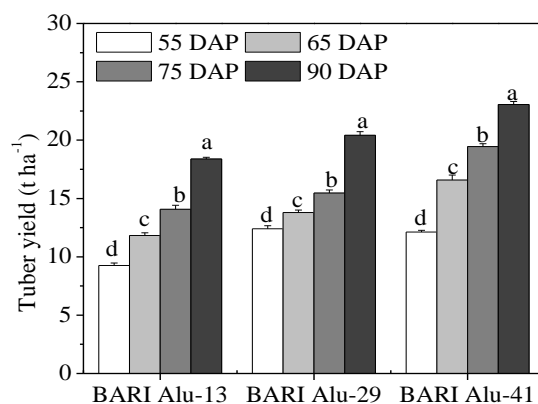


Figure 3: Yield of potato tubers at different stages of maturity. Error bars represent SD values (n=3). Different letters indicate significant differences ($P < 0.05$) among the treatments within each genotype.

Correlation coefficients among the total glycoalkaloid, phenols, tuber yield and stage of maturity

Correlation coefficients among total glycoalkaloid, phenols, tuber yield and stage of maturity shown in the figure below. A highly significant correlation relationship was found among the total glycoalkaloid, phenols, tuber yield and stage of maturity. Total glycoalkaloid, phenols, a strong correlation which depict that these parameters decrease along with the days to maturity. But in case of tuber yield it increase with time. In case of total glycoalkaloid, showed strong negative correlation with days to maturity and slightly negative correlation with total phenol. Moreover, total phenol showed almost same negative and strong correlation with all tested traits. So, it revealed that total glycoalkaloid and total phenols decreases over time i.e. Days after planting while tuber yield was found highest at the highest point of days after planting which indicates full maturity.

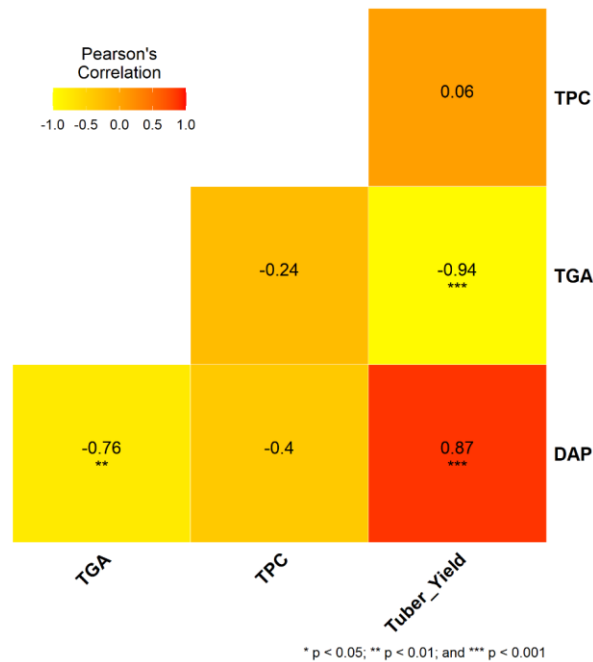


Figure 4: Correlation map of the 4 traits based on Spearman correlation analysis (positive, red; negative, yellow).

CONCLUSION

Variety and stage of maturity influenced the production of phytochemicals in potato tubers. The glycoalkaloid content in fresh mature tubers of the potato varieties studied was found to be well below the upper safety limit of 20 mg 100g⁻¹ Fwt implying that it does not pose as any public health and safety concern. This study also revealed large variations in total phenolic contents among the potato varieties at different stages of maturity. The glycoalkaloid and phenolic levels decreased with advancing maturity of potato tubers. Therefore, harvesting potato at full maturity is essential for consumer safety as well as to maintain good nutritional quality and commercial value.

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SOCIOECONOMIC STUDY ON A LOCAL POTATO CULTIVAR IN CHATTOGRAM DISTRICT OF BANGLADESH

