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EFFECT OF SALINITY STRESS ON EARLY SEEDLING GROWTH AND SOME PHOTO-PHYSIOLOGICAL PROCESSES OF EGGPLANT GENOTYPES

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ABSTRACT

The performance of 11 eggplant genotypes to varying levels of salinity in a water-based nutrient solution was studied in the hydroponics laboratory of the Horticulture Research Centre (HRC) of the Bangladesh Agricultural Research Institute (BARI) during October 2009 to January 2010. Table salt was added to the nutrient solution to derive salinity levels of EC 4, 8 and 12 dS/m. The eggplant genotypes tested were SM 11, SM 12, SM 18, SM 19, SM 20, BARI Begun-1, BARI Begun-4, BARI Begun-6, BARI Begun-7, BARI Begun-8 and Tarapuri. Plant response to salinity stress was assessed in terms of dry matter production, photosynthesis rate and leaf fluorescence. Differences in dry matter production, stomatal conductance and transpiration rate among the genotypes were insignificant before imposing salt stress at transplanting. The pre-salt stress photosynthesis rate decreased, for all genotypes, with increasing salinity in the growth medium. Plant submitted to maximum salinity increasing minimum fluorescence (F_0). The ratio (Fv/Fm) of variable fluorescence (Fv) to maximum fluorescence (Fm) for all genotypes was similar at each level of salinity.

Keywords: Eggplant, fluorescence, photosynthesis, salinity, stomatal conductance

INTRODUCTION

Eggplant (*Solanum melongena L.)*, commonly known as brinjal in Bangladesh, is one of the most popular vegetables in the world. This crop can withstand diverse climates and stress conditions and can be grown in tropical, subtropical, and even temperate countries. Eggplant is a quick growing crop, and ranks among high-valued vegetables with high nutritional value (Liu et al., 2018). In Bangladesh, eggplant is a favorite vegetable of people of all ages and plays an important role in generating household income. Eggplant is classified as a moderately to highly salt-sensitive crop (Bresler et al., 1982; Maas, 1984), and thus, growing eggplant may be a good option to increase cropping intensity in the salt-affected coastal saline zones of Bangladesh where salinity is a serious problem for agriculture (Mondal et al., 2011).

Genotypic diversity is essential in a breeding program to develop varieties with increased resistance to growth inhibition by salinity. Salinity reduces the growth rate of both halophytes and non-halophytes (Flowers et al 1977). Among non-halophytes, there is a large variability among species, ranging from extremely sensitive to tolerant to salinity

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overlapping with halophytes (Greenway and Munns, 1980). Traits impacted by salinity are usually photosynthesis rates (A), stomatal conductance (Gs), evapotranspiration rate (Et), and leaf chlorophyll fluorescence (Kwon et al., 2019). With new knowledge of easily measurable Et, Gs, A, and fluorescence products it is now possible to identify physiological and morphological traits tolerant to many stresses like salinity. Chlorophyll fluorescence is a non-invasive measurement of photosystem II (PSII) activity and its measurement is a commonly used technique in plant physiology.

Excited chlorophyll dissipates the absorbed light energy by driving photosynthesis (photochemical energy conversion), as heat in non-photochemical quenching, or by emission as fluorescence radiation. As these processes are complementary, analyzing chlorophyll fluorescence is an important tool in plant research with a wide spectrum of applications. Considering the aforementioned facts, this study was undertaken to evaluate eggplant genotypes based on gas exchange, stomatal conductance, and leaf fluorescence products under different levels of salinity in the growth media.

MATERIALS AND METHODS

The experiment was conducted in the Hydroponic Laboratory of the Horticulture Research Centre (HRC), Bangladesh Agricultural Research Institute (BARI), Gazipur from October 2009 to January 2010. Eleven eggplant genotypes, SM 11, SM 12, SM 18, SM 19, SM 20, BARI Begun-1, BARI Begun-4, BARI Begun-6, BARI Begun-7, BARI Begun-8 and Tarapuri were included in the study. We collected seeds of the genotypes from the Olericulture Division, HRC. Seedlings were raised in a hydroponics laboratory on a floating foam. The size of each foam was 3m X 2m and the thickness was 2.5 cm. One-centimeter deep furrows were made following the length and breadth of the foam. Furrow to furrow distances in each direction was 2 cm and as such 2 x 2 cm blocks were made. A 0.5 cm deep cut was made at the centre of each block in which seeds were put.

The foams were placed in 3 X 4 m water tanks made of tin. Seedling germination was monitored, and the seeds were considered to have germinated when their radicles reached a length >3 mm. Seedlings after 10-15 days of emergence were transferred into the nutrient solution. Buckets of 10 L capacity were used to grow the seedlings. A cork sheet was placed on the top of the bucket to hold the seedlings in the nutrient solution. The seedlings after 25-30 days of emergence were exposed to salinity levels of EC 4 (T_1), 8 (T_2), 12 (T_3) dS/m and control (T_4, no salt added) developed by adding table salt to the nutrient solution. An electric conductivity (EC) meter were used to measure the salinity of the nutrient solution. The experiment was laid out in a RCBD factorial (salinity as the main factor and genotype as the secondary factor) design with three replications. Three seedlings were planted in each bucket. Each seedling was considered a replication.

Morphological and physiological data including chlorophyll fluorescence values, rate of photosynthesis, and stomatal conductance were measured before exposing the seedlings to salinity. Growth of the seedlings in the nutrient solution was monitored. When symptoms of salt stress such as, chlorosis, burning of leaf margins and necrosis, and reduction in growth were observed in plants, physiological and morphological data were recorded again. Photosynthetic CO_2 assimilation rate (*A*) was measured by using a portable photosynthetic system (Li-Cor 6800; Li-Cor Inc.) with an integrated

fluorescence chamber head of 6 cm^2. Li-Cor 6800 and plants were together moved to a climate cabinet during measurement.

Data recording

Germination percentage: Data were recorded on seed germination and initial growth of seedlings at transplanting in salt solution. Germination percentage (GP) was calculated as follows:

$$GP = Ni / N \times 100$$

Ni = number of germinated seed till i^{th} day

N = total number of seeds

Speed of germination: Speed of seed germination (S) was computed by the formula stated by Wardle et al. (1991):

$$S=N_1/T_1+N_2/T_2+N_3/T_3+\cdots+Nk/Tk$$

Where, N_1, N_2, N_3, ……, N_k are the numbers of germinated seeds observed at time (days or hours) $T_1, T_2, T_3, \cdots\cdots T_k$ after sowing (number of seeds that germinated at the specific time) and K is the total number of time intervals.

Chlorophyll fluorescence

Chlorophyll fluorescence (Chl-fluorescence) was measured in the dark and light-adapted leaves with a portable fluorometer, PAM-2500, Walz, Effeltrich, Germany. After 30 min of dark adaptation, Fv/Fm was calculated as $(F_m - F_o)$/Fm, where F_m and F_0 were the maximal and minimal fluorescence, respectively (Genty et al., 1998). The difference between F_0 and F_m is the variable fluorescence, F_v. It has been shown theoretically and empirically that F_v/F_m gives a robust indicator of the maximum quantum yield of PSII chemistry (Butler, 1978; Genty et al., 1992).

Data analysis

The collected data were summarized and analyzed by the Mstat-c computer package and means were separated by DMRT to interpret the results.

RESULTS AND DISCUSSIONS

Seed germination and initial growth of seedlings

The speed of seed germination of genotypes ranged from 2.06 to 7.35 and the highest germination speed was observed and recorded in *BARI Begun-6* and the lowest in Tarapuri. At the transplanting age, the shoot length of genotypes ranged from 4.00 to 9.83 cm and the longest shoot was recorded for f*SM-11* which was similar as that for SM-18. The shortest shoot was found in *BARI-Begun-1* which was statistically similar as that in *SM-20, Tarapuri, BARI-Begun-8,* and *BARI Begun-7*. The total leaf area varied from 17.14 to 68.82 cm^2 and the highest values for leaf area were recorded for *SM-19, BARI-Begun-6, BARI-Begun-12*, and *BARI-Begun-7,* and the minimum leaf area was recorded for Tarapuri.

Differences among the genotypes in terms of number of leaves per plant and dry matter production of seedlings were statistically insignificant (Table 1). Genetic variability was found in the cultivated eggplant of Bangladesh for plant growth, leaf character, 1000 seed

weight, and seed germination (Solaiman et al., 2015). The authors added that where there is greater variability, there are greater chances of genotype improvement either from the existing variability or through the segregates of a cross-through selection.

Pre-treatment photosynthesis and stomatal conductance

Photosynthesis rates of the genotypes (A) ranged from 31.13 to 8.55 µmol $m^{-2}s^{-1,}$ the highest being in BARI Begun-8, and A in BARI Begun-4 and SM-12 was similar. The lowest A was recorded for SM-11. Stomatal conductance (Gs) and transpiration rates (Et) of seedlings did not differ significantly (Table 2). Arantes (2014) considered Gs and Et the basis for determining the productive potential of cultivars in breeding programs. Ullah et al., (2014) also reported genotypic variation in physiological and photosynthetic properties and subsequent plant growth. Further, Gobu et al. (2017) reported, that variations in seed germination and seedling growth of eggplant genotypes are genetically controlled without environmental effects. Variations in physiological provide an opportunity for selecting germplasm for breeding programs to develop a superior cultivar.

Photosynthesis

Salinity in the growth medium affected photosynthesis rates (A), transpiration rates (Et) and dry matter production of the eggplant genotypes. Stomatal conductance on the other hand did not respond significantly to salinity levels (Table 3). The values of photosynthesis and dry matter production were graphed and trends were calculated for their relations with salinity levels. The linear model revealed a declining trend of A with increasing salinity level. The decrease in photosynthesis (slope) was 1.1855% per unit increase in EC (Figure 1a). A similar declining trend was also observed in dry matter (DM) accumulation (Figure 1b). The reduction of photosynthesis was partly due to a reduced stomatal conductance and consequent restriction of the availability of CO_2 for carboxylation.

The findings are in harmony with those Lima et al. (2015) who reported that salinity above 0.5 dS/m reduced plant growth and fruit production in eggplant. Furthermore, they reported that toxic Na ions might interfere with photosynthates and dry matter accumulation. Latrach et al. (2014) added that in reduced leaf area under saline conditions, the rate of photosynthesis decreased, causing a severe decline in overall growth and yield. According to Donald et al. (2021) Na^+ and Cl^- toxicity was the main reason for reduced leaf area. In a study, Dionisio-Sese and Tobita (2000) observed that the net photosynthetic rate, measured in terms of CO_2 assimilation of the youngest fully expanded leaf of four rice varieties, declined with increasing levels of salinity stress. These authors explained that this might be due to a direct effect of salt on stomatal resistance via a reduction in guard cell turgor.

Photosynthesis rates of genotypes ranged from 11.24 to 18.88 µmol$m^{-2}s^{-1}$ and it was maximum in SM-12 and identical to that of BARI Begun-7. The minimum photosynthesis rate was recorded from SM-11. Stomatal conductance (Gl) ranged from 0.05 to 0.20 $mm^{-2}s^{-1}$ and it was the highest in SM-11 which was identical to that of SM-12, SM-18, and BARI Begun-8 respectively. The lowest Gl was recorded for BARI Begun-7. Leaf evaporation (E) ranged from 1.37 to 2.13 mmol $m^{-2}s^{-1}$ and it was the highest in genotype SM-11 which was statistically similar to that of SM-11 and SM-19

respectively. The lowest E was recorded from BARI-Begun-7 (Table 4). According to the reports of recent studies, the decline in photosynthesis is related to a decrease in chlorophyll contents and variation in chlorophyll ultrastructure. Zhang et al., (2014), reported salinity drought causes leaves to wilt and become less efficient at capturing sunlight for photosynthesis. Nadarajah (2020) and Mhamdi and Van Breusegem (2018) reported that salt-tolerant and/or resistant eggplant varieties activate stress-responsive genes for recovering from stress. Ashraf and Harris (2013) added that water use efficiency could be improved by decreasing stomatal transpiration without causing a reduction in CO_2 uptake under osmotic stress conditions due to salinity induced physiological drought. The genetic manipulation of stomatal density could be one of the most promising strategies for developing stress-tolerant varieties.

The interaction between genotype and salinity level was highly significant in respect of dry matter production, photosynthesis as well as transpiration rate (Table 5). Under the non-saline condition, phytomass accumulation, photosynthesis, and transpiration rates were observed to be maximum in genotypes BARI Begun-7, BARI Begun-7, SM-11, SM-12, and BARI Begun-1 which were negatively affected as the salinity of the growth media increased. The reduction of photosynthesis was greater in Tarapuri. However, SM-11, SM-12, BARI Begun-8, and SM-18 including Tarapuri were able to maintain consistency in dry matter production. A higher CV% of the trait stomatal conductance indicates heterogeneity in stomatal aperture across the leaf surface. According to Brugnoli and Lauteri (1990), this difference in its tolerance could be related to the varieties or cultivars and the environmental conditions in which they grow.

Fluorescence yield

The lowest values for chlorophyll fluorescence (F_0) were recorded from the seedlings grown in the nutrient solution without added salt (control). The F_0 value is minimal fluorescence (arbitrary unit), the fluorescence level of the dark-adapted sample when all reaction centers of the photosystem II (PSII) are open. Murchie and Lawson (2013) previously reported that a rise in F_0 is associated with a photo-inhibitory environment. Changing minimum fluorescence F_0 to maximum value F_m is the F_v component (F_m-F_o) while the ratio of F_v to F_m (F_v/F_m) is the proportion of quantum yield of photochemistry.

According to Demmig and Björkman (1987), in healthy leaves the ratio of F_v to F_m is highly consistent with values of 0.80 and correlates with maximum quantum yield of photosynthesis. A lower value indicates that a proportion of PSII reaction centers is damaged or inactivated, a phenomenon termed photo-inhibition commonly observed in plants under stress conditions (Ashraf and Harris, 2013; Ogaya et al., 2011). F_0 was found to increase with the increase of salinity level of the nutrient solution in the present study. Maximum F_v/F_m was recorded from seedlings grown at EC 4 dS/m which was equivalent to that of seedlings from the control treatment. The decline of F_v/F_m values was noticed in the seedlings grown at EC 8 dS/m and 12 dS/m (Table 6). According to Murchie and Lawson (2013) a rise in F_0 was associated with photo-inhibitory damage of photosynthesis reaction centre PSII due to salinity.

The yields of F_0 (minimal) and F_m (maximum) by genotypes were not significant. However, different varieties yielded F_v (variable fluorescence) and F_v/F_m (quantum

yields) differently. Genotypes SM-18, SM-19, BARI Begun-8, and Tarapuri yielded the highest F_v/F_m of 0.8 which was identical to that of SM-12 and SM-11. The lowest F_v/F_m of 0.76 was recorded from BARI Bagun-7 (Table 7). The result is an indicator of the susceptibility of BARI Begun-7 to salinity. The interaction of genotypes to salinity levels was also found significant. Quantum efficiencies of PSII (F_v/F_m) of genotypes SM-12, SM-18, and SM-18, BARI begun-8, and Tarapuri were unaffected at EC up to 8 dS/m. In contrast, BARI Begun-1, and BARI Begun-7 were quite susceptible to a salinity level as low as 4 dS/m to 12 dS/m (Table 7). These differences in fluorescence levels under salinity conditions could be related to inherent properties of the genotypes.

CONCLUSION

The eggplant genotypes under study varied significantly in their response to salinity in the growth medium in terms of physiological parameters and dry matter production. The study elucidated the physiological mechanisms underlying genetic variability in salt-sensitivity of crops. Chlorophyll fluorescence imaging could be a powerful tool to screen crop varieties for salinity tolerance.

AUTHOR CONTRIBUTION

Conceived, designed, experimented, and wrote the article.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest regarding the publication of this paper.

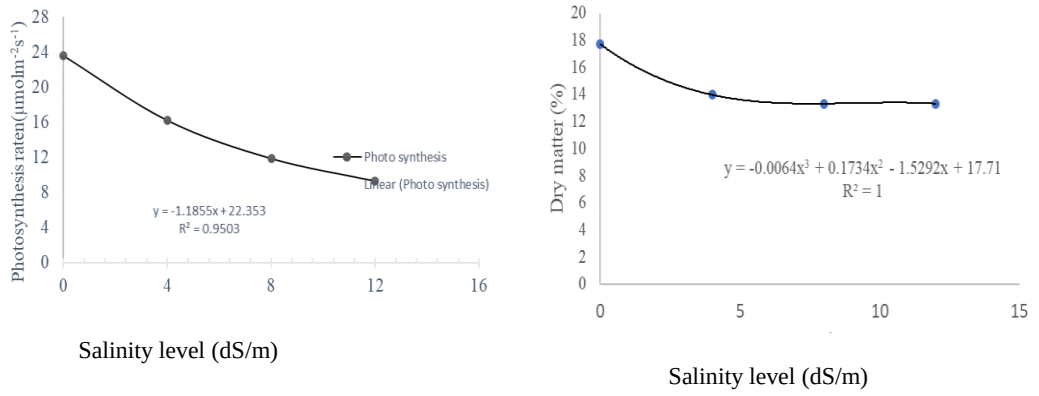

Figure 1. Relationship between (a) photosynthesis (b) dry matter production and salinity level in 11 eggplant genotypes

Table 1: Seed germination and initial growth of seedlings of eggplant genotypes

Genotype	Speed of germination	Seedling length at trans planting (cm)	No. of leaves of seedling at transplanting (no.)	Total leaf area of seedling at transplanting (cm^2)	Dry Matter accumulation (g)
SM-11	5.93bcd	9.83 a	4.67	55.45 bc	17.37
SM-12	6.42abc	7.06 bcd	4.33	66.05 ab	15.51
SM-18	6.91ab	8.50 ab	4.33	32.67 d	15.65
SM-19	5.05d	7.00 bcd	5.00	68.82 a	15.16
SM-20	5.38cd	4.67 ef	4.00	43.29 cd	16.95
BARI Begun-1	5.39cd	4.00 f	3.67	47.16 c	16.69
BARI Begun-4	6.61ab	6.67 bcde	4.00	45.91 c	17.63
BARI Begun-6	7.35a	7.83 bc	4.00	66.60 ab	16.59
BARI Begun-7	6.15bc	6.06 cdef	4.00	61.57 ab	16.69
BARI Begun-8	6.64ab	5.57 def	4.00	42.57 cd	17.00
Tarapuri	2.06e	4.83 ef	5.00	17.24 e	15.7
Level of significance	**	**	NS	**	NS
CV (%)	7.19	12.50	13.32	10.38	16.04

Table 2: Photo-physiological characters of seedlings of 11 eggplant genotypes at transplanting

Genotype	Photosynthesis ($\mu mol\ m^{-2}s^{-1}$)	Stomatal conductance ($mmol\ m^{-2}\ s^{-1}$)	Transpiration rate ($mmol\ m^{-2}s^{-1}$)
SM-11	8.55d	0.28	3.25
SM-12	25.28abc	0.12	2.14
SM-18	22.60bc	0.45	1.60
SM-19	22.36c	0.18	2.54
SM-20	23.27bc	0.09	2.26
BARI Begun-1	24.00bc	0.06	1.56
BARI Begun-4	30.08ab	0.08	2.22
BARI Begun-6	22.95bc	0.12	2.28
BARI Begun-7	23.39bc	0.06	1.45
BARI Begun-8	31.31a	0.06	1.59
Tarapuri	19.04c	0.07	1.53
Level of significance	**	NS	NS
CV (%)	12.52	115.21	35.00

Table 3: Effect salinity on photosynthesis and stomatal conductance of 11 eggplant genotypes

Salinity EC	Photosynthesis			
	Dry matter (g)	Rate of Photo-synthesis ($\mu mol\ m^{-2}s^{-1}$)	Stomatal conductance ($mm^{-2}s^{-1}$)	Evaporation ($mmol\ m^{-2}s^{-1}$)
4 dS/m	13.96 b	16.22 b	0.12	2.03 b
8 dS/m	13.31 b	11.85 c	.08	1.15 c
12 dS/m	13.31 b	9.27 c	0.10	0.91 c
Control	17.61 a	23.62 a	0.9	2.58 a
Level of significance	*	**	Ns	**
CV (%)	14.42	26.15	100.77	22.34