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ABSTRACT

The local potato cultivar, Dohazari Alu is very popular and has a high demand in the Chattogram district of southeastern Bangladesh for its distinct features, but it is largely unknown to agricultural scientists and policy makers. This study was carried out in nine villages participating with 55 farmers in Chandanish upazila (sub-district) of Chattogram to examine the input use patterns, productivity and profitability of this crop at the farm level. In addition, farmers' and consumers' perceptions of this promising potato cultivar were assessed. The multi-stage sampling technique was followed for selecting the specific study area for data collection. Proportionate random sampling was used for determining the sample size and descriptive statistics was employed for analyzing the collected data. Results revealed that farmers used relatively low level of inputs, particularly low fertilizer doses for cultivating Dohazari Alu. The average yield was recorded was 13.75 t/ha with an estimated gross margin of Tk. 1, 71,466/ha. The benefit-cost ration (BCR) was found to be 1.83 on a variable cost basis. The cost of production per kg of potato was calculated at Tk.17.91. The farmers reported a high market demand for Dohazari Alu because of its good taste. The consumers while complaining of high prices agreed that this potato cultivar imparts a fine taste to the curry, its quality remains good and it does not rot even if stored at the ambient temperature and it is readily available at the local markets. Regular training of farmers on fertilizer and pest and disease management will help increase the productivity and profitability of this potato cultivar.

Keywords: Local cultivar, Dohazari Alu, Profitability, Productivity, Chattogram

INTRODUCTION

Potato is the staple food of the people in at least 40 countries, and, as such, it is a major food crop of the world. In terms of production, potato stands fourth after paddy, wheat and maize. Potato is an important tuber crop in Bangladesh, and is widely cultivated in some districts of the country with favorable weather and marketing conditions. It is a short-term high yielding crop that can help increase food production in a land hungry country like Bangladesh. According to BBS (2022), the total production of potato in the country in 2020-21 was 9887242 metric tons (MT) of which 9090810 MT (91.95%) came from high-yielding varieties (HYV) and the rest (8.05%) from local cultivars. In that

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year, the total production of local cultivars of potato in the Chattogram district was 25772 MT with a cultivable area of 2175 ha, the average yield being 11.848 t/ha. On the other hand, in the Chattogram district, only 22032 MT potato came from HYVs cultivated on 1206 ha of land in 2020-21 with an average yield of 18.263 t/ha. According to the existing data, the area under local cultivars of potatoes in Chattogram was larger than that under HYVs.

A local variety of potato, called Dohazari Alu, is a local improved cultivar originating in the Chandnaish upazila (sub-district) of Chattogram district. The yield of this early variety of potato is good everywhere in this region including on the banks of the river Shangu. It is well known in the Chandnaish region as a Rabi (winter) season cash crop. . According to the statistics of Upazila Agriculture Office, potato is cultivated in a total area of 580 ha. There are only two cold storages in the Chandnaish upazila. Sometimes potato farmers become frustrated as they do not get the right price of their produce due to inefficient marketing. Bangladesh has formulated a draft roadmap with a target of exporting 2.5 million MT of potato by 2025. It is learned that 18 varieties of potato suitable for export have been registered.

Hundreds of varieties of potato are cultivated in the world. These differ in appearance, tuber structure, size and color, maturation time, cooking and marketing qualities, yield and resistance to diseases and insects. A variety suitable for one area may not be suitable for another. Potato varieties cultivated in Bangladesh can be divided into two main categories, local and high yielding (HYV). The so-called indigenous varieties are not, in fact, completely indigenous. In the distant past, they were brought to this part of the subcontinent and in the absence of a variety development effort they gradually became degraded to relatively low-yielding cultivars. The Bangladesh Agricultural Research Institute (BARI) has so far developed 100 HYV of potato. About 28 local varieties of potato are cultivated in different parts of the country (The Daily Jaijaidin, 2021). They have familiar local names. Despite being low yielders, some local cultivars are still cultivated across the country for various reasons. Dohazari Alu is one of them. It is mainly cultivated in the Chandnaish area of Chattogram district. The tubers are round and pale, each weighing about 25 g (The Daily Jaijaidin 2021). This potato is in high demand locally as it has a long storage life at normal temperatures and is very tasty. It is possible to increase its current productivity to some extent through improved management. Research initiatives for developing improved production practices need some basic information about this cultivar. Therefore, the present study was undertaken to provide necessary information for commencing on-farm research. The specific objectives of the study were to (i) document the socioeconomic and contextual information of the growers, (ii) estimate the input use, productivity and profitability of the local potato cultivar Dohazari Alu, (iii) assess the farmers' and consumers' perceptions regarding this cultivar and (iv) suggest some policy measures.