Table 4: Dry matter accumulation and photo-physiological traits of genotypes

Genotype	Photosynthesis			
	Rate of photosynthesis ($\mu mol\ m^{-2}s^{-1}$)	Stomatal conductance ($mmol\ m^{-2}s^{-1}$)	Evaporation ($mmol\ m^{-2}s^{-1}$)	Dry matter accumulation (g)
SM-11	11.24 c	0.20 a	2.13 a	15.48
SM-12	18.88 a	0.10 ab	1.62 b	14.43
SM-18	13.42 bc	0.16 ab	1.58 b	14.21
SM-19	12.96 bc	0.06 b	1.81 ab	14.78
BARI Begun-1	14.28 abc	0.06 b	1.64 b	14.78
BARI Begun-7	18.36 a	0.05 b	1.37 b	14.20
BARI Begun-8	17.21 ab	0.09 ab	1.67 b	14.45
Tarapuri	14.36 abc	0.06 b	1.44 b	13.97
Level of significant	**	**	**	NS
CV (%)	26.15	100.77	22.34	14.42

Table 5: Interaction of genotypes and salinity on photo physiological characters of 11 eggplant genotypes

Salinity level (EC)	Genotype	Dry matter (%)	Photosynthesis (µmol $m^{-2}s^{-1}$)	Stomatal conductance (mmol $m^{-2}s^{-1}$)	Evaporation (mmol $m^{-2}s^{-1}$)
4dS/m	SM-11	16.95 bcde	10.18 ghi	0.14	2.71
	SM-12	14.45 cdefg	20.14 bcdehg	0.19	1.67
	SM-18	12.12 gh	10.83 fghi	0.19	1.81
	SM-19	14.99 cdefg	12.53 defghi	0.09	2.41
	BARI Begun-1	13.08 efgh	15.94 cdefghi	0.09	1.84
	BARI Begun-7	12.26 fgh	23.32 abc	0.08	1.97
	BARI Begun-8	14.32 cdefgh	21.02 bcdef	0.07	1.70
	Tarapuri	12.97 efgh	15.77 cdefghi	0.06	1.80
8 dS/m	SM-11	13.13 efgh	10.72 ghi	0.07	1.40
	SM-12	12.91 efgh	16.63 cdefghi	0.05	1.24
	SM-18	14.39 cdefg	12.21 efghi	0.34	1.14
	SM-19	13.82 cdefgh	11.67 fghi	0.07	1.03
	BARI Begun-1	13.16 efgh	11.42 fghi	0.04	0.99
	BARI Begun-7	10.19 h	11.54 fghi	0.04	1.05
	BARI Begun-8	14.20 cdefgh	9.60 hi	0.3	1.45
	Tarapuri	14.72 cdefg	11.00 fghi	0.04	0.89
12 dS/m	SM-11	14.07 cdefgh	10.28 ghi	0.35	1.16
	SM-12	12.99 efgh	7.89 i	0.10	0.94
	SM-18	14.03 cdefgh	8.87 hi	0.07	0.52
	SM-19	14.12 cdefgh	10.11 ghi	0.06	1.06
	BARI Begun-1	13.23 fgh	11.25 fghi	0.04	1.09
	BARI Begun-7	12.34 fgh	7.64 i	0.03	0.42
	BARI Begun-8	12.83 efgh	9.80 hi	0.08	1.07
	Tarapuri	13.18 efgh	8.28 hi	0.07	1.00
Control	SM-11	17.80 bc	13.78 cdefghi	0.13	3.23
	SM-12	17.37 bcd	30.86 a	0.10	2.55
	SM-18	16.31 bcdefg	21.77 abcde	0.05	2.84
	SM-19	16.19 bcdefg	17.52 cdefghi	0.04	2.73
	BARI Begun-1	19.69 ab	18.49 cdefgh	0.06	2.65

Salinity level (EC)	Genotype	Dry matter (%)	Photosynthesis (µmol $m^{-2}s^{-1}$)	Stomatal conductance (mmol $m^{-2}s^{-1}$)	Evaporation (mmol $m^{-2}s^{-1}$)
	BARI Begun-7	22.04 a	30.93 a	0.06	1.05
	BARI Begun-8	16.47 bcdef	28.43 ab	0.19	2.47
	Tarapuri	15.05 cdefg	22.38 abcd	0.07	2.07
	Level of significant	**	**	NS	**
	CV (%)	14.42	26.15	100.77	22.34

Table 6: Fluorescence yield of eggplant genotypes as influenced by salinity

Salinity (EC)	Fluorescence			
	F_0	F_m	F_v	Fv/Fm
4 dS/m	118.3 c	768.67 b	674.5 b	0.866 a
8 dS/m	144.8 b	504.58 c	399.6 c	0.793 b
12 dS/m	237.5 a	407.12 c	219.8 d	0.554 c
Control	88.08 d	1116.16 a	946.8 a	0.847 a
Level of sig.	**	**	*	*
CV (%)	22.03	22.11	24.16	25.23

Fo = minimum fluorescence, Fm = maximum fluorescence, Fv/Fm = maximal quantum yield

Table 7: Fluorescence yield of 11 eggplant genotypes

Genotype	Fluorescence			
	F_0	F_m	F_v	Fv/Fm
SM-11	162.00	728.33	626.41 cd	0.77 ab
SM-12	142.83	660.50	681.50 bcd	0.79 ab
SM-18	141.33	718.08	869.08 a	0.80 a
SM-19	144.91	684.41	698.16 abc	0.80 a
BARI Begun-1	153.33	688.16	504.16 d	0.70 b
BARI Begun-7	148.16	669.667	785.25 ab	0.0.60 c
BARI Begun-8	131.50	724.58	705.33 abc	0.80 a
Tarapuri	153.25	719.33	821.50 ab	0.80 a
Level of significance	Ns	Ns	**	**
CV (%)	22.03	22.11	24.16	15.23

Fo = minimum fluorescence, Fm = maximum fluorescence, Fv/Fm = maximal quantum yield

Table 8: Interaction effect of salinity and genotype on fluorescent yield of 11 eggplant genotypes

Genotype and salinity level (EC)	Fluorescence yield			
	F_0	F_m	F_v	Fv/Fm
4dS/m				
SM-11	157.00 defg	853.33	735.67 abcde	0.82
SM-12	132.67 efgh	645.67	722.33 abcde	0.81
SM-18	157.67 defg	1108.00	908.67 ab	0.83
SM-19	130.00 efgh	802.33	647.33 bcde	0.84
BARI Begun-1	179.33 cdef	644.00	374.67 defg	0.60
BARI Begun-7	172.67 cdefg	481.00	190.67 g	0.40
BARI Begun-8	125.67 fgh	933.00	870.67 abc	0.87
Tarapuri	103.33 fgh	807.00	857.00 abc	0.87
8dS/m				
SM-11	126.00 fgh	488.33	493.33 cdef	0.76
SM-12	118.67 fgh	487.67	731.33 abcde	0.80
SM-18	109.67 fgh	550.00	968.67 ab	0.80
SM-19	115.667 fgh	567.00	630.00 bcde	0.82
BARI Begun-1	122.00 fgh	527.67	552.33 cdef	0.72
BARI Begun-7	104.67 fgh	538.67	210.67 g	0.65
BARI Begun-8	130.00 efgh	389.33	534.00 cdef	0.78
Tarapuri	119.67 fgh	488.00	776.33 abcd	0.83
12dS/m				
SM-11	101.67 fgh	507.67	536.00 cdef	0.76
SM-12	105.00 fgh	388.33	595.00 bcde	0.78
SM-18	89.33 gh	374.00	689.00 bcde	0.79
SM-19	98.00 fgh	399.67	716.00 abcde	0.80
BARI Begun-1	69.00 h	373.67	335.33 efg	0.64
BARI Begun-7	92.67 gh	352.67	203.67 fg	0.61
BARI Begun-8	60.33 h	423.67	674.00 bcde	0.81
Tarapuri	88.67 gh	437.33	889.67 abc	0.81
Control				
SM-11	263.33 ab	1064.00	740.67 abcde	0.70
SM-12	215.00 bcd	1120.33	677.33 bcde	0.77
SM-18	208.67 bcde	1065.33	710.00 abcde	0.80
SM-19	236.00 abcd	968.67	799.33 abc	0.76

Genotype and salinity level (EC)	Fluorescence yield			
	F_0	F_m	F_v	Fv/Fm
BARI Begun-1	243.00 abc	1207.33	754.33 abcd	0.80
BARI Begun-7	222.67 bcd	1206.33	747.00 abcd	0.73
BARI Begun-8	210.00 bcde	1152.33	742.67 abcde	0.73
Tarapuri	301.33 a	1145.00	763.00 abcd	0.69
Level of significance				
CV (%)	22.03	22.11	24.16	15.23

Fo = minimum fluorescence, Fm = maximum fluorescence, Fv/Fm = maximal quantum yield

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DIFFERENCE IN YIELD AND PHYSIOLOGICAL FEATURES IN RESPONSE TO SALINITY STRESS IN WHEAT GENOTYPES

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ABSTRACT

Salinity of the soil is a significant barrier to the cultivation of wheat in the coastal region of Bangladesh. In order to evaluate the salt tolerance of some wheat genotypes, a pot experiment was conducted in the greenhouse of the Plant Physiology Division of the Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur during the rabi season of 2016-2017. Three wheat genotypes viz., BAW 1135, BAW 1157 and BARI Gom 29 were tested for their tolerance of salinity induced by the application NaCl solution with EC 15 dS/m. The genotypes were greatly affected by salinity in respect of days to heading and flowering, leaf area, SPAD value, plant height and plant dry weight. Salinity stress reduced the growth and yield parameters of wheat genotypes. The wheat plants had a higher Na^+ concentration in leaves than in stems. Importantly, BAW 1135 and BAW 1157 had substantially increased leaf K^+ concentrations; BAW 1157 was more efficient in restricting Na^+ loading in leaf. Moreover, a significant decrease in the cell membrane stability index (CMSI) and an increase in malondialdehyde (MDA) were associated with a significant decrease in the total biomass in salt stressed BARI Gom 29. Soluble protein and soluble sugars increased significantly in BAW 1135 and BAW 1157 under salt stress conditions, along with an increase in antioxidant activity. Salt stress significantly reduced grain yield and 1000-grain weight of wheat; however, BAW 1135 and BAW 1157 were less affected than BARI Gom 29. Our results suggest that high tolerance to salinity stress in BAW 1135 and BAW 1157 is closely related to lower Na^+ and higher K^+, enhanced soluble protein and sugar contents band improved antioxidative capacity for scavenging reactive oxygen species during the stress period.

Keywords: Genotypes, salinity stress, wheat

INTRODUCTION

One of the main abiotic factors impacting agricultural output in the semi-arid and coastal regions is soil salinity, which has detrimental effects on plant development and overall crop yield (Munns et al., 2006). The yield of grain crops over large areas of the world's farming

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land is limited by a number of physicochemical constraints in the subsoil including salinity and sodicity (Rengasamy, 2010; Rowson et al. 2011). Attempts to develop viable management options to improve productivity of these problem soils such as, irrigation and drainage, have met with minimal success to date. Wheat as the most important crop for human consumption in the world is grown on saline soils in many parts of the world. Therefore, breeding for improved salinity tolerance would be an effective way for improving yield and yield stability under such conditions (Genc et al. 2007, Barma, 2011).

Increased levels of reactive oxygen species (ROS) contribute to salt damage in plant cells (Monteiro et al. 2011; Gómez et al., 2004). Under normal conditions the production and destruction of ROS is well regulated in cell metabolism. However, disruption of the homeostasis under saline conditions results in the accumulation of ROS, and subsequent changes in normal cellular metabolism through oxidative damage to membranes, proteins, and nucleic acids (Miller et al., 2010). Formation of ROS is also caused by ion toxicity due to accumulation of Na^+ and/or Cl^- that disturbs redox status of the plasma membrane, and different compartments such as chloroplasts and mitochondria of the plant cells. Over-reduction of electron transport in cellular organelles, such as chloroplasts and mitochondria under stress results in formation of ROS such as the superoxide anion ($O_2\cdot^-$) and H_2O_2 which damage lipids, proteins, other vital molecules such as DNA and RNA in the cells (Turkan and Demiral, 2009).

Plants possess non-enzymatic and enzymatic antioxidants such as SOD, POX, CAT, APX and GR. Among these, SOD decomposes $O_2\cdot^-$ to H_2O_2 which is further scavenged by POX in extracellular space and cytosol; and mainly by CAT in peroxisomes; H_2O_2 is also decomposed by APX, one of the Asadae Halliwell enzymes, in different cell compartments. Hence, reacting directly or indirectly with ROS, enzymatic and non-enzymatic antioxidants contribute to maintain the integrity of cell structures and the proper functions of various metabolic pathways (Chaparzadeh et al., 2004). Thus, antioxidant resistance mechanisms, along with other strategies such as ion exclusion and osmotic adjustment may provide a strategy to enhance salt tolerance. Plant height, stem diameter and dry weight decreased with increasing levels of salinity (Azoz et al., 2004, Asha and Dhingra, 2007). Salinity reduced fertile ears, ear length, grain yield, straw yield and harvest index (Francois et al., 1986 and Asha and Dhingra, 2007).

Wheat (*Triticum aestivum* L) is the second most important cereal in Bangladesh. There are 2.85 million hectares coastal and off shore land in the country affected by varying degrees of soil salinity (Barma, 2011). This area remains uncultivated due to salinity and non-availability of salt tolerant crops. Moderate to severe levels of salinity affect, crop growth in this area. There are no suitable varieties of wheat to cultivate in the coastal area which can tolerate moderate and high salinity. In our previous work, we identified two accessions of wheat, i.e. BAW1135 and BAW1157, which showed high tolerance to salt stress (Ahmed et al., 2016). However, the basic physiological and biochemical mechanism involved in stress tolerance is still unclear.

Thus, further studies are needed to test the hypothesis that BAW1135 and BAW1157 are more tolerant to salinity stress than BARI Gom 29, and if yes, whether tolerance mechanism is associated with increasing antioxidant levels, and to gain a better understanding of how plants adapt to the adverse environment. The main objective of the present study was to compare the effects of salinity stress on the advanced wheat lines and cultivated wheat based on morpho-physiological parameters.

MATERIALS AND METHODS

Test breeding lines and salt treatment

A pot experiment was conducted in a greenhouse of the Plant Physiology Division, Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh during November, 2016 to March 2017. Sandy loam soil (pH 6.1) was collected from the Kodda area of Gazipur. The soil was air-dried and mixed daily until 8% water content was reached. Plastic pots (10 L, 30 cm height) were filled with the mixture of air-dried soil and cow dung in a 4:1 volume ratio. Fertilizers at the rates of 120-30-90-15-6-2-1 kg/ha NPKSMgZnB (FRG, 2012) in the forms of urea, triple super phosphate, muriate of potash, gypsum, zinc sulphate and boric acid were incorporated in the soil. Scanty rainfall, low humidity and clear sunny days were the characteristic features of the growing season.

Two wheat genotypes, BAW1135 and BAW1157, earlier identified as salinity-tolerant (Ahmed et al., 2016), were the test breeding lines and a popular variety BARI Gom 29 was used as the check variety. Ten seeds of each genotype were sown in each pot on 23 November 2016. Five plants in each pot were kept by thinning of seedlings 10 days after emergence (DAE).

Two salinity treatments were imposed at the crown root initiation (CRI) stage of wheat: (1) control (no salinity, irrigated with tap water, EC 0.25 dS/m) only), soil moisture maintained 50-60% of the water holding capacity throughout the crop growing period; (2) application of NaCl solution (salt + tap water) with an EC of 15 dS/m during the CRI to reproductive stage of the crop. Electrical conductivity of the soil in salinity treatment was 15 dS/m, as measured by $EC_{1:1}$ soil water suspension method described by Rhoades et al. (1999). The salt solution was applied in excess so that the extra solution dripped down from the bottom of the pots. The pots were arranged in a randomized complete block design (RCBD) with 9 replications.

Agronomic parameters and Na, K contents

At the end of salinity stress treatment four replicates of plants were collected for biomass and leaf area measurements. Leaf area was measured by an automatic area meter (LI 3100, LI-COR, USA). After measurement of plant height, plants were separated into leaves and shoots, and dried at 105°C for 3 hr, followed by 80°C for 48 hr, and then weighed. Dried shoots and roots were powdered and weighed, then dried to 550°C for 12 hr to get the ash. The ash was digested with 30% HNO3 and then diluted using deionized water (Ahmed et al., 2013a). Concentrations of Na^+, K^+ and other mineral elements were determined by a atomic absorption spectrophotometry (SHIMADZU AA-6300, Kyoto, Japan).

At maturity of the wheat plants, spike length, grains per spike, 1000 grain weight and grain yield per plant were measured. Spike length was measured as the length from neck node to tip of the upper most spikelet.

Chlorophyll content

The chlorophyll content was measured as SPAD (soil plant analyses development) value on intact fully expanded leaves (the second from the apex) using a chlorophyll meter-Minolta SPAD- 502 (Feibo *et al.*, 1998). Measurements were made in 5 replicates.

Antioxidant enzyme activity

Flag leaves (0.5 g) harvested after salt treatment were homogenized in 10 ml 50 mM Tris buffer (phosphate buffer saline, pH 7.4) using a pre-chilled mortar and pestle, and then the homogenates were centrifuged at 10,000 × *g* for 15 min and the supernatants were used for malondialdehyde (MDA) content and antioxidant enzyme activity assay. All procedures were carried out at 4 °C using the method as described by Ahmed et al., 2013a.

Guaiacol peroxidase (POD, EC 1.11.1.7) activity was determined at 25 °C with guaiacol. For measurement of POD activity, assay solution (3 ml) containing 50 mM phosphate buffer (pH 7.0), 20 mM guaiacol, 40 mM H_2O_2 and 0.1 ml enzyme extract. The reaction was initiated by adding the enzyme extract. Increase in absorbance (470 nm) of the reaction solution at was recorded once every 20 s. one-unit POD activity was defined as an absorbance change of 0.01 units min^{-1}.

Catalase (CAT, EC 1.11.1.6) activity was measured as disappearance of H_2O_2. For measurement of CAT activity, assay solution (3 ml) containing 50 mM phosphate buffer (pH 7.0), 5.9 mM H_2O_2 and 0.1 ml enzyme extract. Decrease in absorbance of the reaction solution at 240 nm was recorded after every 20 s. An absorbance changes of 0.01 units min^{-1} was defined as one-unit CAT activity.

Ascorbate peroxidase (APX, EC 1.11.1.11) activity was assayed according to the method of Asada (1981). The reaction mixture consisted of 100 µl enzyme extract, 100 µl ascorbate (7.5 mM), 100 µl H_2O_2 (300 mM) and 2.7 ml 50 mM Tris buffer solution (pH 7.30). The oxidation of ascorbate was determined by the change in absorbance at 290 nm ($\varepsilon = 2.8\ mM^{-1}\ cm^{-1}$).

Lipid per oxidation and cell membrane stability index

The level of lipid peroxidation in the leaf tissue was measured in terms of malondialdehyde (MDA, a product of lipid peroxidation) content determined by the thiobarbituric acid (TBA) reaction. In 1 ml aliquot of the supernatant, 4 ml of 20% TCA containing 0.5% TBA was added. The mixture was heated at 95°C for 30 min and then quickly cooled in an ice bath. After centrifuging at 10,000 × *g* for 10 min, the absorbance (532 nm) of the supernatant was read and the value for the non-specific absorption at 600 nm was subtracted. The MDA content was calculated by using the extinction coefficient of 155 $mM^{-1}cm^{-1}$.

Cell membrane stability index (CMSI) was determined by estimating ion leaching from leaf tissue into distilled water. Plant material (1.0 g) was placed in 20 ml of distilled water in two sets. The first set was subjected to a temperature of 25°C for 12 hr, and its EC was recorded with a conductivity meter (C1). The second set was kept in a boiling water bath (100°C) for 10 min, and its conductivity was also recorded (C2). CMSI was calculated from the formula: CMSI = 1-C1/C2 (Ahmed et al., 2013b).

Soluble protein and total soluble sugar

Soluble protein content was measured according to Bradford (1976) using bovine serum albumin as a standard. Total soluble sugars were estimated by the anthrone reagent (Yemm and Willis, 1954). An aliquot (0.05 ml) was taken in test tubes and the volume

was made up to 1 ml. To this solution 4 ml of anthrone reagent was added and the mixture was heated in boiling water bath for 10 min followed by cooling. The optical density of green to dark green color was read at 630 nm.

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 salt 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

Phenological development and crop duration

The genotypes BAW 1135 and BAW 1157 were found to reach heading and flowering earlier than BARI Gom-29 in both control and salinity stress conditions (Table1). However, the obtained results revealed that crop duration in terms of days to heading and flowering was decreased due to salinity stress Irrespective of genotypes/variety

Table 1: Effect of salinity on days to heading and flowering of selected wheat genotypes/varieties

Genotype/variety	Days to heading		Days to flowering	
	Control	Salinity	Control	Salinity
BAW-1135	54	50	68	66
BAW-1157	57	53	67	64
BARI Gom-29	60	57	70	67
Mean	57.00	53.33	68.33	65.67
SD	3.00	3.51	1.53	1.53

Leaf area

At heading stage, the highest leaf area, 501.68 cm^2/plant, was found in BARI Gom-29 which was followed by BAW-1157 (388.25 cm^2/plant) under non-saline condition (Table 2). The lowest leaf area was recorded for BAW-1135 (371.88 cm^2/plant). After salinity treatment, BAW-1157 produced the maximum relative leaf value (75.53%) and BARI Gom-25 the minimum value (62.68%) compared with control (501.68 cm^2/plant).

At anthesis, the maximum leaf area (589.46 cm^2 /plant) was obtained in BARI Gom-29. The lowest leaf area was recorded from the genotype BAW-1157 but it was statistically similar with BAW-1135 under non-saline condition (Table 3). The maximum relative leaf area (61.26%) was obtained for the genotype BAW-1157 followed by BAW-1135, i.e. 55.24 % under salinity compared with control. The minimum relative leaf area, 48.50%, was obtained for BARI Gom-29.

Table 2: Effect of salinity on leaf area (LA) per plant of selected wheat genotypes/varieties at different growth stages

Genotypes/varieties	LA (cm^2) at heading		LA (cm^2) at anthesis	
	Control	Salinity	Control	Salinity
BAW-1135	371.88	256.69 (69.02)	537.13	296.72 (55.24)
BAW-1157	388.25	293.25 (75.53)	426.67	261.36 (61.26)
BARI Gom-29	501.68	314.43 (62.68)	589.46	285.84 (48.50)
$LSD_{(0.05)}$	7.93		11.33	
CV (%)	4.4		5.6	

Values in parenthesis show relative value compared with LA in control, calculated as [(value of parameter under stress/value of control) x 100]

SPAD value

The SPAD value varied among the genotypes. At heading stage, SPAD value differed significantly among the genotypes both under control and saline conditions (Table 3). In control condition, BAW 1157 gave the highest SPAD value and BAW 1135 the lowest. Under salinity condition, the maximum relative SPAD value was recorded in BARI Gom 29 (109.91%) followed by BAW 1157 (107.86%) and BAW 1135 (103.19%). At anthesis stage, the highest SPAD value was recorded in BAW 1157 in control condition and the lowest in BARI Gom 29, the SPAD values for BAW 1157 and BAW 1135 being statistically similar (Table 3). The highest relative SPAD value was obtained in BARI Gom-29 (121.68 %) followed BAW-1157 (107.51%) under salinity relative to control. The lowest relative value was recorded in BAW-1135 (104.06%) compared with control. The results showed that the relative SPAD value increased with increasing salinity level at both the stages due to decrease in leaf area and increase in thickness and greenness of leaves.

Table 3: Effect of salinity on SPAD value of selected wheat genotypes/varieties at different growth stages

Genotype/variety	Heading		Anthesis	
	Control	Salinity	Control	Salinity
BAW-1135	45.96	47.43 (103.20)	48.76	50.22 (104.06)
BAW-1157	49.96	53.96 (107.86)	50.11	53.53 (107.51)
BARI Gom-29	47.86	52.6 (109.90)	44.98	53.98 (121.68)
$LSD_{(0.05)}$	2.36		1.98	
CV (%)	4.3		5.6	

Values in parenthesis show relative value compared with LA in control, calculated as [(value of parameter under stress/value of control) x 100]

Plant height and dry weight

Plant height was apparently reduced by salinity (Fig. 1). Significant genotypic differences were noticed as follows: decreased by 14.2% in BAW-1135; 10.4% in BAW-1157 and

17.7% in BARI Gom 29 under salinity treatment (Fig. 1). Similar results were reported by Francois et al. (1986), Mass and Poss (1989) and Rawson (1986) in wheat. Under condition of salinity while adjusting to salinity stress vigorous growth and continual replacement of lost leaves results in dilution of salt concentration in the plant system. The obtained results are in agreement with the findings of Flower et al. (1998). Meanwhile, plant dry weight under salinity decreased by 26.6% in BAW-1135; 10.8% in BAW-1157 and 28.2% in BARI Gom 29. The results are in agreement with the findings of Azoz et al., (2004), Asha and Dhingra (2007). The reduction in plant dry weight might be due to decrease in CO_2 uptake in leaves (Fedina and Popova, 1996) resulting less CO_2 available for carboxylation reaction in the photosynthetic apparatus (Yadav et al., 1996) and NaCl also decreases stomatal conductance.

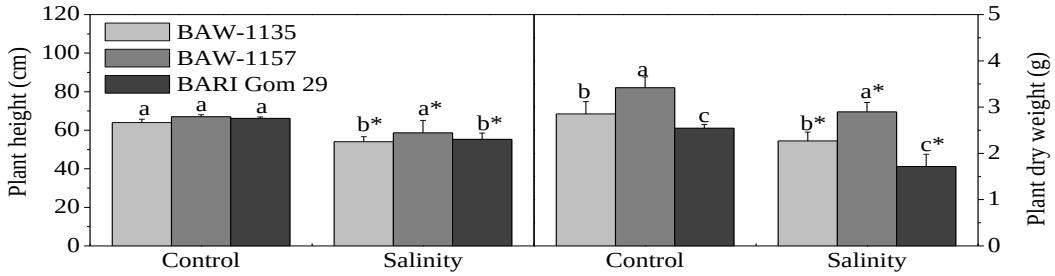

Fig. 1: Effect of salinity stress on plant height and dry weight of three wheat genotypes. Data are mean ± standard error of four replicates. Different letters indicate significant differences ($P < 0.05$) among the genotypes within treatment *Indicates significant differences ($P < 0.05$) between genotypes.

Table 4: Root and shoot Na^+ and K^+ contents of 3 wheat genotypes exposed to salinity

	Control	Salinity	Control	Salinity
Genotype	Stem		Leaf	
	Na^+ concentration (mg g^{-1} DW)		Na^+ concentration (mg g^{-1} DW)	
BAW-1135	3.63 b	16.32 a	3.17 b	32.36 a
BAW-1157	3.62 b	15.16 a	4.15 b	31.33 a
BARI Gom 29	3.99 b	19.93 a	4.61 b	35.41 a
LSD.05 between genotypes	0.46	0.62	4.82	4.65
	K^+ concentration (mg g^{-1} DW)		K^+ concentration (mg g^{-1} DW)	
BAW-1135	28.85 a	23.22 b	24.30 a	19.55 b
BAW-1157	30.69 a	24.15 b	25.63 a	22.21 b
BARI Gom 29	27.92 a	25.63 b	24.90 a	17.23 b
LSD.05 between genotypes	1.22	0.69	0.68	0.41

Results are presented as means of five measurements. Different letters indicate significant differences ($P < 0.05$) among the treatments within each genotype

Na^+ and K^+ concentrations

Salinity treatments caused significant (P<0.05) increases in Na^+ concentration and Na^+/K^+ ratio in stems and leaves in all genotypes, compared with control (Table 4). Moreover, a preferential accumulation of Na^+ in leaf rather than in stem was observed under salinity stress. In plants subjected to salinity leaf Na^+ concentration increased by 676.02% in BAW-1135, 654.93% in BAW-1157 and 880.88% in BARI Gom 29; stem Na^+ concentration increased by 349.58%, 318.78% and 399.50%, respectively, compared with control. The leaf and stem K^+ concentrations decreased in BARI Gom 29; but leaf K^+ increased in BAW-1135 and BAW-1157, whereas no significant difference was observed in the stems of these genotypes. An increase in Na^+ ion concentration and a decrease in K^+ ion uptake interrupts ionic balance as observed in most species exposed to salinity stress (Qiu et al., 2011).

Antioxidant enzyme activities

Antioxidant responses in wheat genotypes to salinity treatments are presented in Fig. 2. POD activity increased under salinity treatment in all genotypes. The highest increases in POD activity were seen in BAW-1157 and in BAW-1135 under salinity treatment. CAT activity also increased in all genotypes under salinity stress. Compared to control, APX activity decreased in BARI Gom 29 and increased in BAW-1135and BAW-1157 under salinity treatment (Fig. 2). Several studies demonstrated that salt-tolerant species increase their antioxidant enzyme activities in response to salt stress, while salt-sensitive species failed to do so (Shalata et al., 2001; Jbir et al., 2001). Plants have several mechanisms to defend themselves against salinity-induced stress in the form of antioxidant enzymes (APX, POD, CAT, SOD, etc.) that act in concert to alleviate cellular damage under oxidative stress conditions (Foyer and Noctor 2000).

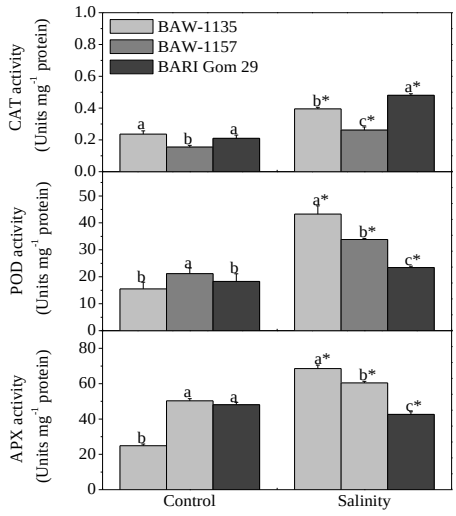

Fig. 2: Effect of salinity stress on CAT, POD and APX of 3 wheat genotypes. Data are mean ± standard error of four replicates. Different letters indicate significant differences (P < 0.05) among the genotypes within treatment *Indicates significant differences (P < 0.05) between genotypes

Lipid peroxidation content and cell membrane stability index

Exposure to salinity stress induced a genotype-independent in MDA content in flag leaves (Fig. 3); greater accumulation was seen in BARI Gom 29 than that in BAW-1157 and BAW-1135. It was found that a salt-tolerant mulberry variety showed little change in MDA content under salt stress (Sudhakar et al., 2001). CMSI changed slightly, when plants were exposed to salinity stress relative to control. Under salinity treatment, CMSI was markedly reduced in BARI Gom 29 compared with BAW-1157 and BAW-1135. For example, CMSI under salinity treatment decreased by 18.4%, 15.1% and 25.7% in BAW-1135, BAW-1157 and BARI Gom 29, respectively, compared with control (Fig. 3). Huang et al., (2005) showed that CMSI changed a little when plants were exposed to 50 mM NaCl, but it declined sharply under 300 mM NaCl, indicating the decline in CMSI stands for the injury caused by stress.

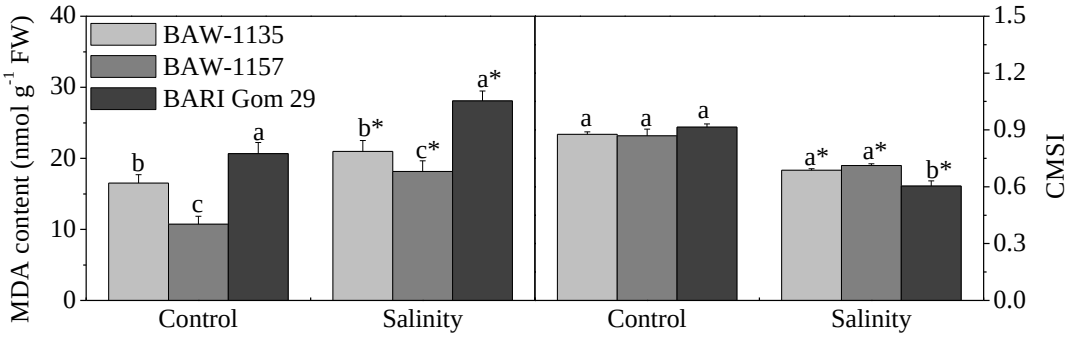

Fig. 3: Effect of salinity stress on MDA content and CMSI of 3 wheat genotypes. Data are mean ± standard error of four replicates. Different letters indicate significant differences ($P < 0.05$) among the genotypes within treatment *Indicates significant differences ($P < 0.05$) between genotypes.

Soluble sugars and soluble protein

As shown in Fig. 4 soluble sugar (SS) content increased significantly ($P<0.05$) in BAW-1157 and BAW-1135 (with a larger increase in BAW-1157) under salinity treatment. The SS content also increased in BARI Gom 29 under salinity treatment compared to the control plants. Soluble protein content also increased in flag leaves of BAW-1157 and BAW-1135 under salinity treatment, while in the case of BAW-1157 and, a slight increase in soluble protein content was observed. Accumulation of protein and soluble sugars under stress protect the cell by balancing the osmotic strength of cytosol with that of vacuole and external environment (Greenway and Munns, 1980).

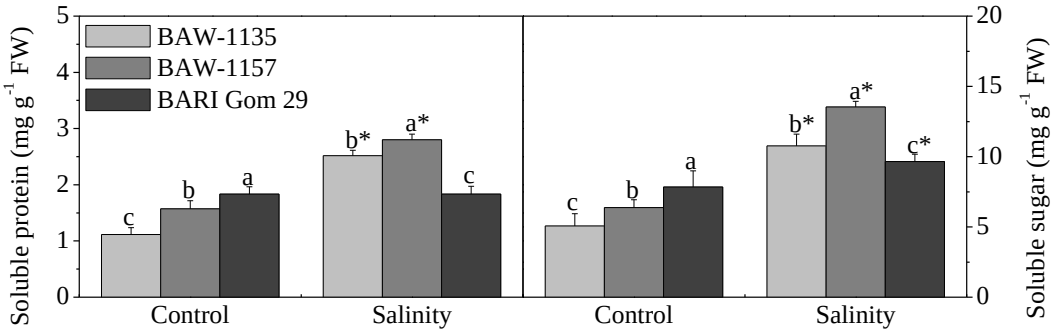

Fig. 4: Effect of salinity stress on soluble protein and soluble sugar of 3 wheat genotypes. Data are mean ± standard error of four replicates. Different letters indicate significant differences (P < 0.05) among the genotypes within treatment *Indicates significant differences (P < 0.05) between genotypes.

Agronomic parameters, yield components and yield

The phenotypic changes in spikes of BAW-1157, BAW-1135 and BARI Gom 29 during salinity stress are shown in Table 5. Compared with control, spike length significantly (P<0.05) decreased in BARI Gom 29 under salinity stress conditions, while no significant difference was observed between BAW-1157 and BAW-1135. The grains per spike were significantly reduced in all genotypes; this component was less affected in BAW-1157 and BAW-1135 under salinity treatment compared to control. The salinity stress treatment significantly reduced percentage of grain-setting in BARI Gom 29, while BAW-1157 and BAW-1135 remained unaffected (Table 5). Genotype-specific growth inhibition under salinity was observed in barley by Ahmed et al. (2013b).

Table 5: Effects of salinity stress on yield and yield components of four wheat genotypes

Treatment	Spike length (cm)	Filled grains per spike	Unfilled grains per spike	Rate of filled grains per spike (%)	1000 grain weight (g)	Grain yield per plant (g)
	BAW-1135					
Control	9.83 a	61.67 a	9.00 b	87.26 a	45.95 a	30.80 a
Salinity	8.33 a	48.02 b	11.01 a	81.35 b	32.11 b	18.48 b
	BAW-1157					
Control	9.07 a	65.33 a	6.00 a	91.59 a	43.84 a	31.94 a
Salinity	8.16 a	54.33 b	7.33 a	87.06 b	36.23 b	21.65 b
	BARI Gom 29					
Control	9.43 a	55.00 a	11.00 b	83.33 a	40.59 a	33.02 a
Salinity	6.53 b	39.00 b	17.43 a	69.11 b	26.97 b	16.60 b
$LSD_{0.05}$	2.4	4.5	2.9	3.9	4.3	3.8
CV (%)	4.2	12.0	10.2	11.8	8.9	8.6

Different letters indicate significant differences (P<0.05) among three genotypes within each treatment (n=4).

Wheat plants subjected to salinity exhibited significant decreases in grain yield and 1000-grain weight, but the effect was very small effect on the genotypes BAW 1157 and BAW 1135. Although BARI Gom 29 gave the highest grain yield and 1000-grain weight under the non-saline conditions, it showed substantial reduction of both traits under salinity stress compared with BAW 1157 and BAW 1135. Decreases in grain yield/1000-grain weight was 32.22%/ 17.36% in BAW-1157, 40.0%/ 30.11% in BAW-1135 and 48.16%/ 33.56% in BARI Gom 29, respectively, as compared with control (Table 5). These findings are supported by Ahmed et al. (2013b) who observed similar phenomena in barley genotypes.

CONCLUSION

This study revealed that the wheat genotypes BAW 1157 and BAW 1135 are comparatively tolerant to salinity treatment as high as EC 15 dS/m compared with the variety BARI Gom 29. Salinity tolerance of the two lines appeared to be related to comparatively lower leaf and stem Na^+ concentrations within the plants. In addition, enhanced contents of soluble sugars and soluble proteins were also noticed in these two wheat genotypes which could results in a greater adaptation to salinity than the cultivated wheat variety. Furthermore, enhanced activities of antioxidant enzymes CAT, POD and APX were beneficial in antagonizing oxidative stress, as indicated by the lower accumulation of MDA and CMSI. These two wheat genotypes could be of value in understanding the mechanisms of salt tolerance in wheat and identification of specific genes related to salinity resistance and for future breeding of wheat for salt tolerance.

COMPETING INTERESTS

The authors declare that they have no competing interest.

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AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: NM IMA. Performed the experiments: IMA NM AHMMRT SNM BA. Analyzed the data: IMA AFMSA KMMR PPD. Wrote the paper: NM IMA FA

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DEVELOPMENT OF *STEMPHYLIUM* BLIGHT DISEASE OF LENTIL UNDER INOCULATED CONDITION AT DIFFERENT SOWING DATES

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ABSTRACT

Field experiments were conducted at the Pulses Research Centre, Ishurdi, Pabna, of the Bangladesh Agricultural Research Institute (BARI) in rabi (winter) seasons of 2013-14 and 2014-15 to study the development of *Stemphylium* blight disease of lentil. The experimental plots were artificially inoculated with spores of *Stemphylium botryosum* in each cropping season, and seeds were sown on November 01, 10, 20, 30 and December 10 of each year to achieve variable crop ages. A favorable environmental condition especially temperature was the predisposing factor for disease initiation rather than crop age. Disease initiation noticed when the prevailing temperature was consistently more than 24^0C irrespective of age of the crop plant in both the years. The first disease symptoms observed between February 6 to 12, 2014 when crop reached 94, 88, 81, 71 and 63 days of age in the 2013-2014 cropping season. In 2014-2015, the first disease symptoms were observed between February 2 to 9, 2015 when crop was 91, 82, 73, 68 and 59 days old. Disease severity became lower and reduction in number of pods plant^{-1}, number of seeds pod^{-1}, weight of seeds plant^{-1} and drastic yield reduction of lentil also recorded from delayed sowing in both years.