METHODOLOGY

Study area selection: A multi-stage sampling technique was followed to select the study area. In the first stage of sampling, the Chattogram district was selected purposively to address the local cultivars scenario in the district. In the second stage, Chandanish

Upazila was selected purposively as the targeted local potato cultivar is widely/extensively cultivated in this area. In the third stage, nine villages were selected purposively from the aforementioned Upazila for data collection. Based on the availability of local cultivars, specific locations were selected in consultation with Upazila Agriculture Officer (UAO) and Sub-Assistant Agriculture Officer (SAAO). The selected villages were Moddham Hasimpur, Dakkin Joara, Fatorkul, Chagachar, Jamijori, Diakul, Purbo Dohazari, Hazipara, and Purbo Hasandondi.

Sampling techniques: There is no safe general rule as to how large the sample size must be for the use of normal approximation in computing the confidence limit (Cochran, 1999). When the population size is known and the researchers are careful of the heterogeneity problem, any number (equal to or) greater than the statistically large sample (of 30 sample units) may be appropriate (Freund and Williams, 1983). However, a proportionate random sampling technique was adopted for selecting the sample size. In this process, a sampling frame was constructed by 100 growers of Dohazari Alu. After that, the proportionate sampling (55%) was done equally for this crop. By applying this technique, due to resource limitations, a total of 55 sample households were selected for the study. Uddin et al. (2010) followed the same sampling procedures for another study.

Data collection procedure: Both primary and secondary data were used in this study. Secondary data and information were collected through reviewing related literature, relevant documents/reports, BBS, and the internet. Primary data were collected from sample households using a semi-structured survey schedule. Five experienced SAAO's were engaged for data collection. Before starting data collection, a discussion meeting on the survey schedule was held among data collectors for better understanding of the survey schedule. The data collection period was from February to April 2022. The researcher himself and Upazila Agriculture Officer monitored data collection so that the actual growers of local cultivar (Dohazari Alu) were interviewed. .

Analytical techniques: Descriptive statistics such as mean and percentage were used to analyze the primary data. The mean comparison and significance test were done by One-Way ANOVA using SPSS. In the ANOVA technique, the F-value was used to judge whether there is a significant difference or not among the locations and samples. The productivity was measured by the average yield of this crop per hectare basis. The average farm-gate price of potato was used for estimating the gross return. The profitability of crop production was analyzed based on gross return, gross margin, and benefit-cost ratio. Land use cost was calculated based on the per hectare value of leased land for four months. The following equations were used for working out gross return, net return and gross margin.

$$GR_{ij} = Y_{ij}P_{ij} \text{-----} (1)$$

Where,

- GR_{ij}= Gross return (Tk/ha) of jth crop for ith farmer
- Y_{ij}= Quantity of jth crop produced (kg/ha) by the ith farmer
- P_{ij}= Price of jth crop (Tk/kg) received by the ith farmer

Net return= Gross return – Total cost (total variable cost+ total fixed cost) ----- (2)

Gross margin= Gross return- Total variable cost ----- (3)

RESULTS AND DISCUSSION

Socioeconomic characteristics of the respondents

The socioeconomic characteristics of the respondent farmers are presented in Table 1. The average age of the respondent of Dohazari Alu was 45.95 years indicating that farmers involved in local cultivar production were fairly young. A similar result was obtained by Uddin et al. (2020) who reported an average age of 45.4 years for farmers in Hathazari, Chattogram. The average education level of the respondent was found to be 6.09 years of schooling. This might enhance their adoption of local cultivars. Kehinde (2005) noted that education was the key to enhancing productivity among farming households as it promotes their understanding of modern technologies. The major occupation of the respondents was agriculture (100%) in the study areas. Occupations of the respondents may influence the adoption of new technologies. The secondary occupation was reported to be business (15%) and service (2%) for all locations. The average household size was 6.75 persons per family which were higher than the national average of 5.0 persons (BBS, 2018). The mean difference the household size varied insignificantly among the locations (villages) ($F= 1.131$; $p \leq 0.203$). The average cultivated land per household was recorded as 0.927 hectares irrespective of locations. The highest 51% of farmers were found to be tenants followed by owner cultivators 45% in all locations (Table 1).

Table 1: Socioeconomic and demographic characteristics of the respondents

Sl. No.	Particulars	
1.	Average age of the respondents (years)	45.95
2.	Level of education [year of schooling]	6.09
3.	Occupational status (%): (multiple response)	
	- Agriculture	100.0
	- Business	14.5
	- Service	1.8
	- Day labour	-
4.	Family size (person/family)	6.75
5.	Cultivated land (ha/household)	0.927
6.	Farmer's category (%):	
	- Owner cultivators	45.5
	- Tenants	50.9
	- Owner cum tenant	3.6

Production-related information

Production-related information includes plot size, seed used, sources of seed, seed treatment, sowing period, number of spraying, harvesting period, duration of harvests, and yield (Table 2). Results revealed that the average plot size of the cultivar was found to be 0.379 ha which was (40.88%) of the total cultivated land of the respondent. The use of seed rate is an important issue for raising the productivity of the crop. The average seed used was 1046.9 kg/ha. Most of the seeds (52.7%) were bought from local bazaar (market) and some portions were kept themselves (40.0%). Farmers did not maintain recommended spacing for this cultivar. Table 2 shows that the highest 87.3% of respondents did not treat seeds before sowing. The prevalence of diseases and pests in cultivars is a common phenomenon particularly late/early blight and cut worm at the seedling stage. Controlling these problems, farmers usually spray different types of pesticides. The frequency of sprayed pesticides as per recommended SAAO's and dealer shopkeepers was 2.41 times for controlling pests & diseases. The average duration of crops was estimated at 96.21 days. The period of harvest of Dohazari Alu were ranged from February to March.

Table 2: Production-related information about local cultivar Dohazari Alu

Sl. No.	Particulars	Amount
1.	Average plot size (ha/farm)	0.379
2.	Seed used(kg/ha)	1046.9
3.	Sources of seeds (%):	
	- Own source	40.0
	- Neighbors	5.5
	- Local bazaar	52.7
	- Dealer's shop	1.8
4.	Whether the seeds are treated before sowing (%)?	
	-Yes	12.7
	- No	87.3
5.	Number of spraying per season	2.41
6.	Crop duration (days)	96.21

Input use pattern in Dohazari Alu production

The rationale and efficient use of inputs are prerequisites for enhancing the productivity of crops. Respondent farmers applied cowdung (as organic fertilizer), chemical fertilizer (i.e. Urea, TSP, MoP & Gypsum), and pesticides to the potato crop. The applied amounts of inputs were found to be lower than the recommended doses for HYV Potato (BARI, 2019). Farmers used a lower amount of fertilizers due to a lack of capital and unknown the effect of recommended doses of fertilizer (Table 3).

Table 3. Quantity of inputs used in producing Dohazari Alu

Sl. No.	Type of Inputs	Amount used in local potato(kg/ha)	Amount used in HYV potato (kg/ha)*
1.	Seed	1047	1500-2000
2.	Cowdung	4032	10,000
3.	Urea	162.4	325-350
4.	TSP	321.7	200-220
5.	MoP	161.8	250-300
6.	Gypsum	86.6	100-120
7.	Boron	-	6-9

*potatoarocal cultivar costs and fixed cost---- gross return ar le.h *Source: BARI, 2019

Production cost of local cultivar (Dohazari Alu)

The cost of production included both variable costs and fixed cost in the process of Dohazari Alu production. The variable costs included human labour used for land preparation, tractor use, cost of seeds, weeding cost, manure & fertilizer cost, insecticide cost, irrigation, and harvesting cost. The study found that the total variable cost of this local cultivar was estimated at Tk. 205463 per hectare which was 83.4% of the total cost of production. The leased value of land was considered as the fixed cost of production of the local cultivar. The land-use cost was estimated at Tk. 22344per hectare which is 9.1% of the total cost (Table 4).

Table 4. Cost of production of Dohazari Alu in the study areas

Sl. No.	Cost items	Amount (Tk/ha)	
		Taka	% of total
A.	Total Variable Cost		
1.	Labour cost for land preparation	20182	8.2
2.	Cost of tractor use	19983	8.1
3.	Seed cost	52638	21.4
4.	Weeding cost	20091	8.2
5.	Fertilizer cost:		
	- Cowdung	5121	2.1
	- Urea	3616	1.5
	- TSP	11177	4.5
	- MoP	2893	1.2
	- Gypsum	1264	0.5
6.	Insecticide cost	15396	6.3
7.	Irrigation cost	12733	5.2
8.	Harvesting cost	40370	16.4

Sl. No.	Cost items	Amount (Tk/ha)	
		Taka	% of total
	Total Variable Cost (TVC)	205463	83.4
10.	Interest on operating capital @9%	18492	7.5
B.	Fixed Cost (land use rent for potato season)	22344	9.1
C.	Total Cost [A+B]	246299	100

Note: Seed cost @ Tk 50.27/kg, Cowdung= Tk.1.27/kg, Urea =Tk.22.26/kg; TSP= Tk.34.74/kg; MoP= Tk. 17.88/kg; and Gypsum= Tk.14.58/kg. Labour wages= Tk. 550/man-day