Keywords: Crop age, lentil, *stemphylium* blight and yield

INTRODUCTION

Lentil (*Lens culinaris* Medik.) is one of the oldest crops in the world, which has been grown for over 8000 years. It is a good source of protein and vitamin A and also provides fiber, potassium, B vitamins, and iron (Kochhar, 2009) for human consumers. Lentil is the most important pulse crop in Bangladesh in terms of area (1.445 lakh ha) and production (1.91 lakh t), and ranks first in consumer preference and total consumption (BBS, 2022). *Stemphylium* blight of lentil caused by *Stemphylium botryosum* Wallr, one of the major diseases of much economic importance, has been reported in lentil in Bangladesh, Egypt, Syria and the USA (Bayaa, and Erskine, 1998). It is a foliar fungal disease and attacks the crop in the early pod setting stage. *Stemphylium* blight causes

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severe leaf drop, resulting in defoliated plants. Diseased plants are often left with only terminal leaves, thereby severely affecting the assimilation of photosynthates, crop yield and seed quality. In severe infestation, it may cause yield losses of up to 92% (Bakr, 1991 and Bakr and Ahmed., 1992). In India, the intensity of the disease was 82.55% and the yield loss was recorded to be as high as 93.4% (Singh *et al.*, 1990). Environmental factors play an important role in the development of this disease. High temperature favoured the germination of conidia of *S. botryosum* and under controlled conditions, the optimum temperature for conidial germination was between 25°C and 30°C (Mwakutuya *et al*., 2004). Bakr (1991) and Erskine and Sarker (1997) reported that in Bangladesh, *S. botryosum* initiated infection on lentil when the night temperature remained above 8°C with an average day temperature above 22°C and relative humidity in the plant canopy exceeded 95%. Sinha and Singh (1993) reported that an average mean temperature of 18±2°C and RH of 85 to 90% in the morning were favorable for the appearance, development and spread of *Stemphylium* blight of lentil in India, whereas RH of >50% in the afternoon was essential. There is debate on initiation and development of this disease in lentil. Some researchers think *Stemphylium* infection initiates at growing stage, some think that it may initiate at flowering while some others are of the opinion that it may occur at any stage of plant growth if congenial conditions for the fungus prevail. This piece of research work was undertaken to study the scenario of the development of this disease in lentil.

MATERIALS AND METHODS

The experiment was carried out in field conditions at the Pulses Research Centre (PRC) of the Bangladesh Agricultural Research Institute (BARI), Ishurdi, Pabna, Bangladesh during the rabi (winter) season of 2013-14 and 2014-15.The unit plot size was 1.0 m x 1.0 m and distance between the rows was 30 cm. The experiment was laid out in a randomized complete block design with 3 replications. Seeds of BARI Mashur-1 (susceptible to *Stemphylium* blight) were sown on November 01, 10, 20, 30 and December 10, 2013 and 2014. Continuous seed sowing was done in rows. All the plots were artificially inoculated with spores of *Stemphylium botryosum* (Fig.1) on January 29 of each year when the crop attained 88, 78, 68, 58 and 48 days of age. The concentration of the inoculum was 18000 conidia/ml. A proper humid condition was ensured by covering the seedbeds with poly hoods for 48 hours after inoculation (Fig.2). Fertilizers and manure were applied as per recommendations. Irrigation and other cultural practices were performed as and when necessary. The experiment was monitored regularly. Disease initiation date, age of plant when disease was initiated, disease scores10 days before harvest were recorded. Disease was scored on a 0-5 scale (Bakr and Ahmed 1992). where 0 = no infection, 1 = a few scattered leaf infections but no twig blighted, 2 = 5-10% leaflet infection and/or a few scattered (1%) twigs blighted, 3 = 11-20% leaflet infection and/or 1-5% twigs blighted, 4 = 21-50% leaflet infection and/or 6-10% twigs blighted, and 5 = 51% leaflet infection and/or more then 10% twigs blighted. The ambient temperature during the experimental period was recorded. The crop was harvested on March 2 and March 4 when fully matured in 2013-14 and 2014-15, respectively, and grain weights per plot were measured. The data were analyzed

statistically and the variations among the treatments were compared following the Duncan's New Multiple Range Test (DMRT).

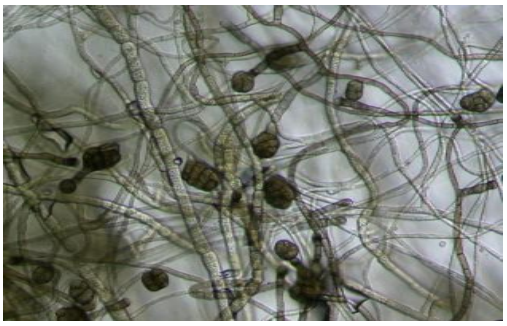

Fig. 1: Conidia of *Stemphylium* on conidiophore

Fig. 2: Poly hoods after artificial inoculation of the lentil plots by *Stemphylium*

RESULTS AND DISCUSSION

Stemphylium blight initiation

There was no remarkable difference among the sowing dates in terms of *Stemphylium* blight initiation in any year. In the 2013-2014 cropping season, the first disease symptom was observed between February 6 to 12, 2014 when the crop attained the age of 94 days (Nov.01), 88 (Nov.10), 81 (Nov.20), 71 (Nov.30), and 63 (Dec.10) (Table 1). In the 2014-2015 cropping season, the first disease symptom was observed between February 2 to 9, 2015 when the crop became 91 (Nov.01), 82 (Nov.10), 73 (Nov.20), 68 (Nov.30), and 59 (Dec.10) days old (Table 1). In the 1st year (2013-14) up to January 31, 2014, the maximum day temperature at Ishurdi remained mostly below 22^{0}C and fluctuated, which was not congenial for disease development but since the start of February, 2014, the maximum day temperature was constantly >24^{0}c which was very favorable for *Stemphylium* blight (Fig. 3). A similar temperature regime prevailed in the 2nd year (2014-15) (Fig. 4). In both years it was found that the first symptom of *Stemphylium* blight disease of lentil showed up when the temperature exceeded 24^{0}C indicating that the environment, especially temperature, rather than the age of crop, is a crucial factor in the development of *Stemphylium* blight in lentil. These results are in agreement with the findings of Bakr (1991) and Erskine *et al.* (1997). They observed that in Bangladesh, *S. botryosum* initiated infection in lentil when the night temperature remained above 8°C with an average day temperature above 22°C and the relative humidity in the plant canopy exceeded 95%. The results of this study are also in line with the findings of Huq *et al* (2008) who reported atmospheric factors such as a maximum temperature of 23.7^{0}C (range 20.5-29.0^{0}C) and minimum temperature of 14.2^{0}C (range 5.4-13.3^{0}C) and relative humidity ranging from78.0-84.5% (mean 81.0%) were the predisposing factors for disease initiation. Sinha and Singh (1993) opined that an average mean temperature of 18±2°C and RH of 85 to 90% in the morning are favorable for the appearance, development and spread of *Stemphylium* blight of lentil in India, but RH >50% in the afternoon is essential.

Table 1: Initiation of *Stemphylium* blight in relation to crop age during the rabi season of 2013-2014 and 2014-2015

Sowing date	2013-2014		2014-2015	
	Disease initiation date	Age of plant (days)	Disease initiation date	Age of plant (days)
November 01	Feb. 6, 2014	94 a	Feb. 2, 2015	91 a
November 10	Feb. 8, 2014	88 b	Feb. 2, 2015	82 b
November 20	Feb. 10, 2014	81 c	Feb. 3, 2015	73 c
November 30	Feb. 10, 2014	71 d	Feb. 8, 2015	68 d
December 10	Feb. 12, 2014	63 e	Feb. 9, 2015	59 e
LSD ($p \geq 0.05$)		0.5477		0.4595
CV (%)		0.84		0.75

Means in a column followed by the same letter(s) do not differ significantly at 1% level of significance by DMRT

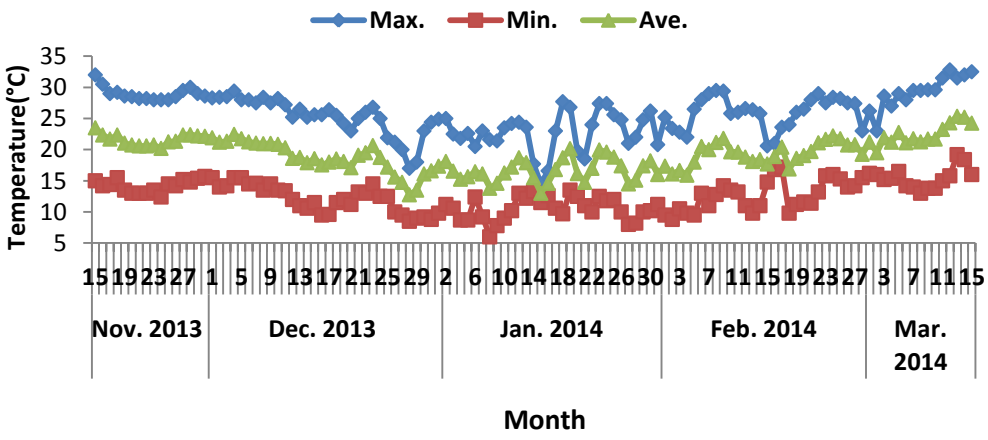

Fig. 3: Maximum, minimum and average daily air temperatures from 15 November/2013 to 15 March/2014 at Ishurdi, Pabna

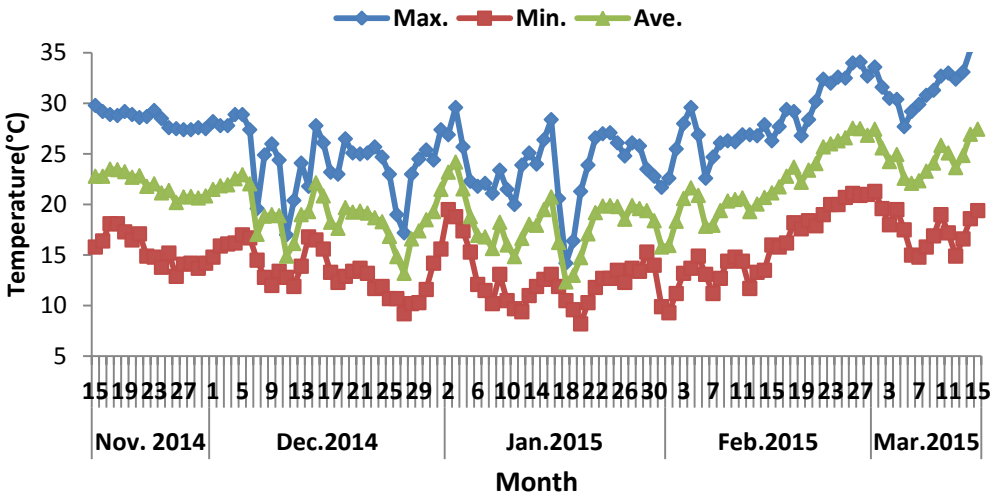

Fig. 4: Maximum, minimum and average daily air temperatures from 15 November/2014 to 15 March/2015 at Ishurdi, Pabna

Effect of sowing time on the severity of *Stemphylium* blight

Severity of *Stemphylium* blight disease showed significant variations among the different sowing times in both the years. In 2013-2014, the severity of *stemphylium* blight disease was 1.67-4.67on the 0-5 scale. The highest disease severity was recorded with the Nov 01 sowing date which was statically similar as the Nov. 10, 2013 sowing and they differed significantly from the other sowing times (Table 2). The result was similar in the next year (Table 3). Findings both the cropping seasons showed that disease severity could be lower with late sowing (Fig. 5).The results are agreement with the findings of Salam *et al.* 2016 who reported that time of sowing strongly affected disease severity, and in general, late sowing reduced the disease severity.

November 1 November 10 November 20 November 30 December 10

Fig. 5: Severity of *Stemphylium* blight disease of lentil 10 days before harvest with different dates of sowing

Effect of sowing time on yield and yield contributing characters

Grain yield and yield contributing characters of lentil were influenced significantly by sowing time in both the cropping seasons (Tables 2 and 3).In 2013-2014, the number of pods per plant ranged from 9.47-48.67, the highest being recorded for the Nov.10 sowing date which was statistically similar to that for the Nov. 01 sowing and the lowest was found for the Dec.10 sowing date which was statistically similar as that for the Nov. 30, 2013 sowing date (Table2). In2014-2015, the number of pods per plant ranged from 9.33-36.27 and highest and lowest values for the sowing dates compared well with those in the previous year. [[In the 2013-2014 cropping season, the number of seeds per pod ranged from 1.47-1.80. The highest number of seeds per pod was recorded for plants sown on Nov. 20 followed by that for the Nov. 01sowing date. The lowest seeds per pod were observed for the Nov. 30 and Dec. 10 dates but the differences with the values for the Nov. 01 and 10 sowing dates were statistically insignificant. In 2014-15, the results and trends were similar as those in the previous year (2013-14).The highest weight of seeds per plant (1.02g) was found for Nov. 10 sowing and the lowest (0.41g) for Dec. 10 sowing in the 2013-2014 cropping season. Similarly, in 2014-2015, the highest weight of seeds per plant (1.12g) was found for Nov. 01 sowing while the lowest (0.44g) was observed for Dec. 10 sowing.

The lentil yield which ranged from 417-1373 kg/ha in the 2013-2014 cropping season was significantly influenced by sowing time (Table 2). The highest yield of 1373 kg/ha was obtained from Nov. 01 sowing and it differed significantly from that with any of the other sowing dates. Low yields were obtained from the Nov 30 (837 kg/ha) and Dec 10 (417 kg/ha) which were statistically significant from each other. Similarly, in 2014-2015, the highest yield of 1433 kg/ha was recorded for Nov. 01 sowing which was significantly higher than that with any other sowing date later in the season (Table 3). Low yields were obtained from the Nov 30 (657 kg/ha) and Dec 10 (477 kg/ha) which did not differ significantly from each other. These results indicate that sowing time is one of the more important factors that determine yield of lentil. Reductions in seed yield and yield attributes of lentil with delayed sowing occurred due to shortening of the growth period and the time available for the later-sown crops to mature. Similar results was reported by Tawaha and Turk (2001).

Table 2: Effect of sowing date on severity of *Stemphylium* blight, yield and yield contributing characters of lentil during rabi season 2013-2014

Sowing date (2013)	Blight score	No. of pods/ plant	No. of seeds/ pod	Wt. of seeds/plant (g)	Seed yield (kg/ha)
November 01	4.67 a	45.93 a	1.67 ab	0.83 b	1373 a
November 10	4.00 a	48.67a	1.50 b	1.02 a	1030 b
November 20	2.67 b	19.33 b	1.80 a	0.81 b	1057 b
November 30	2.00 b	12.40 c	1.47 b	0.58 c	837 c
December 10	1.67 b	9.47 c	1.47 b	0.41d	417 d
LSD (p≥0.05)	1.031	3.620	0.2306	0.1191	108.7
CV (%)	18.26	7.08	7.88	8.24	6.12

Means in a column followed by the same letter(s) do not differ significantly at 1% level of significance by DMRT

Table 3: Effect of sowing date on severity of *Stemphylium* blight, yield and yield contributing characters of lentil during rabi season 2014-2015

Sowing date (2014)	Blight score	No. of pods/ plant	No. of seeds/ pod	Wt. of seeds/plant (g)	Seed yield (kg/ha)
November 01	5.00 a	36.27 a	1.93 a	1.12 a	1433 a
November 10	5.00 a	35.00 ab	1.93 a	1.10 a	1230 b
November 20	4.33 b	30.53 b	1.87 a	1.02 a	1167 b
November 30	4.00 b	13.67 c	1.20 b	0.57 b	657 c
December 10	3.00 c	9.33 c	1.13 b	0.44 b	477 c
LSD (p≥0.05)	0.4874	5.377	0.2663	0.1975	189.2
CV (%)	6.05	11.44	8.47	12.40	10.12

Means in a column followed by the same letter(s) do not differ significantly at 1% level of significance by DMRT

CONCLUSION

This study conducted in two consecutive years revealed that the yield contributing factors and yield of lentil could be significantly reduced by delayed sowing. Initiation of the *Stemphylium* blight disease of lentil could occur at any age of the crop. Favorable environment factors especially temperature $>24^0$C is a predisposing factor for the *Stemphylium* blight disease development in lentil. However, the disease may be less severe in case of late sowing.

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EFFECT OF GROWING MEDIA ON GROWTH, FLOWER YIELD AND QUALITY OF DUTCH ROSE (*ROSA HYBRIDA* L.) CV. TOP SECRET

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ABSTRACT

An experiment was conducted at the field of Landscape, Ornamental and Floriculture Division of Horticulture Research Centre (HRC), BARI at Joydebpur, Gazipur during January 2020 to June 2021 to determine the effect of growing media for growth, flowering, yield, and quality improvement of commercial Dutch rose cv. Top Secret in pot culture. Seven treatment combinations were T_1 - Soil + Farmyard manure (1:1, v/v), T_2 - Soil + cocodust (1:1, v/v), T_3 - Soil + vermicompost (1:1, v/v), T_4 - Soil + perlite (1:1, v/v), T_5 -Soil + cocodust + vermicompost + leaf compost (1:1:1:1, v/v), T_6 - Soil + cocodust + vermicompost + perlite (1:1:1:1, v/v) and T_7 -Soil (control) following Completely Randomized Design (CRD) with five replications. Results revealed that plant height (52.0 cm), leaf area (16.4 cm^2), plant spread (33.5 cm), number of leaf per flowering stalk (41.0), number of branches per plant (9.0) and stalk length of cut flower (33.5cm) were significantly higher in combination of soil + cocodust+ vermicompost + leaf compost media (T_5). The lowest days to flowering (55.0 days) was found in T_5. Significantly higher number of petal per flowers (21.0), petal size (4.5 cm^2), flower size (9.8 cm^2) and number of flowers per plant (20.0) were also found in T_5. Flowering duration (21.0 days) and vase life of flower (17.0 days), fresh weight of cut flower (2.5 g) and dry weight of cut flower (1.3 g) were observed significantly higher in the same T_5 treatment. The result suggested that combination of soil, cocodust, vermicompost and leaf compost mediDutch rose (*Rosa hybrida* L.) cv. Top Secret.

Keywords: Dutch rose cv. top Secret, flower quality, growing media, growth, yield

INTRODUCTION

Rose is one of the leading cut flowers in the global floriculture trade (Salem et al., 2019). Dutch roses are extremely scented and used in festivals and religious or social functions (Khandaker et al., 2020). For its fragrance and color, the Dutch rose is also very popular as potted plants in urban households (Rajasekar and Suresh, 2015). Potted plants are the only group of plants which can provide freshness even in a small space decreasing the air

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pollution indoors (Tariq et al., 2012). Growing media play a significant role in the growth and quality of potted plants like rose. Rose requires a good medium for better growth and quality of flower (Rajasekar and Suresh, 2015). Physio-chemical properties determine the nutritional status, water holding capacity and aeration of the growing media which regulate the plant growth rate (Atif et al., 2008). A light, rich, porous and well drained media is considered ideal for rose cultivation (Younis et al., 2015). For this, garden soil, leaf compost, vermicompost, cocodust, perlite are the growing media preferably used singly or combined for the container production both annual and perennial ornamental plants (Sunhainan, 2018). These materials have no harmful effects on soil, they increase microbial activities in soil which improve soil health and they fulfill the nutritional requirements of numerous pot grown crops including cut flowers like the Dutch rose (Younis et al., 2015; Chavada et al., 2017; Majid et al., 2017; Richardville et al., 2022).

In Bangladesh, the commercially available growth media are becoming expensive day by day but demand are increased higher, so research is required to evaluate the growth media for potted flower plants. This study was undertaken to find out appropriate media for improving growth, yield and quality of Dutch rose (*Rosa hybrida* L.) cv. Top Secret.

MATERIALS AND METHODS

The experiment was conducted by in the research field of the Landscape, Ornamental and Floriculture Division of Horticulture Research Centre (HRC), Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur during January 2020 to June 2021. A commercial variety of Dutch rose cv. Top Secret was collected from Moumita Flower Products Private Limited, Gazipur and plants (six months old) were transplanted singly in 10 cm × 12 cm earthen pots filled with different growing media comprising T_1 = soil + farmyard manure (1:1, v/v), T_2 = soil + cocodust (1:1, v/v), T_3= soil + vermicompost (1:1, v/v), $T_{4=s}$oil + perlite (1:1, v/v), $T_{5=s}$oil + cocodust + vermicompost + leaf compost (1:1:1:1, v/v), T_6= soil + cocodust + vermicompost + perlite (1:1:1:1, v/v) and T_7= soil only (control). The pots were arranged in a completely randomized design (CRD) with five replications. Irrigation was applied as per requirement. The test growth media were prepared two months before planting. The growth media were decomposed for two months with watering twice weekly. Treatment-wise initial (before planting) and postharvest growth media samples were analyzed for pH, organic matter (OM), EC and available N, P and K contents before planting and after harvest by standard methods (Page et al., 1982). Washed and cleaned pots were filled with the growth media. Fertilizer such as, N, P and K were applied @ 15-15-10 g/plant as recommended by Bose et al. (2015). Commercial micronutrient grade Ormichem @ 0.5 g/plant was sprayed at 30-day intervals. Weeds were removed manually. The growth media were loosened once a month for good aeration. Irrigation, disbudding, staking etc. were done properly as per requirement. Diseases like leaf spot and powdery mildew were controlled by 3 sprays of the fungicide Ridomil Gold (a.i. Metalaxyl+Mancozeb) @ 2.0 g/L water at 15-day intervals starting from 30 days after transplanting, and insects like aphids, thrips, leaf miners etc. were controlled by 3 applications of Ripcord (a.i. Cypermethrin) @ 2.0 ml/L in the same spraying protocol. Flowers were harvested in the morning at the tight bud stage (2 to 3 petals unfurled). Five plants were randomly selected from each treatment and tagged for data recording. Data on

plant height, plant spread, leaf area, number of leaves per flowering stalk, number of branches per plant, stalk length, days to flowering, number of petals per flower, size of petals, size of flower, number of flowering, fresh weight of cut flower, dry weight of cut flower, flowering duration and vase life were recorded. Dutch rose cut flowers and cut stems were collected from pots in the morning to avoid excessive heat and brought to the laboratory in a bucket containing 3 to 4 liters of water for observing vase life. Stems were cut (slanting) to a uniform length 30 cm and leaves near the bottom of cut stems were removed avoiding few leaves below the inflorescence for placing in vase water. Cut stems were placed in 250 ml conical flasks containing 200 ml of water and kept in a laboratory room temperature 18±2ºC and relative humidity 70±5%. Five flowers were taken randomly from each treatment and vase life was recorded by counting number of days from the time when cut flowers began to lose their decorative value after complete opening or shedding of petals.

The data were statistically analyzed by the MSTAT software and treatment means were separated by Duncan's Multiple Range Test (DMRT) at 5% level of probability according to Steel et al. (1997).

RESULTS AND DISCUSSION

Growth parameters and flower yield

Growth parameters like plant height, leaf area, plant spread, number of leaves per plant, number of branches per plant and stalk length of Dutch rose were significantly influenced by different growing media (Table 1). Among the treatments, T_5 (soil + cocodust + vermicompost + leaf compost) gave the tallest plants (52.0 cm) followed by T_6 (soil + cocodust+ vermicompost + perlite) (48.0 cm) and T_7 (garden soil only) gave the shortest plants (35.5 cm). Treatment T_5 might have more organic matter which released essential plant nutrients particularly nitrogen that accelerated plant growth. Barman et al. (2006) opined that N enhance cell division and formation of more plant tissues resulting in luxuriant vegetative growth and increased plant height. Similar results were reported Younis et al. (2015) for rose and Ysmeen et al. (2012) for carnation.

Leaf area is an important agronomic parameter determining the capacity of a plant to trap solar energy for photosynthesis which has a marked effect on plant growth. Leaf area of Dutch rose in this experiment was markedly influenced by growing media. The highest leaf area (16.4 cm^2) was found with T_5 treatment which was statistically similar to that with T_6 (14.5 cm^2), T_3 (16.4 cm^2) or T_2 (16.4 cm^2) (Table 1). An increase in the leaf area enhanced the net assimilation rate ultimately augmenting plant growth. This result was in agreement with the findings of Younis et al. (2015) for rose.

In terms of plant spread, too, T_5 outperformed the other growth media giving the highest plant spread (32.4 cm) in contrast with the lowest value 19.0 cm) in control (T_7) (Table 1). Treatment T_5 contributed in increasing the plant spread by a greater nutrient supply to the plants as well as by enhancing the higher water holding capacity of the soil. This is supported by findings of Ysmeen et al. (2012) for rose and Jawaharlal et al., (2011) for anthurium.

The treatment T_5 produced the highest number of leaves per plant (41.0) followed by T_6 (38.0), and T_7 (control) the lowest number (26.0) (Table 1). Similar results were reported

by Younis et al. (2015) and Ahmed et al. (2012) who observed that adding cocodust, vermicompost and leaf compost to soil media increased the number of leaves in rose. In the present study, the highest number of branches per plant (9.0) was recorded for T_5 which compared well with most of the other treatments except T_7 (control) which produced the lowest number of branches per plant (4.5) (Table 1). This beneficial effect of the growth medium (T_5) consisting of soil with cocodust, vermicompost and leaf compost can be attributed to then storage and release of nutrients to plants for a relatively long period which resulted in the intensification of leave growth (Sunhainan, 2018).

From a marketing point of view, length of the flower stalk is an important flower growth parameter. Treatment T_5produced the longest stalk (33.5 cm) followed by T_6 (29.5 cm), T_3 (28.6 cm) and T_2 (28.0 cm) (Table 1). A good amount of leaves coupled with conducive root development might have led to proper nutrient uptake by the plants may have led to an increase in the rose stalk length as observed by Younis et al. (2015). High nutrient contents in cocodust, vermicompost and leaf compost contribute to good plant growth which was also observed by Ahmad et al. (2012) in rose and Ysmeen et al. (2012) in carnation.

Table 1: Effect of growing media on growth parameters of Dutch rose cv. Top Secret

Growth medium	Plant height (cm)	Leaf area(cm^2)	Plant spread (cm)	Number of leaves/plant	Number of branches/plant	Stalk length (cm)
T_1	40.0 c	9.20 bc	23.5 c	30.0 c	6.0 ab	24.5 bc
T_2	42.5 bc	12.4 ab	25.2 bc	33.0 bc	6.0 ab	28.0 ab
T_3	43.0 bc	12.8 ab	26.0 bc	35.0 b	6.0 ab	28.6 ab
T_4	45.5 b	10.9 b	25.0 bc	32.0 bc	6.0 ab	26.0 b
T_5	52.0 a	16.4 a	32.4 a	41.0 a	9.0 a	33.5 a
T_6	48.0 ab	14.5 ab	28.2 b	38.0 ab	7.0 ab	29.5 ab
T_7	35.5 d	7.00 c	19.0 d	26.0 d	4.5 b	22.2 c
CV (%)	11.5	8.4	11.7	10.3	9.8	12.6

Means within a column followed by a common letter(s) do not differ significantly ($P \leq 0.05$) by DMRT; $T_{1=}$ soil + FYM (1:1, v/v), T_2= soil + cocodust (1:1, v/v), T_3= soil + vermicompost (1:1, v/v), T_4= soil + perlite (1:1, v/v), T_5= soil + cocodust + vermicompost + leaf compost (1:1:1:1, v/v), T_6= soil + cocodust + vermicompost + perlite (1:1:1:1, v/v) and T_7= soil alone (control)

Growing media significantly influenced days to flowering, petal size, number of petals per flower, flower size and number of flowers per plant of Dutch rose (Table 2). The days to flowering was the lowest (55.0 days) with T_5 followed by T_6 (58.0 days) (Table 2). The shortening of the time to flower with T_5 might have been due to vigorous growth of the plant, rapid uptake of nutrients and water resulted early flowering. Moreover, leaf number increased by T_5 may have facilitated greater accumulation of photosynthates which could have induced early flowering. Also augmented protein formation from carbohydrate deposits in vegetative parts and greater protoplast formation may also have been responsible for relatively early flowering. Rajasekar and Suresh (2015) and Jawaharlal et al. (2011) observed that a minimum period was required for flowering of

rose and athurium, respectively, with a combination of soil, cocodust, vermicompost and leaf compost as the growing medium.

In the study, T_5 produced the largest petals (4.5 cm^2) comparable with most of the treatments and the smallest petals (2.4 cm^2) were found with T_7 (control) (Table 2). An increase in the petal size with could have been related to an increase leaf area with T_5 as noted for rubisco by Ysmeen et al. (2012). A higher K content in the growing medium meant good physical properties like high porosity, good water holding capacity and higher retention of moisture that increased the size of petal (Khandaker et al., 2020). The number of petals per flower varied significantly among the treatments where the highest number of petals (21.0) was counted for T_5 followed by T_6 (18.0), and the lowest was for soil alone (control) (11.0) (Table 2). Appropriate quantities of vermicompost in growing media has synergistic effects and cocodust and leaf compost improve physical properties of the media, decrease compaction and enable better growth of plant that was beneficial for increasing the number of petals per flower. Results of this study agreed well with the findings of Younis et al. (2015) and Ahmed et al. (2012) for rose and Atif et al. (2008) for zinnia. Growing media markedly influenced the size of flower (Table 2). The size of flower was maximum (9.8 cm) with T_5 and minimum (6.5 cm) with T_7 (control). Proper amounts of N and K in T_5 helped obtain large flowers. Similar results were reported by Barman et al. (2006) for rose.

Flower production was markedly influenced by growing media (Table 2). The highest number of flowers per plant (20.0) was recorded for T_5 and the lowest (13.0) for T_7 (control). This might have been due to higher water retention by cocodust, vermicompost and leaf compost (T_5) which decreased the media temperature in pots and also might have had the at optimum EC and pH resulting in a vigorous plant growth and enhanced flower production. The T_5 treatment stimulated nutrient uptake and it had positive effect on protein synthesis and vegetative growth, hence the increased flower yield. Similar findings were reported by Ysmeen et al. (2012) for rose and Jawaharlal et al. (2011) for anthurium.

Table 2: Effect of growing media on flower characteristics in Dutch rose cv. Top Secret

Growing media	Days to flowering	Petal size (cm^2) /flower	Number of petals/flower	Flower size (cm^2)	Number of flowers/plant
T_1	66.0ab	3.0 ab	14.0 bc	8.0 ab	15.0ab
T_2	64.0 b	3.1 ab	15.0 bc	8.1 ab	15.0 ab
T_3	60.0 c	3.20 ab	16.0 b	8.2 ab	16.0 ab
T_4	62.0 bc	3.3 ab	14.0 bc	8.0 ab	15.0 ab
T_5	55.0 d	4.5 a	21.0 a	9.8 a	20.0 a
T_6	58.0 cd	3.5ab	18.0 ab	8.6 ab	17.0 ab
T_7	70.0 a	2.4 b	11.0 c	6.5 b	13.0 b
CV (%)	9.60	10.8	10.9	9.3	10.2

Means within a column followed by a common letter(s) do not differ significantly ($P \leq 0.05$) by DMRT; $T_{1=}$ soil + FYM (1:1, v/v), T_2= soil + cocodust (1:1, v/v), T_3= soil + vermicompost (1:1, v/v), T_4= soil + perlite (1:1, v/v), T_5= soil + cocodust + vermicompost + leaf compost (1:1:1:1, v/v), T_6= soil + cocodust + vermicompost + perlite (1:1:1:1, v/v) and T_7= soil alone (control)

Flower quality parameters

Flower quality parameters such as, fresh and dry weights of cut flower, flowering duration and vase life of cut flower were significantly influenced by the different growing media (Table 3). Fresh weight of cut flower was the highest (2.5 g) with T_5 which was statistically similar as that with the other treatments except T_7 (control) which gave the lowest (0.7g) fresh weight. This indicated that T_5 ensured good plant health owing to adequate supplies of nutrients and water from the media to the plants. Rajasekar and Suresh (2015), Barman et al. (2006) and Chavada et al. (2017) got similar results in rose grown in soil, cocodust, vermicompost and leaf compost growing media. The highest dry weight of cut flower (1.3 g) was achieved with T_5 while T7 (control) gave the lowest (0.3 g) dry weight (Table 3). This result is in close conformity with findings of Hasan (2017), Younis et al. (2015) and Barman et al. (2006) for rose. The longest flowering duration (21 days) was recorded for T_5 followed that for T_6 and T_3 treatments (Table 3). The increased flowering duration could be related to the internal carbohydrate content of flowers responsible for flowering duration. The soil + cocodust + vermicompost + leaf compost combination of T_5 facilitated good absorption of nutrients by the plants which ultimately resulted in the production of long stems and turgid flowers. Dutta et al. (2002) and Ahmad et al. (2012) reported similar results for rose. In the present study, the longest vase life (17 days) of cut flower was noted for T_5 which was statistically similar with that for T_6 (15 days) and T_3 (14 days) (Table 3). This result was in agreement with that reported by the findings of Hasan (2017) and Younis et al. (2015) for rose.

Table 3: Effect of growing media on quality parameters of Dutch rose cv. Top secret

Growing media	Fresh wt. of cut flower (g)	Dry weight of cut flower (g)	Flowering duration (days)	Vase life of cut flowers (days)
T_1	1.2ab	0.5ab	14.0 bc	11.0 bc
T_2	1.3ab	0.6ab	16.0 b	13.0 b
T_3	1.4ab	0.7ab	17.0 ab	14.0 ab
T_4	1.3ab	0.6 ab	16.0 b	13.0 b
T_5	2.5 a	1.3 a	21.0 a	17.0 a
T_6	1.6 ab	0.8 ab	18.0 ab	15.0 ab
T_7	0.7 b	0.3 b	11.0 c	9.0 c
CV (%)	7.5	8.3	7.2	8.4

Means within a column followed by a common letter(s) do not differ significantly ($P \leq 0.05$) by DMRT; $T_{1=}$ soil + FYM (1:1, v/v), T_2= soil + cocodust (1:1, v/v), T_3= soil + vermicompost (1:1, v/v), T_4= soil + perlite (1:1, v/v), T_5= soil + cocodust + vermicompost + leaf compost (1:1:1:1, v/v), T_6= soil + cocodust + vermicompost + perlite (1:1:1:1, v/v) and T_7= soil alone (control)

Nutrient status of growing media

The pH of growing media after harvest was almost acidic except T_1 and T_7 treatment (Table 4). The lowest pH (5.80) was noted from T_5 followed by T_6 (5.87) treatment (Table 4). The pH of different media after harvest showed a decreasing trend from that

before planting. The EC was also lowest (0.22) in the media soil + cocodust +vermicompost + leaf compost T_5 followed by 0.25 (T_6). Organic matter, N, P and K contents were the highest (2.95%, 3.50%, 0.97% and 2.35%) in T_5 (soil + cocodust + vermicompost + leaf compost) followed by T_6 (2.86%, 3.38%, 0.83% and 2.24%) treatment (Table 4). Rose requirs an acidic condition for good growth and yield, and T_5 was suitable in this regard. These results are in conformity with the earlier findings of Rajasekar and Suresh (2015) for rose. The soil + cocodust + vermicompost + leaf compost medium had higher N, P and K contents which increased the N, K uptake and an increase in the availability of P could result from a higher activity of P-solubilizing organisms (Swetha et al., 2014).

Table 4: Postharvest change of nutrient status in growing media with reference to initial status

Treatments	pH	OM (%)	EC (dS/m)	N (%)	P (%)	K (%)
Initial						
T_1	7.40	0.98	0.70	0.60	0.51	0.56
T_2	6.50	1.80	0.56	0.95	0.75	1.13
T_3	6.80	1.70	0.60	1.18	0.85	0.98
T_4	6.60	2.00	0.58	1.10	0.81	0.90
T_5	6.10	2.48	0.35	1.61	1.32	1.42
T_6	6.20	2.30	0.42	1.50	1.24	1.29
T_7	7.20	0.67	0.77	0.42	0.45	0.52
Post-harvest						
T_1	7.20	1.70	0.65	1.90	0.40	0.52
T_2	6.30	2.24	0.50	2.30	0.60	1.25
T_3	6.50	2.29	0.48	2.89	0.64	1.20
T_4	6.20	2.20	0.45	2.70	0.62	1.18
T_5	5.80	2.95	0.22	3.50	0.97	2.35
T_6	5.87	2.84	0.25	3.38	0.83	2.24
T_7	7.16	0.72	0.61	0.41	0.37	0.55

$T_{1=}$ soil + FYM (1:1, v/v), T_2= soil + cocodust (1:1, v/v), T_3= soil + vermicompost (1:1, v/v), T_4= soil + perlite (1:1, v/v), T_5= soil + cocodust + vermicompost + leaf compost (1:1:1:1, v/v), T_6= soil + cocodust + vermicompost + perlite (1:1:1:1, v/v) and T_7= soil alone (control)

CONCLUSION

In the light of the results obtained from this investigation, it can be concluded that among the various growing media, a combination of soil, cocodust, vermicompost and leaf compost (1:1:1:1, v/v) would make a very good growth medium for Dutch rose cv. Top Secret in terms of plant growth, flower yield and flower quality.

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EFFECTS OF BREWER'S YEAST ON BLOOD PARAMETERS AND LIVER MORPHOMETRY OF NILE TILAPIA, *Oreochromis niloticus*

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ABSTRACT

The effect of a probiotic brewer's yeast, *Saccharomyces cerevisiae*, on hemato-biochemical indices and liver morphometry of the Nile tilapia (*Oreochromis niloticus*) was studied in a 60-day trial in aquaria where Nile tilapia fingerlings were fed with *S. cerevisiae* at the rates of 0, 1, 2 and 4 g/kg diet. Various haemato-biochemical parameters such as, hemoglobin (Hb; g/dl), red blood cells (RBC; $\times 10^6/mm^3$), white blood cells (WBC; $\times 10^3/mm^3$) and glucose (Glu; mg/dl) were measured. The values of Hb, RBC, WBC and Glu ranged from 7.60 to 8.70, 117 to 141, 1.21 to 1.97 and 3.21 to 3.62, respectively in blood samples of fish fed with supplements of different amount of the yeast. The probiotic yeast supplemented fish had a significantly higher number of regular shaped nucleus in the liver hepatocytes than that in fish from the control group (no yeast supplement). Also, in comparison with fish from the control group, less vacuolation was found in the liver sections of fish fed with diet containing the probiotic yeast (4 g kg^{-1}).

Keywords: Aquaculture, feed, fish, hematology, liver health, *Saccharomyces cerevisiae*

INTRODUCTION

The Nile tilapia (*O. niloticus*) has grown phenomenally in popularity and has become the second most abundantly farmed fish, behind carps (FAO, 2020).This fish is produced in large quantities in aquaculture worldwide due to its modest requirement for artificial feeds, its tolerance of water quality and environmental variation, high resistance to most pathogens, short crop cycle and absence of intermuscular bone. According to the Fishery Statistical Yearbook of Bangladesh (DoF, 2022), tilapia production in Bangladesh was about 3,92,095 metric tonnes in 2020-2021. Due to the rapid expansion of hatcheries producing monosex all male seed, within a span of two years (2019-2020), tilapia production increased by more than three folds in Bangladesh.

The expansion of tilapia culture, accompanied by a shift to more high-input, intensive systems, has made farmed tilapia more vulnerable to stress and disease outbreaks, leading to severe mortality and economic loss (Subasinghe, 2005). This shift has also led to massive use of antimicrobials for disease control and growth promotion, with varying, and sometimes controversial results (Carlet et al., 2012). Residues of these drugs may

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persist in fish tissue, and may also encourage the natural emergence of bacterial resistance which will become difficult to control and eradicate (Austin and Austin 2007). Therefore, the search for alternative, unconventional and more environmentally friendly disease treatments and control strategies has become imperative (Cabello, 2006). In this regard, the contribution of probiotics to aquaculture development is receiving considerable attention. Probiotics (especially antagonistic probiotics) can reduce pathogenic bacteria by competitive exclusion, provide nutrients and enzymes to promote host growth, enhance immune response by immune stimulation, and they do not cause secondary pollution problems. In view of this, obtaining *S. agalactiae* antagonizing probiotics suitable for tilapia culture is of great practical significance for improving the resistance of tilapia and reducing the use of antibiotics. Baker's yeast is a source of nucleic acids and β-1,3-glucans which have been recognized to effectively enhance immune functions of the African catfish (Yoshida et al., 1995), Atlantic salmon (Engstad et al., 1992), rainbow trout (Siwicki et al., 1994), and shrimp *Penaeus monodon* (Thanardkit et al., 2002). Moreover, Sakai et al. (2001) reported that the nucleotides from brewers' yeast RNA were capable of enhancing the phagocytic and oxidative activities of kidney phagocytic cells, serum lysozyme in common carp as well as resistance to *A. hydrophila*.