Productivity and profitability of local cultivar (Dohazari Alu)

The average marketable yield of *Dohazari Alu* was recorded to be 13.75 tons/ha which is very much lower than that of HYV potatoes (BARI, 2016). The average farm-gate price of Dohazari alu was Tk.27.4/kg. The gross return, gross margin, and net return were calculated at Tk. 3,76,929, Tk.1,71,466, and Tk. 1,30,631 respectively for this local cultivar. The BCR of this crop was found to be 1.53 and 1.83 based on total cost and variable cost, respectively. It implies that the production of this crop is comparatively profitable at the farm level. However, the average cost of production per kilogram of this cultivar was estimated at Tk. 17.91 (Table 5). The daily JaiJaidin (2021) reported that the yield of Dohazari Ali was 80-85 mounds per 40 decimal lands and per kg potato was sold at a Tk. 21.25 per kg at farm-gate level in 2021. But in the year 2022, the yield was found to be lower than in the year 2021 hence the farm-gate price was higher compared to the previous year. The mean difference in yields varied significantly at a 5% level among the locations ($F= 5.237$; $p \leq 0.0513$).

Table 5. Productivity and profitability of the local cultivar (Dohazari Alu)

Sl. No.	Particular	Amount (Tk/ha)
1	Marketable yield (ton/ha)	13.75
2	Gross return	3,76,929
3	Total variable cost	2,05,463
4	Total fixed cost	22,344
5	Total cost [3+4]	2,46,299
6	Gross margin[2-3]	1,71,466
7	Net return[2-5]	1,30,631
8	Benefit-cost ratio (BCR)	
	Total cost basis	1.53
	Variable cost basis	1.83
9	Cost of production per kg	17.91

Note: Average farm-gate price of Dohazari Alu is Tk.27.4/kg

Farmer's perceptions for cultivating Dohazari Alu

Respondent farmers stated various reasons for cultivating this local cultivar in the study areas (Table 6). According to the farmer's opinion, the market demand of this cultivar is high because of its good taste. The price is higher than the other varieties of potatoes due to its higher demand. But the yield is medium to low due to poor management and less use of fertilizers. The infestations of diseases and insects are high. The storage capacity of this cultivar is also good at normal temperatures.

Table 6: Reasons for cultivating local cultivar (Dohazari Alu)

Sl. No.	Type of response	% of responses			
		Very high	High	Medium	Low
1.	Higher local demand	16.4	83.6	-	-
2.	Market price is high	10.0	72.7	17.3	-
3.	Very tasty	40.1	50.9	9.0	-
4.	High pest & disease infestation	52.7	27.1	20.2	-
5.	Storability at room temperature	-	76.4	23.6	-
6.	Poor to medium yield	-	-	63.7	36.3

Consumer's perceptions to Dohazari Alu

The consumers of local potato cultivar opined that its buying price is high due to market demand. This potato gives a different taste to the curry. The potato quality is high in terms of storing at normal temperatures. It is available in the local market. It does not rot easily if kept at room temperatures. The level of consumers' responses regarding this local cultivar is shown in Table 7.

Table 7: Consumer's responses to the local cultivar (Dohazari Alu)

Sl. No.	Type of response	In % of responses			
		Very high	High	Medium	Low
1.	Very high price	66.2	73.8	-	-
2.	Tasty	20.0	72.3	7.7	-
3.	Attractive colour	-	40.0	49.1	10.9
4.	High storage capacity at room temperature	52.7	67.1	32.9	-
5.	Available in the local market	-	76.2	23.8	-

Problems of Dohazari Alu cultivation

The highest percentage of respondents opined that they faced the problems of different pests and diseases attacked in the potato field. For example, viruses, blight, and fungus are the main diseases. In the case of pest attack, cutworm, etc most of the respondents claimed that they paid the high price of fertilizer i.e TSP. The other problems were stated

by the respondents as lack of irrigation and lower yield of the local cultivar (Table 8). Farmers attributed to the low yield of this cultivar to low fertilizer application and disease attacks.