Assessment of hemato-biochemical parameters is an essential tool to evaluate the physiological changes caused by the environmental factors and gain an insight into fish health status (Fazio et al., 2019). Hemato- biochemical parameters such as Hb and blood glucose levels have been used as indicators of physiological stress responses of fish i to intrinsic or extrinsic factors (Jahan et al., 2019; Shahjahan et al., 2018; Sharmin et al., 2016).

Use of antibiotics for fish growth promotion has been banned and public awareness of healthy fish production has also increased leading to an increased interest in the potential of functional feeds as health promoters. The use ofrobiotics improves fish feed efficiency, growth, immune status, digestive enzyme activities, gut and liver morphology, disease resistance and stress responses. As an alternative to antibiotics, functional ingredients such as probiotic, prebiotic and symbiotic organisms are becoming increasingly popular in aquaculture and other animal production industries to promote animal health and well-being (Rohani et al., 2022). The present study was designed to study the effect of probiotic yeast on hemato-biochemical parameters and morphological changes of liver in the Nile tilapia fish fed with diet containing varying concentrations of a probiotic yeast supplement.

MATERIALS AND METHODS

Experimental fish

Active and apparently healthy tilapia (*O. niloticus*) fingerlings were purchased from Sharnalata Agro Fisheries Ltd. Fulbaria, Mymensingh. The fingerlings were carefully

transported to the Laboratory of Fish Ecophysiology, Department of Fisheries Management, Bangladesh Agricultural University, Mymensingh. The mean initial length and weight of fingerlings were recorded as 7.6 ± 0.48 cm and 7.55 ± 1.25 g, respectively. The fingerlings were randomly stocked into 12 glass aquaria (75 cm × 45 cm × 45 cm) and acclimatized for 15 days in the ambient temperature and environment. The fingerlings were fed twice daily with diet at the rate of 5% of the total body weight at 9.00 AM and 5.00 PM. During the acclimatization period, the behavior and apparent health status of each fingerling was monitored.

Experimental scheme

After acclimatization fingerlings were transferred into twelve new glass aquaria (length 75 cm × width 45 cm × height 45 cm) with 100 L of clean tap water. Baker's yeast, *S. cerevisiae* (Angel Yeast Co. Ltd., China) was incorporated into basal diets at different concentrations of 0 (control), 1, 2 and 4 g kg^{-1} and fed to the quadruplicate group of tilapia fingerlings. Twenty fish were randomly stocked in each aquarium for each treatment with three replications per treatment in this experiment. The duration of the experiment was 60 days. The experiment was managed in order to prevent any potential cross-contamination among different group of probiotics. Adequate aeration was provided for all aquaria to maintain saturated dissolved oxygen throughout the experimental period. Feeding was done twice daily, at 9.00 AM and 5.00 PM, throughout the study period and unutilized feed and faeces were siphoned off to maintain good water quality. The rations were adjusted weekly considering increasing body weights of the fish. The aquaria were cleaned every week to minimize the potential of bacterial growth and ammonia toxicity.

Diet preparation and feeding program

In the experiment, four diets with graded levels of probiotic yeast were prepared from fresh fish feed ingredients following the Pearson's Square Method (Wagner and Stanton, 2012). Dietary ingredients (Table 1) were obtained from the local fish market. Probiotic yeast was incorporated into the diets at levels of 0 g kg^{-1}, 1 g kg^{-1}, 2 g kg^{-1} and 4 g kg^{-1}. All dry ingredients were measured and mixed homogenously with molasses using a food mixer. Cold distilled water was added to make dough, which was formed into pellets with a commercial pellet machine. Finally, the formulated pellets were dried in open air and maintained at -20°C in airtight polythene bags before being used for the experiment. A standard protocol (AOAC, 2005) was followed to analyse the proximate composition of the formulated diets (Table 1) in the Fish Nutrition Laboratory, Department of Aquaculture, Bangladesh Agricultural University, Mymensingh.

Table 1: Formulation and proximate composition of experimental diets (% dry matter basis)

Ingredients (%)	Diet 1	Diet 2	Diet 3	Diet 4
Feed Formulation				
Fish meal	45	45	45	45
Soybean meal	25	25	25	25
Mustard oil cake	13	13	13	13
Wheat bran	10	10	10	10
Molasses	5	5	5	5
Vitamin-Mineral premix	2	2	2	2
Probiotic yeast (g kg^{-1}) supplemented	0	1	2	4
Proximate composition				
Moisture (%)	12.09	12.11	12.22	12.29
Crude lipid (%)	7.45	7.12	7.42	7.38
Crude protein (%)	40.32	40.27	40.26	40.86
Ash (%)	8.46	8.23	8.15	8.37
Crude fibre (%)	5.20	4.88	5.28	4.77
Nitrogen free extract/Carbohydrate	26.48	27.39	26.67	26.93

Hemato-biochemical parameters

After 60 days of the feeding trial, 10 fish from each of the treatments were anesthetized with clove oil (5 mg/l) and sacrificed, and blood samples were collected from the caudal vein using a heparinized plastic syringe to determine the level of hemoglobin (Hb; g/dl), red blood cells (RBCs; $\times 10^6/mm^3$), white blood cells (WBCs; $\times 10^3/mm^3$) and blood glucose (Glu; mg/dl). After collecting blood, the levels of Hb and Glu were directly determined by a digital EasyMate®GHb (Model ET232, Hb/Glu double monitoring system, Bioptic Technology Inc. Taiwan 35,057) using hemoglobin and glucose strips, respectively. In order to count red blood cell (RBC), 5 µl blood was mixed with 995 µl Hayem's fluid (RBC thinning liquid). After adding a drop of Giemsa stain, 10 µl of RBC solution was taken in a hemocytometer. For counting white blood cell (WBC), 5 µl of blood was mixed with 995 µl Turk's fluid (WBC thinning liquid). Subsequently, by adding a tiny quantity of Giemsa stain, 10 µl of WBC solution was taken and drawn in a hemocytometer. Both RBCs and WBCs were measured according to standard practice using a Neubauer hemocytometer under the light microscope. For calculating RBC and WBC, respectively, the following formulas were used:

Red blood cell (RBCs) count = $\left(\frac{\text{sum of RBC} \times 4000 \times 200}{5 \times 16}\right) \text{cells/mm}^3$

White blood cell (WBCs) count = $\left(\frac{\text{sum of WBC} \times 40}{0.1}\right) \text{cells/mm}^3$

Histopathology of liver

For the histo-pathological study, a microtome machine (Model Leica JUNE RM 2035), wax dispenser (Leica EG 1120) and water bath (Leica HI 1210) were used. The chemicals used in this experiment were ethyl alcohol, chloroform, and melted wax. High temperature was used as an environmental parameter. Alcohol (70%) was used to preserve the sample. Ethyl alcohol, xylene and eosin were used to prepare the slides. The DPX was used as a mounting agent.

The collected fish were dissected out carefully by scissor starting from anus to the lower jaw, and the belly was opened. Then the muscles of the abdomen were cut out vertically from the anus towards the vertebral column. Muscles, fat tissue, digestive organs, blood vessels and kidney were removed properly and liver samples were collected. The samples were preserved in previously prepared 70% alcohol and kept in a refrigerator to conduct a future histopathological study.

The preserved liver tissues were passed through graded alcohol series to dehydrate them. The dehydrated liver tissue samples were cleaned by chloroform and embedded into paraffin. Sectioning was done using a microtome. The liver sections were then stained with hemato-xylene eosin (HE) stains and mounted with DPX. Finally, the liver sections were observed under a microscope. This microscopic observation helped identify the histopathological changes in liver tissues of *O. niloticus* exposed to different probiotic yeast supplements.

Statistical analysis

Values are expressed as means ± standard deviation (SD). Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to assess statistically significant differences among treatments. Statistical significance was set at $P<0.05$. Statistical analyses were performed using the PASW Statistics 18.0 software (IBM SPSS Statistics, IBM and Chicago, USA).

RESULTS AND DISCUSSION

Effect of probiotic yeast on blood hemoglobin

The values of hemoglobin ranged from 7.60 to 8.70 g dl^{-1} in the fish blood supplemented with different graded levels of probiotics. There was no distinct difference in the values of Hb among different levels of probiotic yeast (0, 1, 2 and 4 g kg^{-1}) supplemented feed fed fish (Figure 1). Hemoglobin in blood carries oxygen from the lungs or gills to the rest of the body (i.e. the tissues). There it releases the oxygen to permit aerobic respiration to provide energy to power the functions of the organism in the process called metabolism. Data showed that the blood hemoglobin of Nile tilapia was not affected with probiotic inclusion levels, and this observation agreed well t with the other recorded values in tilapia (Akinrotimi et al., 2012). An increase in levels of hemoglobin was reported in Nile tilapia fed with *S. cerevisiae* and *Bacillus* spp. treated diets (Dahiya et al., 2012; Mehrabi et al., 2012).

Effect of probiotic yeast on the RBC count

The RBC counts ($\times 10^6$ mm^3) were 3.45, 3.36, 3.21 and 3.62 in t blood samples collected from fish supplemented with *S. cerevisiae* at 0, 1, 2 and 4 g kg^{-1} diet, respectively. The highest RBC counts were found in the fish fed with 4 g kg^{-1} probiotic incorporated diet followed by 0, 1 and 2 g kg^{-1} probiotic-containing diets. However; no

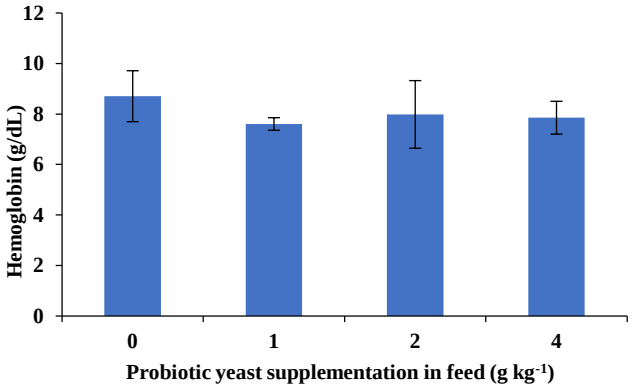

Fig. 1: Changes in blood hemoglobin levels of Nile tilapia fed with probiotic yeast supplemented diets for 60 days

significant differences were recorded for RBC between fish fed on diets supplemented with probiotic yeast 1 g kg^{-1} and 2 g kg^{-1} (Figure 2). Erythrocyte cell contains Hb and maintains the hematopoietic system in fish. Red blood cells are essential components in both innate and adaptive immune response and a higher RBC count indicates a stronger immune system (Standen et al., 2013). In the present study, the RBC count of Nile tilapia was not affected by inclusion levels which was similar as earlier the recorded values in tilapia (Akinrotimi et al., 2012). An increase in levels of hemoglobin was reported in Nile tilapia fed on *S. cerevisiae* and *Bacillus* spp. treated diets (Dahiya et al., 2012; Mehrabi et al., 2012). In the present study, there was no significant ($p<0.05$) difference between the probiotic yeast (*Saccharomyces cerevisiae*) supplemented diet treatments and the control.

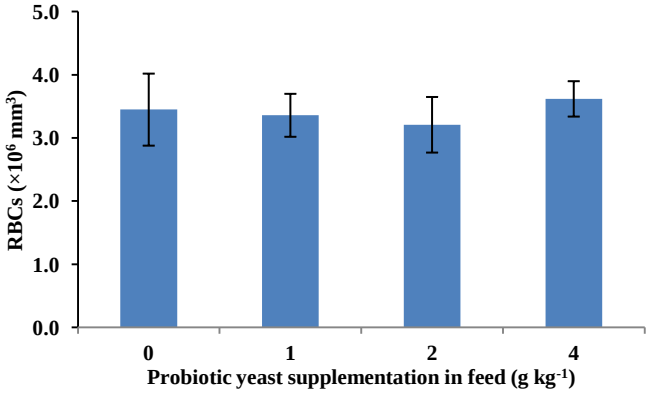

Fig. 2: Changes in RBC counts of Nile tilapia fed with probiotic yeast supplemented diets for 60 days

Effect of probiotic yeast on the WBC count

The WBC counts increased with an increase in the probiotic level but declined at the highest level of inclusion. The highest value of WBC (1.97×10^3 mm^{-3}) was recorded in fish fed on diet containing probiotic yeast and the lowest (1.21×10^3 mm^{-3}) in the control group (Figure 3). However, no significant differences ($P<0.05$) were recorded for WBC among fish fed on diets supplemented with probiotic yeast 3 g kg^{-1} and 4 g kg^{-1}. The practice of using probiotic supplemented diets was found to be capable of increasing the WBC levels of fish. In the current study, an increase in WBCs with increased level of probiotic inclusion was observed. In contrast to the fish fed control diets, *L. sporogenes* as probiotic in *C. batrachus* (Dahiya et al., 2012), the application of a mixed probiotic species *L. sporogenes*, *L. acidophilus*, *B. subtilis*, *B. licheniformis* and *S. cervirial* in *C. mrigala* (Sharma et al., 2013), *B. subtilis* in rainbow trout (Kamgar and Ghane, 2014), mixed probiotic species of *B. subtilis* and *S. cerevisiae* in *C. mrigala* (Ullah et al., 2018), *L. acidophilus* and *B. subtilis* in Nile tilapia (Aly et al., 2008), *S. cerevisiae* in Nile tilapia (Jahan et al., 2021) had been reported to increase the WBC levels. Fish fed with probiotic supplemented diets may have a better immune response than those with no probiotic supplement. Feeding fish with probiotic-supplemented diets enhanced immune defense (Munir et al., 2018).

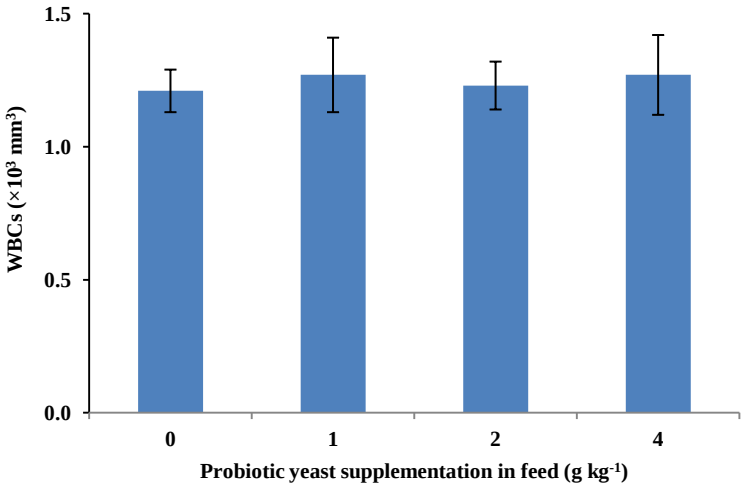

Fig. 3: Changes in WBC counts of Nile tilapia fed with probiotic yeast supplemented diets for 60 days

Effect of probiotic yeast on glucose levels

The values of glucose levels were 117, 120, 135 and 141 mg/dl in blood samples of fish fed with *S. cerevisiae* supplement at 0, 1, 2 and 4 g kg^{-1} diet, respectively. The highest glucose level was found in the fish fed with 4 g kg^{-1} probiotic incorporated diet followed by 2, 1 and 0 g kg^{-1} probiotic-containing diets (Figure 4). In the present study, the levels of glucose increased significantly with the increase of yeast probiotics in the diet in

parallel with improvement of growth. Reports of significantly increased glucose level in Nile tilapia, *O. niloticus* (Abdel-Tawwab et al., 2008), mrigal, *C. mrigala* (Sharma et al., 2013), Asian seabass, *Lates calcarifer*, silver barb, *Barbonymus gonionotus* (Salam et al., 2021) and rohu fish *Labeo rohita* (Jahan et al., 2021) fed with diet incorporated with *S. cerevisiae* support the findings of the present study. Various types of probiotics species enhanced the Glu content of walking catfish, *Clarias batrachus* (Dahiya et al., 2012), Caspian kutum, *Rutilis frisii* (Azarin et al., 2015) and *O. niloticus* (Garcia-marengoni et al., 2015).

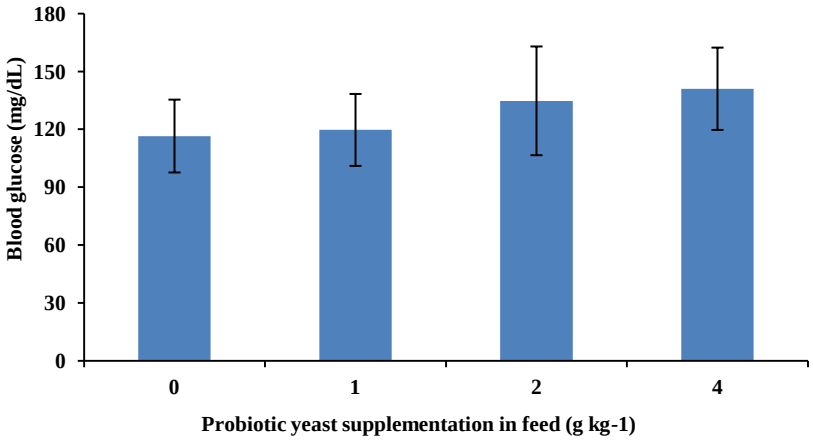

Fig. 4: Changes in blood glucose levels of Nile tilapia fed with probiotic yeast supplemented diets for 60 days

Effect of probiotic yeast on liver histology

Probiotic yeast improved the liver cells of Nile tilapia (Figure 5). It was found that the probiotic yeast supplemented fish had a significantly ($p<0.05$) lower number of irregular shaped nucleus in the hepatocytes compared with control (Table 2). The numbers of irregular shaped nucleus in hepatocytes of fish fed with 0, 1, 2, 4 g kg^{-1} probiotic incorporated diets were 15.67 ± 2.73, 12.67 ± 2.73, 11.00 ± 1.79 and 8.33 ± 2.25, respectively (Table 2). The liver section from the control showed large vacuolization and the space among the hepatocytes was 6.67 ± 0.93 µm. The liver sections from fish groups, fed *S. cerevisiae* at 1 and 2 g kg^{-1} diet, showed moderate vacuolation and the spaces among the hepatocytes were 5.83 ± 0.93 µm and 5.33 ± 0.68 µm, respectively. Less vacuolation was found in the liver section of fish group fed diet containing yeast *S. cerevisiae* 4 g kg^{-1} and the space among the hepatocytes was 2.17 ± 0.68 µm (Table 2). An increase in probiotic yeast (*S. cerevisiae*) incorporation in the feed of Nile tilapia reduced vacuolation in the hepatocytes.

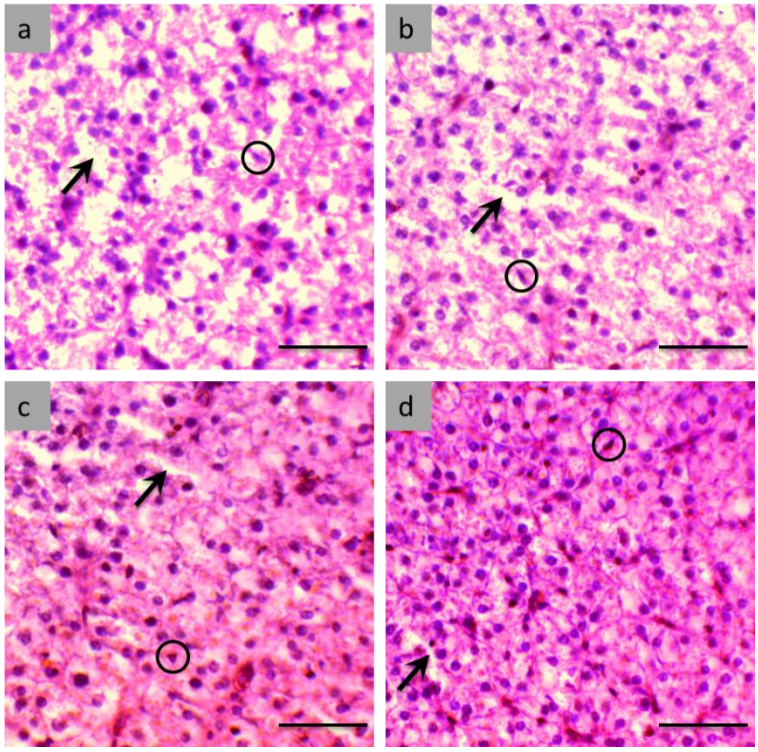

Fig. 5: Histological section of liver of Nile Tilapia (*O. niloticus*) supplemented with probiotic yeast diets (a, 0; b, 1; c, 2; and d, 4 g kg^{-1} feed) at 60 days shows space (arrow) among the hepatocyte cells (a = large, b, c = moderate and d = less) and irregular shaped nucleus (circle). Image 40x; scale bar = 50 µm; Hematoxylin and Eosin staining.

Table 2: Changes in hepatocyte cells of Nile tilapia fed with probiotic yeast supplemented diets for 60 days

Parameters	Probiotic yeast supplementation in the feed (g kg^{-1})			
	0	1	2	4
Irregular shaped nucleus	15.67 ± 2.73^{a}	12.67 ± 2.76^{b}	11.00 ± 1.79^{b}	8.33 ± 2.25^{c}
Distance of liver tissue (µm)	6.67 ± 0.93^{a}	5.83 ± 0.93^{b}	5.33 ± 0.68^{b}	2.17 ± 0.68^{c}

Statistically significant ($p<0.05$) variations among different groups in a row are indicated by different alphabetical superscripts. The values are expressed as means ± SD (n = 6)

The liver is the key organ of metabolism and excretion, being responsible for detoxification, removing toxic substances from the blood, and excreting them (Surai, 2015). The present study resulted in a higher number of hepatocytes with regular shaped nuclei in Nile tilapia fed with diets supplemented with the probiotic yeast compared with control, and vacuolation decreased significantly ($p<0.05$) with the increase of probiotic yeast *S. cerevisiae* incorporation in the diets. Dietary administration of probiotics yeast *S.*

cerevisiae significantly increased the villus length, villus width and crypt depth in Nile tilapia (Jahan et al., 2021 and Salam et al., 2021). Ruiz et al. (2020) reported that the probiotic bacterium *Lactobacillus plantarum* supplemented fish's liver showed less damage of the cordonal aspect and lower congestion degree when compared with non-supplemented ones and it also improved hepatic function and promoted liver restoration in Nile tilapia. Moreover, histological analysis of the liver of sea bass fed with different levels of yeast probiotic extract showed steatosis with fat degeneration, whereas liver morphology was considerably improved with yeast probiotic extract supplementation (Panagiotidou et al., 2016). Histological analysis of liver of Nile tilapia supplemented with a probiotic yeast (*S. cerevisiae)* showed unclear hepatocytes with irregular shaped nucleus in the control and hepatocytes with regular shaped nucleus in the treatments (Ran et. Al.2015; Salam et al., 2021). The data obtained from the histopathology examination in the present study were in agreement with all the above findings, and yeast showed a significant ability to maintain the liver. Light microscopic observations revealed that the groups treated with yeast had normal hepatocytes with prominent round nuclei and relatively few spaces among the hepatocytes.

CONCLUSION

Hemato-biochemical parameters of fish can be used to evaluate the health condition of the organism. The present study indicated that fish blood parameters could be affected by probiotics. Increased blood glucose level in fish that received probiotics supplementation indicated that probiotics did not induce metabolic stress in the fish. Supplementation of yeast (*S. cerevisiae*) in diets had a positive impact on liver histology. The dietary supplementation of yeast may constitute a valuable nutritional approach towards a sustainable tilapia aquaculture. Fish performance could be improved considerably by the prophylactic use of probiotics as biological control agents. However, the exact relation between probiotics and fish immune system is not yet well understood. Further investigations into the effects of probiotics on physiological and immunological responses in Nile tilapia are necessary. In future research, emphasis should be given on molecular analyses of endogenous microbiota and subsequent effects on gene expression in response to probiotics application.

CONFLICT OF INTEREST

The authors have no conflict of interests. The authors themselves are responsible for the content of paper.

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FISH DIVERSITY IN THE RIVER NARSUNDHA AT TARAIL, KISHOREGANJ, BANGLADESH

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ABSTRACT

The River Narsundha is a tributary of the River Old Brahmmaputra and is connected with a number of Rivers, Beels and *Haors* of Kishoreganj district. The diversity of fish fauna in the River Narsundha was studied during January 2012 to December 2017. About 114 fish species which could be grouped in 10 Orders, 27 Families and 68 Genera were recorded and identified in this investigation. Among the 114 species, 78 were found to belong to the Order Cypriniformes, 17 to Perciformes, 5 to Clupeiformes, 4 to Channiformes, and 2 to Synbranchiformes, Tetraodontiformes and Beloniformes and a single species was found from the Order Syngnathiformes, Anguilliformes and Cyprinodontiformes. No exotic species was recorded. Only 19 endangered fish species were recorded. The number of commercial fish species was 33 that of rare species was 19. Among the 114 fish species, 26 spawn in the river, and the remaining 88 in the floodplain. Fishes from seven trophic guilds like planktivore, omnivore, carnivore, herbivore, invertivore, molluscivore and benthivore were recorded. Diversity of the reproductive guild of fishes was six that included broadcaster, guarder, nest builder, phytophil, phytolithophil and psammophil. Thus, fish species diversity is high in River Narsundha comprising members from diverse trophic and reproductive guilds.

Keywords: Fish, guild, habitat, trophic, reproduction, river

INTRODUCTION

Bangladesh, a land of rivers, is criss-crossed by 761 rivers that cover about 8% of the area of the country. These rivers and their tributaries total about 24,140 km (Rashid 1991) in length having rich and extensive fishery resources. The main rivers are the Meghna, Brahmaputra-Jamuna and Ganges-Padma, which have numerous tributaries and distributaries. The country abounds in large varieties of fish species including 260 freshwater fish species, 24 species of freshwater prawns, 475 species of marine fish, 36 species of marine shrimps, and 18 species of exotic fishes (Rahman & Akhter 2015). Inland water resources are the main sources of fish production, but unfortunately the total open water production is coming in a fixed cycle with the continuous decreasing of most common fish species. In this context, it is highly essential to measure the fisheries

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diversity of the open water. Although river is the prime habitat for all freshwater fishes, there has been no notable research yet regarding fish diversity in the rivers of Bangladesh (Table 1). The River Narsundha is a tributary of the River Old Brahmaputra.

It rises from the River Old Brahmaputra near Hossainpur upazila of Kishoreganj district and flows easternly until it finally falls into the River Kalni/ Ghurauttra at Chowganga of Itna upazila. Length of the River Narsundha is about 57 km and width ranges from 140 to 300 m. Upstream of the river is narrow and meandering having several bends. The river is connected to *Beels*, *Haors* and rivers of Sylhet-Mymensingh basin of the River Meghna catchment. Water flow is about 7000 m^3/s and 05 m^3/s during rainy and winter seasons, respectively. There are several deep pools locally called "*Kur*" in the river. These "*Kurs*" are used as fish refuse during winter season. In the early 1960s, it was a spawning ground of many fish species like major carps, minor carps, barbs, minnows, loaches, snakeheads, perches and different species of catfishes. Running males and oozing females of *Catla*, *Cirrhinus mrigala*, *Labeo calbasu*, *Labeo gonius* and *Labeo rohita* were observed during May-June while spawning males and females of *Wallago attu* were found in the newly inundated and weed-infested shallow areas of the river. Spawning pits (*Atail*) of *Mystus aor* and *M. seenghela* on the weed-infested hard bottom surface near the bank of the river were found during the month of April-May. *Clupisoma garua*, *Eutropiichthys vacha* and *Silonia silondia* and also large *Wallago attu*, *Channa striatus* and *C. marulius* during July-August. During the winter season, *Mystus bleekeri*, *M. cavasius*, *M. tengera*, *Mastacembelus armatus*, *Macrognathus aculeatus* and *Nandus* used to be caught from the "*Kurs*" by hook and line. Moreover, many fish species like *Puntius sarana*, *W. attu*, *M. armatus*, *M. aculeatus*, *N. nandus*, *L. rohita*, *C. catla*, *C. mrigala*, *L. calbasu*, *L. gonius*, *Cirrhinus reba* and different species of *Puntius* used to be harvested by a fish trap locally called "*Hogra*" during winter. During November-December when water currents became sluggish, fixed lift nets were used to catch *Amblypharyngodon mola*, *Chela bacila*, *Chela phulo*, *Rohtee cotio*, *Puntius* spp., *Mystus* spp., *Gudusia chapra*, *Corica soborna*, *Aila coila*, *Pseudotropius arthenoides* and many other small fish species. During winter season, another fixed fish trap called "*Chunga*" made of bamboo was used to harvest *M. armatus*, *M. aculeatus*, *N. nandus*, *Heteropneustes fossilis* and *Glossogobius giuris*. During late February and March when the water level became very low, people in groups used to catch different types of fish with bare hands and an fish trap called "*Polo*". In the last 50 years, the scenario changed drastically as fish species diversity and density decreased critically. Spawning grounds of major carps and catfishes have been lost due to siltation and low flow conditions, and no large carps and catfishes are found in the river. Therefore, it is imperative to study fish species diversity in the River Narsundha to assess the current status of fish. Results of the study might help policy makers and development partners to formulate conservation strategies for the River Narsundha.

Table 1: Fish diversity in Inland waters of Bangladesh

Area/Waterbody/River	Family	Genus	Species	Source
River Madhumati, Kalna	29	68	92	Rahman *et al*. 2014
Rivers & Estuaries of Khulna Division	80	182	281	Rahman & Akhter 2009
Sundarban	67		225	Akhter & Rahman 2008
River Jamuna-Brahmaputra	27	83	156	Rahman & Akhter 2007
Exotic fish species	16	57	93	Akhter & Rahman 2007
Rivers & Estuaries of Chittagong Division	72		240	Rahman & Akhter 2006
River Halda, Hathazari	22	53	72	Rahman *et al*. 2006
Lake Kaptai, Rangamati	25	47	73	Haldar *et al*. 2002
Rivers Rajshahi Division	-	-	158	Hossain *et al*. 2002, Halls 1998
River Karatoa, Bagura	24	55	104	Hossain *et al*. 2002
River Chickli, Rangpur	19	39	69	Hossain *et al*. 2002
River Atrai, Dinajpur	31	74	158	Halls 1998
River Barnai, Rajshahi	30	66	119	Hossain *et al*. 2002
River Padma, Rajshahi	28	59	110	Hossain *et al*. 2002
River Gorai, Kushtia	26	54	96	Hossain *et al*. 2002
Haors and Beels, Sylhet Division	-	-	148	Ali 1997
Rivers of Bangladesh	55	145	260	Rahman 2005
River Surma, Sylhet	29		104	FAP-17, 1994
Rivers Greater Mymensingh & Tangail Districts	-	-	106	Doha 1974
Rivers of Bangladesh	-	-	133	Qureshi 1965
Greater Dhaka district	-	-	71	Bhuiya 1964
Rivers Bangladesh	-	-	107	Ahmad 1953

MATERIALS AND METHODS

Depending on the location of "*Kur*" (deep pool), nine sites were selected to collect data on fish species in River Narsundha (Table 2). Data on fish species diversity were collected mainly from the fishermen on the spot and also from the retail fish markets near the study sites. Visits to the different spots and fish markets were made in both rainy and winter seasons to collect data. Focus group discussions (FGD) were also done to collect data on fish species diversity and density from the local people. The fish species were identified preliminarily on the spot. Unidentified fish species were brought to the laboratories of the Bangladesh Fisheries Research Institute (BFRI), Mymensingh. Identification of fish species were made using a guidebook authored by Rahman (2005)

and also with the help of books authored by Bhuiyan (1964), Talwar and Jhingran (1991) and Shafi and Quddus (2001). Moreover, different reports (Jhingran 1997, Hossain 1997, BCAS 1989, Ali 1989, Haque 1989, Aguero *et al*. 1989, MPO 1987, Qureshi 1965 and Menon 1974 & 1954), journals (Hossain *et al*. 1988, Tsai & Ali 1987, Pillai & Yazdani 1977, Doha 1973, Jayaram 1962 and Hora 1951, 1935, 1921a & b) and books (Rahman & Akhter 2015, Rahman 2005, Ali 1997, Tsai & Ali 1997, Rashid 1991, Talwaer & Jhingran 1991, Ahmad 1989, Ameen 1987, Jayaram 1981, Jhingran 1975, Bhuiyan 1964, Ahmed 1953 and Hamilton 1822) were consulted to obtain information on fish species diversity, habitat use, trophic guild, reproductive guild and other aspects of fisheries of the River Narsundha.

Table 2: Details of data collection sites on the River Narsundha, Tarail, Kishoreganj during early winter

Sampling Sites	Depth (m)	Width (m)	Tidal influence	Erosion	Vegetation
Chowganga	3.0-3.5	300	X	√	X
Dikdair	3.0-3.5	300	X	X	X
Damiha	2.5-3.0	250	X	√	X
Tarail	2.0-2.5	200	X	X	X
Sachail	2.0-2.5	180	X	X	X
Karamshi	3.0-3.5	250	X	√	X
Guzadia	1.0-1.5	250	X	√	X
Chartaljanga	1.0-1.5	200	X	√	X
Nilganj	0.8-1.0	200	X	X	X

RESULTS AND DISCUSSION

A check list of collected fish species was prepared. One hundred and fourteen (114) riverine fish species belonging to 68 Genera, 27 Families and 10 Orders were recorded and identified from the River Narsundha during the study period (Table 3). Out of them, one species belonged to the Family Syngnathidae, Anguillidae, Belonidae, Hemirhamphidae, Cyprinidontidae, Psilorhynchidae, Clariidae, Heteropneustidae, Chacidae, Amblycipitidae and Mugilidae, two to the Family Synbranchidae, Tetraodontidae, Notopteriodae and Nandidae; three to the Family Clupidae, Mastacembelidae, Gobiidae and Centropomidae; four to the Family Channidae and Siluridae; five to the Family Anabantidae; six to the Family Schilbeidae; seven to the Family Sisoridae; nine to the Family Cobitidae; twelve to the Family Bagridae and 36 to the Family Cyprinidae.

The total number of carps, barbs and minnows was 36, while that of catfishes was 33 (Table 3). The River Narsundha harbors 33 fish species of commercial importance while number of rare species was 19. The numbers of riverine, floodplain and migratory species were 34, 30 and 50, respectively. Among the 114 fish species, 26 use river as the spawning habitat while the remaining species use floodplain as the spawning habitat.

Fishes from seven trophic guilds were recorded from the River Narsundha, such as, planktivore (39), omnivore (15), carnivore (40), herbivore (2), invertivore (8), molluscivore (, 2) and benthivore (8). Diversity of reproductive guild of fishes was six that included broadcaster (17), guarder (11), nest builder (9), phytophil (30), phytolithophil (35) and psammophil (12). Results revealed that the fish species diversity, thus, was found to be high in River Narsundha comprising members from diverse trophic and reproductive guilds (Table 3).

Table 3: Fish species diversity (n=114) in River Narsundha, Tarail, Kishoreganj, Bangladesh

Sl.	Family/scientific name	Bangla name	Fish-base name	NH	Status	BG	RG	TG
	Syngnathiformes 1*							
	Syngnathidae (Pipe Fishes) 1**							
1	*Microphis deocata* (Bleeker) 1***	Kumirer Khil	Deocata pipe fish	RS	NC	RB	Bc	C
	Anguiliiformes 2							
	Anguillidae (Freshwater eels) 2							
2	*Anguilla bengalensis* Gray& Hardwicke 2	Bamosh	Indian longfin eel	FS	NC/R	FB	Ph	M
	Synbranchiformes 3							
	Synbranchidae /Amphipnoidae (Mud eels) 3							
3	*Monopterus /Amphipnops cuchia* (Hamilton) 3	Kuchia	Cuchia/ Mud eel	FS	NC/R	FB	Ph	C
4	*Ophisternon bengalensis* McClelland 4	Bamosh	Cuchia/ Mud eel	FS	NC/R	FB	Ph	C
	Tetraodontiformes 4							
	Tetraodontidae (Puffer fishes) 4							
5	*Tetraodon cutcutia* (Hamilton) 5	Potka	Common puffer fish	FS	NC	FB	Pl/Gr	C
6	*Tetraodon fluviatilis* (Hamilton)	Potka	Common puffer fish	FS	NC	FB	Pl/Gr	C
	Beloniformes 5							
	Belonidae (Gars) 5							
7	*Xenentodon cancila* (Hamilton) 6	Kaikka/ Kakila	Niddle fish	FS	Ci	RB	Ph	C
	Hemirhamphidae (Half-beaks) 6							
8	*Hyporhamphus gaimardi* (Valenciennes) 7	Ek Thota	Niddle fish	FS	NC/R	RB	Ph	C
	Cyprinodontiformes 6							
	Cyprinodontidae (Top-Minnows) 7							
9	*Aplocheilus panchax* (Hamilton) 8	Kanpona	Blue panchax	FS	NC	FB	Ph	I
	Channiformes 7							
	Channidae (Snakeheads) 8							
10	*Channa marulius* (Hamilton) 9	Gajar/ Gajal	Great snakehead	FS	Ci	FB	Gr	C
11	*Channa orientalis* (Schneider)	Raga/ Cheng	Walking snakehead	FS	NC/R	FB	Gr	C

Sl.	Family/scientific name	Bangla name	Fish-base name	NH	Status	BG	RG	TG
12	*Channa punctatus* (Bloch)	Taki/Lata/ Okol	Spotted snakehead	FS	Ci	FB	Gr	C
13	*Channa striatus* (Bloch)	Shol	Snakehead	FS	Ci	FB	Gr	C
	Cypriniformes 8							
	Psilorhynchidae 9							
14	*Psilorhynchus balitora* (Hamilton) 10	Balitora	Balitora minnow	FS	NC	RB	Ps	B
	Cyprinidae (Carps, barbs, minnows, etc) 10							
15	*Amblypharyngodon mola* (Hamilton) 11	Mola	Mola carplet	FS	Ci	FB	Ph	P
16	*Aspidoparia jaya* (Hamilton) 12	Piali/Jaya	Jaya	RS	NC	RB	Ph	P
17	*Aspidoparia morar* (Hamilton)	Piali/Morar/Morari	Minnow	RS	NC	RB	Ph	P
18	*Barilius barila* (Hamilton) 13	Barali/Koksa	Barilius	RS	NC	RB	Ph	I
19	*Barilius barna* (Hamilton)	Bani koksa	Barilius	RS	NC	RB	Ph	I
20	*Barilius bendelisis* (Hamilton)	Joia/Hiralu/Chedra	Barilius	RS	NC	RB	Ph	I
21	*Barilius tileo* (Hamilton)	Tila Koksa/ Patharchata	Barilius	RS	NC	RB	Ph	I
22	*Brachydanio rerio* (Hamilton) 14	Anju	Zebra danio	FS	NC	FB	Pl	O
23	*Catla* (Hamilton) 15	Catla	Catla	MS	Ci	RB	Br	P
24	*Chela laubuca* (Hamilton) 16	Kash khaira	Indian glass barb	MS	NC	RB	Br	O
25	*Chela cachius* (Hamilton)	Chep chela	Glass barb	FS	NC	FB	Br	O
26	*Cirrhinus mrigala* (Hamilton) 17	Mrigel/Mirka	Mrigal	MS	Ci	RB	Br	B
27	*Cirrhinus reba* (Hamilton)	Raik/Laasu/Tatkini	Minor carp	MS	Ci/R	FB	Br	B
28	*Danio dangila* (Hamilton) 18	Napati	Danio	RS	NC	RB	Ph	O
29	*Danio devario* (Hamilton)	Debari/Chebli/ Chapchela	Sind danio	FS	NC	FB	Ph	O
30	*Danio rerio* (Hamilton)	Anju	Zebra danio	FS	NC	FB	Ph	O
31	*Esomus danricus* (Hamilton) 19	Darkina/Darka	Flying barb	FS	NC	FB	Ph	O
32	*Labeo bata* (Hamilton) 20	Bata	Minor carp	MS	Ci/R	RB	Br	P
33	*Labeo calbasu* (Hamilton)	Kalbaus/Baus/ Kalia	Orange fin labeo	MS	Ci	RB	Br	B
34	*Labeo gonius* (Hamilton)	Goni/Goinnya/ Kurchi	Karia labeo	MS	Ci/R	FB	Br	P
35	*Labeo rohita* (Hamilton)	Rui/Rohu	Major carp	MS	Ci	RB	Br	P
36	*Osteobrama cotio* (Hamilton) 21	Keti/Lohasura/ Dhipali	Minnow	FS	NC	FB	Ph	P
37	*Puntius chola* (Hamilton) 22	Chala punti	Swamp barb	FS	Ci/R	FB	Ph	H
38	*Puntius conchonius* (Hamilton)	Canchan punti	Rosy barb	FS	Ci/R	FB	Ph	H
39	*Puntius gelius* (Hamilton)	Gili punti	Golden barb	FS	Ci/R	FB	Ph	O
40	*Puntius guganio* (Hamilton)	Mola punti	Glass barb	FS	Ci	FB	Ph	O
41	*Puntius phutunio* (Hamilton)	Phutani punti	Pigmy barb	FS	Ci	FB	Ph	H
42	*Puntius sarana* (Hamilton)	Sarpunti	Olive barb	FS	Ci/R	FB	Ph	O