Table 8: Problems faced by the respondent farmers during cultivation of local cultivar (Dohazari Alu)

Sl. No.	Types of Problems	In % of respondents
1.	Infection of virus diseases	72.7
2.	Occurrence of blight disease	67.3
3.	Attack of cut worm	52.7
4.	High price of fertilizer	63.3
5.	Lack of irrigation facility	52.7
6.	Lower yield	36.3

CONCLUSIONS

The local potato cultivar “Dohazari Alu” is cultivated in Chandnaish upazila of the Chattogram district of southwestern Bangladesh. It is very popular and has a high market demand due to its fine taste and ability to maintain good quality in storage at normal temperatures. This potato cultivar fetches good market prices which encourage local growers to cultivate it in preference over other cultivars. However, Dohazri Alu is grown with low fertilizer doses and it is also susceptible to diseases which result in low yields. Improved fertilizer management and disease and pest control measures can markedly increase the yield of Dhazari Alu and substantially enhance farmers’ incomes. Crop scientists may consider possibility of conducting research on transforming this local potato cultivar into an improved variety or maintain it as a breeding material for future potato breeding work.

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Crops: Agronomy, Soil Science, Plant Breeding and Genetics, Plant Pathology, Entomology, Horticulture, Agricultural Economics, Agricultural Engineering, Post-harvest Processing, Soil and Water resource Management, Agricultural Biotechnology, Agro-forestry, Plant Physiology Microbiology, Molecular Biology, Appropriate Technology, Crop Modeling, Forestry, Climate Change etc.

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Fisheries: Aquaculture, Fish Nutrition, Aquatic Animal Health, Fish Processing and Value Addition, Climate Change, Fisheries Economics, Fisheries Resource Management, Fish Genetics and Bio-technology, Aquaculture Engineering, etc.

Cross-Cutting Issues: Gender issue, Agricultural Market, Value Chain System, Food Systems, Nanotechnology, Precision Agriculture etc.

Criteria for Publication

1. The Manuscript is not submitted to other any Journal or not under consideration for publication elsewhere
2. Research-problem statement, experiments, data analyses and interpretation are stated with technical standard and are described in details.

3. Conclusions are presented precisely and are supported by the data.
4. The article is presented coherently and is written in Standard English (UK).
5. The research meets internationally accepted standards for the ethics and integrity in publishing.
6. The manuscript should be written as per guidelines of the Journal.

Manuscript Submission and Review

Categories of Manuscripts

Manuscripts may be submitted in any of the following categories:

1. **Original Research Articles:** Full length reports with data from original research.
2. **Review Papers:** Comprehensive, unbiased and authoritative descriptions existing literatures of any subject within the scope of the Journal.
3. **Case Studies:** Reports of live examples of farming community/enterprises demonstrating the best practices for sustainable agriculture
4. **Short Communications:** Short papers that present original and significant results of a problem as well as new finding that is expected to have a significant impact and rapid dissemination.

Submission of the Articles

Manuscripts and associated documents should be submitted On-line to the Executive Editor in the E-mail (ID kgfjournal21@gmail.com). The corresponding author has to submit following file separately not embedded in the main manuscript. File formats are DOC or DOCX.

1. Cover Letter

The cover letter will include:

- Broad topic (Crop Science or Livestock or Fisheries or Agroforestry or Cross-Cutting issues) and article category;
- All the authors have given consent to submit the manuscript to the Journal;
- It is the original work of the authors and all authors have contributed significantly for the pursuance of the research;
- The manuscript has not been published previously (except in the form of an abstract) and is not under consideration for publication elsewhere;
- State the novelty in results/findings/output or significance of the results (two to three bullet points);
- Three potential referees' name, affiliation, E-mail ID and reason for their selection.

2. Title Page

Title of the article, list of authors (full names, affiliations, E-mail) indicating the corresponding author. Present the authors' affiliation addresses (where the actual

work was done) below the names. Indicate all affiliations with a lower-case superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name.

3. **Authorship and Its Sequence**

Before submission, authors are anticipated to consider carefully the list and order of authors and mention the definitive order of authors at the time of the original submission based on their contribution. Any addition, deletion or rearrangement of author names will lead to rejection of the manuscript by the Editorial Board of the Journal. Only in exceptional circumstances, the corresponding author can submit such request through their head of the organization stating the reason.

4. **Main Manuscript**

Title, Abstract, Keywords, Introduction, Materials and Methods, Results and Discussions, Conclusion, Acknowledgment. Figures and Tables should be inserted and numbered in text in the order in which they are referenced in the text.

5. **Style and Format**

- Prepare manuscript in single sided A4 paper using the font 'Times New Roman' with font size of 12 pt in MS word (DOC/DOCX) format.
- Manuscript text should be double-line spaced with justified margins (2.54 cm) on each side, without hyphenating words at line breaks. Format the text in a double column.
- Limit manuscript sections and sub-sections to 3 heading levels. Make sure heading levels are clearly indicated in the manuscript text.
- Include number of all pages.
- Manuscripts must be submitted in English.
- Define abbreviations upon first appearance in the text. Do not use non-standard abbreviations unless they appear at least three times in the text. Keep abbreviations to a minimum.
- Using Math Type for display and inline equations is recommended, but Equation Editor or Microsoft's Insert→Equation function is acceptable.
- Use correct and internationally established nomenclature wherever possible. Follow SI units and write name of species in italics.
- JAID uses APA reference style as outlined in the <https://pitt.libguides.com/citationhelp/apa7>

6. **Manuscript Length**

The maximum words count of the manuscript should be restricted to 5000, 4000, and 2500 for Review, Original Research Article and Case-Study/Short-Communication, respectively.

7. Tables and Figures

The number of tables and figures should be restricted to 4 each. Table should have title above and figure caption below the figure. Table title and figure captions are inserted immediately after the first paragraph in which they are cited. Citation and table as well as figures should appear within the main manuscript chronologically. Avoid large tables and figures which are not comprehensible. Data should not be repeated in tables and figures.

For any queries regarding the submission, potential authors are requested to contact with Executive Editor of **JAID** through E-mail.

Review Process

All submitted manuscripts are initially evaluated by the Editorial Board for its scientific merit and suitability of publication in the Journal. Manuscripts deemed appropriate are then sent to two experts in the relevant discipline(s) for review and overviewed by one of the Journal's Editors. A manuscript may be returned to its author without review if it is judged to be of poor quality or inappropriate for this Journal. The reviewer's comments are communicated to the corresponding author and she/he is expected to revise the manuscript in response to the critiques, marking in the text using either tracking changes or highlighting with different colour. If there is any written response to the reviewers' comments, indicate the page number(s), paragraph(s), and/or line number(s) where each revision was made. Author(s) to provide reasons for suggested changes that were not implemented, and identify any additional changes that were made. Revisions not received within **1 (one) month** will be administratively withdrawn. Electronic proofs will be sent as an E-mail attachment to the corresponding author as a PDF or DOC/DOCX file. The Editorial Board is responsible for the final decision regarding the publication of the manuscript.

Article Processing Charge

Articles of any type (research, review, short communication and case study) are accepted based on scientific merit and are published free of cost.

Author Contributions

An author statement file outlining their individual contributions to the paper roles (e.g. conceptualization, experiment designing and methodology, investigation, data analysis, supervision, writing/editing, etc.) should be submitted.

Submission Checklist

Before sending the manuscript to the KGF, the authors are requested to undertake final check of the prepared manuscript:

- (i) Cover letter as has been mention above;
- (ii) Title page as has been mentioned above
- (iii) Entire file should have two parts:

- (iv) The manuscript includes:
 - keywords;
 - all figures with relevant captions;
 - all tables with relevant titles, description, footnotes (if required);
 - ensure all figure and table citations in the text match the files provided;
 - clear indication of color should be used for any figures in print.
- (v) Manuscript has been spell and grammar checked;
- (vi) Permission is taken for the use of copyright material;
- (vii) Authors contribution letter
- (viii) All references mentioned in the body of the manuscript are being cited in the reference list and vice versa.

Article Structure (Research Articles)

Authors are requested to divide their manuscript into clearly defined sections as mentioned below:

1. **Title:** Concise and informative as it is often being used for the scientific information retrieval. Title should be self-explanatory to convey message about the article. Avoid stale words. A small running title may be provided and should not more than two lines.

Running Head: A suggested running head (shortened version of the title) may also be included.

Abstract: It should be self-contained, citation-free and consist of one paragraph, not exceeding 300 words. An ideal abstract should contain main objectives of the study, how the study is done, summary of the most important results and their significance. Avoid abbreviations, but if required that must be defined before they are first used.

Keywords: Just after abstract, provide up to 5 keywords in alphabetical order and separated by comma. The first letter of each of the keyword should be in upper case. Avoid general terms and multiple concepts.

Introduction: The introduction should provide a context for the work to be reported, particularly defining problem addressed and its importance. Present the topic with relevant and appropriate background information evading detailed latest literature survey or results. Note any relevant controversies or disagreements in the field and conclude with a brief statement of the overall aim of the work. This should not exceed three pages.

Materials and Methods: This section provides the experiment design, raw materials, and tools and methods of how the research has been conducted. Describe the methods in such a way that an independent researcher could reproduce the same during their future investigation. Methods which are already published should be mentioned in brief citing the reference. Any modifications or changes made on the established protocol should be described in detail.

Results and Discussions: Results should be elaborated clearly and briefly. This section should describe the results of the experiments, the interpretation of these results, and the conclusions that can be drawn. Discuss significance of the results, relating to the hypothesis presented as the basis of the study and explain implications of findings, particularly in relation to relevant published literature. Repeated citation and discussion should be avoided.

Conclusion: The main conclusions arrived from results should be presented briefly (maximum 150 words). This section may also come up with potential future directions for the area of research interest. Authors should avoid repetition of sentences.

Acknowledgement: It should appear at the end of the article, before the references. Provide funding details with project or sanction number. Please acknowledge the individuals (if any) who have extended help during the research, assisted in language editing, helped in procurement of research materials etc.

Article Structure (Short Communication)

It should restrict the word count to 2500 including -

- title,
- names of authors and affiliation,
- abstract (150 words),
- brief introduction,
- materials and methods,
- results and discussion,
- references,
- relevant tables and figures.

Article Structure (Review)

It should restrict the word count to 5000 including -

- title,
- name of authors and affiliation,
- abstract (250 words), introduction with aim,
- discussion with headings and sub-headings (context specific),
- references,
- relevant tables and figures. The authors of the review should have adequate number of research publications on the subject.

Reference Style in Text

1. Single author: the author surname followed by year of publication (Alam, 2021).
2. Two authors: both authors' surnames followed by the year of publication (Alam and Karim, 2019).

3. Three or more authors: first author surname followed by 'et al.' and the year of publication (Alam et al., 2018).

References

- All references quoted in the text must appear at the end of the article and vice-versa. The spelling of names of author and date or year at the two places should be carefully checked;
- Restrict the references which are relevant to the particular topic. Recent and relevant, not more than 20 years old references are encouraged;
- The references should include names of all authors, year (within brackets), full title of the article, full name of the journal (in italic, no abbreviation), volume number, issue number in bracket, and pages. For book or monograph, the name of the publisher should also be given as well as its volume, edition and relevant pages;
- The references cited together in the text should be arranged chronologically. The list of references should be arranged in alphabetical order. A few examples for correct citation of references are mentioned herewith.

Article in Print Journal

Rahim MMH (2019). Abundance, damage severity and management of guava mealybug, *Ferrisia virgata* CkII. *KGF Journal of Agriculture*, 16(2): 73-82.

Alam MMH, Islam MM, Asaduzzaman M & Uddin MN (2019). Mutants and weather parameters affecting the population dynamics of three major insect pests of mungbean. *SAARC Journal of Agriculture*, 16(2): 1-12.

Article in an Electronic Journal

Grady JS, Her M, Moreno G, Perez C & Yelinek, J (2019). Emotions in storybooks: A comparison of storybooks that represent ethnic and racial groups in the United States. *Psychology of Popular Media Culture*, 8(3), 207–217. <https://doi.org/10.1037/ppm0000185>

A Book/Policy Brief

Sapolsky RM (2017). *Behave: The biology of humans at our best and worst*. Penguin

Gyeltshen K and Sharma S (2019). *Integrated plant nutrition system modules for major crops and cropping systems in South Asia*. SAARC Agriculture Centre, Dhaka, Bangladesh. Pp. 176.

George G and Hassan MS (2020). Policy brief on “*Guidelines for ensuring sustainability of fisheries and aquaculture in South Asia*”. SAARC Agriculture Centre, Dhaka, Bangladesh. Pp. 1-4.

Chapter in an Edited Book

Dillard JP (2020). Currents in the study of persuasion. In MB Oliver, AA Raney, & J Bryant (Eds.), *Media effects: Advances in theory and research* (4th ed., pp. 115–129). Routledge.

Conference/Symposium/ Proceedings

Joshi PK (2004). Crossing frequency and ancestors used in developing Nepalese mid and high hill rice cultivars: Possible criteria for yield improvement and rice genes conservation. *Proceedings of 4th National Conference on Science and Technology* held at Kathmandu, Nepal. Pp. 502-523.

Reference to a Website

Cancer Research UK (2004). *Cancer statistics reports for the UK*. <https://www.cancerresearchuk.org/about-cancer/bowel-cancer>. Accessed on 8th June, 2019.



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