Sl.	Family/scientific name	Bangla name	Fish-base name	NH	Status	BG	RG	TG
43	*Puntius sophore* (Hamilton)	Jait punti/ Vadipunti	Pool barb	FS	Ci/R	FB	Ph	O
44	*Puntius ticto* (Hamilton)	Tit punti	Tic-Tac-Toe barb	FS	Ci	FB	Ph	O
45	*Rasbora elanga* (Hamilton) 23	Sephatia/ Along	Bengala barb	FS	Ci/R	FB	Ph	P
46	*Rasbora daniconius* (Hamilton)	Darkina	Slender rasbora	FS	NC	FB	Ph	P
47	*Rasbora* (Hamilton)	Leuzza darkina	Gangetic scissortail rasbora	FS	NC/R	FB	Ph	P
48	*Salmostoma bacaila* (Hamilton) 24	Katari	Large razorbelly minnow	MS	Ci	RB	Br	O
49	*Salmostoma phulo* (Hamilton)	Ful chela	Finescale razorbelly minnow	MS	Ci	RB	Br	O
50	*Securicula gora* (Hamilton) 25	Ghora chela	Minor carp	MS	CN	RB	Br	O
	Cobitidae (Loaches) 11							
51	*Botia dario* (Hamilton) 26	Rani	Bengal loach	RS	NC/R	RB	Ph	O
52	*Botia lohachata* Chaudhuri	Beti/ Lohachata	Reticulate loach	RS	NC	RB	Ph	O
53	*Lepidocephalus berdmorei* (Blyth) 27	Puiya	Burmese loach	FS	NC	FB	Pl	B
54	*Lepidocephalus guntea* (Hamilton)	Gutum	Guntea loach	FS	NC/R	FB	Pl	B
55	*Nemacheilus botia* (Hamilton) 28	Balichata/ Natwa	Mottled loach	RS	NC	RB	Pl	O
56	*Nemacheilus savona* (van Hasselt)	Savon Khorka	Loach	MS	Ci	FB	Pl	B
57	*Nemacheilus corica* (Hamilton)	Koirka	Loach	MS	NC	FB	Pl	O
58	*Nemacheilus scaturigina* (McClelland)	Dari	Loach	MS	NC	FB	Pl	O
59	*Somileptes gongota* (Hamilton) 29	Ghar puiya	Gongota loach	RS	NC	RB	Pl	O
	Clariidae (Air-breathing catfish) 12							
60	*Clarias batrachus* (L.) 30	Magur	Walking catfish	FS	Ci	FB	Pl	D
	Siluridae (Butter catfishes, Freshwater sharks) 13							
61	*Ompok bimaculatus* (Bloch) 31	Kani/Boali pabda	Butter catfish	FS	Ci/R	RB	Pl	C
62	*Ompok pabda* (Hamilton)	Madhu pabda	Pabda catfish	FS	Ci/R	RB	Pl	C
63	*Ompok pabo* (Hamilton)	Pabda	Pabo catfish	FS	Ci/R	RB	Pl	C
64	*Wallago attu* (Bloch & Schneider) 32	Boal	Wallago	MS	Ci	FB	Pl	C
	Heteropneustidae (Stinging catfish) 14							
65	*Heteropneustes fossilis* (Bloch) 33	Shing/Shingi	Stinging catfish	FS	Ci	FB	Pl/Gr	D
	Chacidae (Square-head catfishes) 15							
66	*Chaca* (Hamilton) 34	Cheka	Squarehead catfish	FS	NC	RB	Nb	C
	Schilbeidae 16							
67	*Ailia coila* (Hamilton) 35	Kajuli/Baspata	Gangetic ailia	RS	Ci/R	RB	Pl	C
68	*Ailiichthys punctata* Day 36	Kajuli	Jamuna ailia	RS	Ci/R	RB	Pl	C

Sl.	Family/scientific name	Bangla name	Fish-base name	NH	Status	BG	RG	TG
69	*Clupisoma garua* (Hamilton) 37	Ghaura	Garu bacha	RS	Ci	RB	Pl	C
70	*Eutropiichthys vacha* (Hamilton) 38	Bacha	Schlbeid catfish	MS	Ci/R	RB	Pl	C
71	*Pseudeutropius atherinoides* (Bloch) 39	Batasi	Silurid catfish	MS	Ci/R	RB	Pl	C
72	*Silonia silondia* (Hamilton) 40	Shilong	Silond catfish	RS	Ci/R	RB	Pl	C
	Amblycipitidae (Torrent catfishes) 17							
73	*Amblyceps mangois* (Hamilton) 41	Kata Maach	Indian Torrent Catfish	RS	NC/R	RB	PS	C
	Bagridae 18							
74	*Aorichthys aor* (Hamilton) 42	Ayre	Long whiskered catfish	MS	Ci	RB	Nb	C
75	*Aorichthys seenghala* (Sykes)	Guizza ayre	Giant river catfish	MS	Ci	RB	Nb	C
76	*Batasio* (Hamilton) 43	Tengra	Bagrid catfish	MS	Ci	FB	Pl	C
77	*Batasio tengara* (Hamilton)	Tengra	Bagrid catfish	MS	Ci/R	FB	Pl	C
78	*Mystus bleekeri* (Hamilton) 44	Gulsha tengra	Day's mystus	MS	Ci	FB	Pl	C
79	*Mystus cavasius* (Hamilton)	Kabashi tengra	Gangetic mystus	MS	Ci/R	FB	Pl	C
80	*Mystus menoda* (Hamilton)	Ghagla/Arwari /Gang Magur	Menoda catfish	MS	NC/R	FB	Pl	C
81	*Mystus punctatus* (Hamilton)	Gagur	Bagrid catfish	RS	Ci	FB	Pl	C
82	*Mystus tengara* (Hamilton)	Bujuri tengra	Pearl catfish	FS	Ci	FB	Pl	C
83	*Mystus vittatus* (Bloch)	Tengra	Striped dwarf catfish	FS	Ci	FB	Pl	C
84	*Rama chandramara* (Hamilton) 45	Laia/Rama	Bagrid catfish	FS	Ci	RB	Pl	C
85	*Rita* (Hamilton) 46	Rita	Rita catfish	RS	Ci	RB	Pl	C
	Sisoridae 19							
86	*Bagarius* (Hamilton) 47	Baghair	Dwarf goonch	RS	Ci	RB	Nb	C
87	*Erethistes pusilus* (Müller & Troschel) 48	Kuta kanti	Sisorid catfish	RS	NC	RB	Pl	C
88	*Gagata cenia* (Hamilton) 49	Kauwa/Jungla	Sisorid catfish	RS	Ci	RB	Pl	C
89	*Gagata nangra* (Bleeker)	Gang tengra	Sisorid catfish	RS	Ci	RB	Pl	C
90	*Glyptothorax telchitta* (Herre) 50	Tel chitta	Sisorid catfish	RS	NC	RB	Pl	C
91	*Hara* (Hamilton) 51	Kuta kanti	Sisorid catfish	RS	Ci	RB	Pl	C
92	*Sisor rhabdophorus* (Hamilton) 52	Senoa/Sisor	Sisorid catfish	RS	NC/R	RB	Nb	C
	Clupeiformes 9							
	Notopteridae (Feather backs) 20							
93	*Notopterus chitala* (Hamilton) 53	Chitol	Clown knife fish	MS	Ci/R	RB	Nb/Gr	C
94	*Notopterus* (Pallas)	Foli	Bronge featherback	FS	Ci	RB	Nb/Gr	C
	Clupeidae (Shads, Herrings etc.) 21							
95	*Corica soborna* Hamilton 54	Kachki	Ganges river spral	MS	Ci	RB	Br	P
96	*Gudusia chapra* (Hamilton) 55	Chapila	Indian river shad	MS	Ci	RB	Br	P
97	*Gonialosa manminna* (Hamilton) 56	Goni chapila	Ganges river gizzard shad	RS	Ci	RB	Br	P

Sl.	Family/scientific name	Bangla name	Fish-base name	NH	Status	BG	RG	TG
	Perciformes 10							
	Mastacembelidae (Spiny eels) 22							
98	*Macrognathus aculeatus* (Bloch) 57	Tara baim	Lesser spiny eel	FS	Ci/R	FB	Ps	B
99	*Mastacembelus pancalus* (Hamilton) 58	Guchi	Barred spiny eel	FS	Ci/R	FB	Ps	B
100	*Mastacembelus armatus* (Lacèpède)	Shal/ Baral baim	Zig-zag eel	FS	Ci	FB	Ps	B
	Mugilidae (Mullets) 23							
101	*Rhinomugil corsula* (Hamilton) 59	Khorsul/ Kholla	Freshwater mullet	RS	Ci/R	RB	Pl	P
	Anabantidae (Climbing perches, Goramies) 24							
102	*Anabas testudineus* (Bloch) 60	Koi	Climbing perch	FS	Ci	FB	Ph	I
103	*Colisa fasciata* (Bloch & Schneider) 61	Kholisha	Giant gourami	FS	Ci/R	FB	Nb	I
104	*Colisa labiosus* (Day)	Kholisha	Thicklipped gourami	FS	Ci/R	FB	Nb	I
105	*Colisa lalia* (Hamilton)	Lal kholishal/ Boicha	Dwarf gourami	FS	Ci/R	FB	Nb	I
106	*Ctenops nobilis* (McClelland) 62	Neftani	Frail gourami	FS	NC	FB	Nb	I
	Gobiidae (Gobies, Mudskippers) 25							
107	*Brachygobius nunus* (Hamilton) 63	Bele	Goby	RS	NC	RB	Ps	C
108	*Awaous stamineus* (Cuvier) 64	Bele	Goby	RS	NC	RB	Ps	C
109	*Glossogobius giurus* (Hamilton) 65	Bailla/ Bele	Tank goby	FS	Ci	RB	Pl	C
	Nandidae (Mud perch) 26							
110	*Badis* (Hamilton) 66	Napit/ Koi bandi	Badis/Leaf fish	FS	NC/R	FB	Nb/Gr	C
111	*Nandus* (Hamilton) 67	Bheda/Mani/ Royna	Mud perch	FS	Ci/R	FB	Pl	C
	Centropomidae (Giant perch, Glass perch) 27							
112	*Chanda baculis* (Hamilton) 68	Chanda	Glassfish	FS	Ci/R	FB	Ph	O
113	*Chanda nama* (Hamilton)	Nama chanda	Elongate glassy fish	FS	Ci/R	FB	Ph	O
114	*Chanda ranga* (Hamilton)	Ranga/ Lalchanda	Indian glassy fish	FS	Ci/R	FB	Ph	O

B= Benthivore, Bc= Brood carer, BG= Breeding Group, Br= Broadcaster, C= Carnivore, Ci= Commercial, D= Detritivore, FB= Floodplain Breeders, FS= Floodplain species, Gr=Guarders, H= Herbivore, I= Invertivore, Li= Lithophils, M= Molluscivore, MS= Migratory species, NC= Non-commercial, Nb= Nest builders, NH= Natural Habitat, O= Omnivore, P= Planktivore, Pe = Pelagophils, Ph= Phytophils, Pl= Phytolithophils, Ps= Psammophils, R= Rare/Threatened species, RB= Riverine Breeders, RG= Reproductive guild, RS= Riverine species, TG= Trophic guild, * = Serial number of Order, ** = Serial number of Family, *** = Serial number of Genus.

Islam and Hossain (1983) recorded 110 species of fish from the River Padma near Rajshahi. Mortuza (1992) recorded 126 fish species from the River Barnai, Rajshahi. No previous statistics is available for the River Narsundha near Tarail, Kishoreganj, and conditions of riverine fisheries catering to this river were obviously poor condition in comparison with those of the Padma, Brahmaputra-Jamuna, Halda and other rivers of Bangladesh. The number of species recorded in the present study was less than the species recorded by Rahman and Akhter (2007) and Halls (1998) probably due to the unavailability of some vulnerable and endangered species. Again, this reduction of fish species was also associated with the siltation of the river (BWAPDA 2002, UN 1995), irrigation and over-fishing (Ali 1997). The Narsundha hugely affected by water fluctuation every year. In the rainy season the water level increases to up to 10 m and in the dry season it declines sharply to 0.5-2.0 m, which affect the fish species diversity and density in this river. Besides, only 19 endangered fish species were found in River Narsundha among which 54 which were in the IUCN declared list of endangered fish (IUCN Bangladesh 2000). In these circumstances, it is essential to take immediate action for habitat improvement of River Narsundha to protect fish biodiversity.

Due to loss of breeding and nursery habitats, many fish species are unable to breed in the wild, consequently fish diversity has decreased in the River Narsundha. Disappearance of fish species is the ultimate consequence of habitat loss and over-fishing (Ali 1997). Results on threatened and disappeared fish species agree with the reports of IUCN (2000), Hussain (1997), Ali (1997) and Rahman (2005). Reports stated that 63 fish species disappeared locally from 38 rivers of the Dhaka Division. It is also reported that 10 fish species are going to disappeared from the rivers and *Haors* of Netrakona district very soon (Ali 1989 and1997).

The River Narsundha does not get sufficient water flow from its source and water flow decreases to a critical level during winter season. Low water flow, siltation, sand bars, over-exploitation and obstructed migration routes are the main causes of fish disappearance from the River Narsundha. Fish fauna of the River Narsundha is poorly explored and hence an intensive survey is necessary. Fish diversity is decreasing due to habitat degradation and thus restoration of degraded habitats is needed. The water quality parameters of the River Narsundha are suitable for living, thriving, growing and spawning of different species of fishes (Table 4). No sign of water pollution was observed during the study period but siltation and low water flow were recorded. During the winter season, water of the River Narsundha is used for crop irrigation with the help of a large number of low lift pumps.

Table 4: Water quality parameters of the River Narsundha, Tarail, Kishoreganj, Bangladesh

Site	O_2 (mg/l)	CO_2 (mg/l)	NH_3 (mg/l)	Alkalinity (mg/l)	Hardness (mg/l)	Conductivity (μS/m)	pH	Temp. (^oC)
Chowganga	5.6	0.30	0.00	6.0	4.3	150	7.2	29
Dikdair	5.8	0.20	0.00	7.1	4.6	110	7.6	28
Damiha	5.8	0.20	0.00	7.0	4.5	120	7.5	28
Tarail	4.2	0.20	0.00	7.0	4.5	120	7.3	28
Sachail	5.1	0.20	0.00	6.1	4.4	120	7.4	28
Karamshi	5.6	0.30	0.00	5.6	4.2	110	7.1	28
Guzadia	5.8	0.30	0.00	5.5	4.1	120	7.2	28
Chartaljanga	5.5	0.20	0.00	5.5	4.0	110	7.1	28
Nilganj	5.0	0.20	0.00	5.1	4.2	110	7.0	28

CONCLUSION

Fish diversity in Bangladesh has been declining gradually in the last five decades and the situation is critical now due to habitat degradation, siltation, low water flow and over-fishing. This is true also for the River Narsundha in Kishoreganj district. Erosion and siltation in the rainy season and low flow in the dry season are the main causes of fish habitat degradation. Considering the cost of dredging, it is recommended to dredge a stretch of about 10 to 15 km of the river, which will act as a natural fish sanctuary. Natural water flow is a critical factor in conserving fish habitats. Therefore, it is necessary to maintain natural water flow to prevent drying up of the river. In this connection, it is recommended to implement Water Policy for Transboundary Rivers (UN Water Convention 1997, United Nations Environment Programme 1993, World Bank Water Resources Management 1993, Biodiversity Convention 1992, UNESCO World Heritage Convention 1972, Helsinki Rules 1966) to get natural flow from shared rivers originating in India or elsewhere. For the rehabilitation of threatened fish species and conservation of germplasm, it is suggested to undertake research on induced breeding of endangered fish species. To rehabilitate and enhance over-exploited wild stocks, it is also suggested to stock resident fish species in the respective rivers. It is further recommended to undertake detailed studies on geo-morphological, ecological and fishery status of the River Narsundha in order to formulate guidelines for the management, protection and, conservation of fish habitats, fish and fisheries.

DISCLAIMER

The views expressed in this paper represent those of the authors and not necessarily those of the Bangladesh Fisheries Research Institute (BFRI) and the Krishi Gobeshona Foundation (KGF).

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Short Communications

IMPACT OF ORGANIC AMENDMENTS AND SHADE LEVELS ON THE GROWTH OF MINT

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ABSTRACT

The effect of three organic amendments and shading on mint growth was studied in an experiment at Sylhet Agricultural University. Three organic amendments such as cow dung, vermicompost and trichocompost and two shade levels, full shading and semi-shading were the treatments. The treatment combination of trichocompost and semi-shading produced the tallest plants, highest leaf and stem numbers in mint.

Keywords: Cowdung, mint, shade level, trichocompost**,** vermicompost

INTRODUCTION

The mint (*Mentha arvensis* Linn) plant, a member of the Lamiaceae family, is used as a medicinal herb containing terpenoids, alcohol, rosmarinic acid, and phenolics. The plant's leaves also have a pleasant scent (e.g., Akram *et al.*, 2011; Wei et al., 2023). It serves as a spice and flavoring ingredient for food, as a home medicine, and in some industries.

There has been a lot of research in how light affects the production of essential oils in plants (Dudai et *al.*, 1992). Mint thrives in shade, while it can handle morning heat, the foliage can wilt in the hot afternoon sun. Mint plants require little care to be strong and healthy. According to Li *et al.* (1995), thyme's highest concentration of essential oil and thymol and myrcene occurred in direct sunshine. Philippine mint (*Mentha cordfolia*) plants cultivated under 2% full sunlight produced 28% less oil (Cantoria and Cuveas, 1974).

According to Kaewseejan *et al*. (2015), using vermicompost coupled with chemical fertilizer increased plant height, oil percentage, and essential oil in two species of mint, *Mentha piperitha* (pepper mint) and *M. arvensis* (Japanese mint). Plant height and shoot weight were maximum with vermicompost (Roy *et al.*, 2010). In comparison to vermicompost alone, the combination of chemical fertilizer and vermicompost increased the leaf area and shoot weight of common beans. In terms of root and shoot dry weight, the number of leaves per plant, the area of the leaves, and the mean plant height, Chand *et al.* (2004) found that organic fertilizer had a significant impact on the growth of mint.

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Rahman and Asad (2013) found that cow dung application resulted in the maximum plant height, number of branches per plant, number of leaves per plant, leaf length, 1000-fresh leaf weight, and fresh yield and the lowest with no manures applied (control). According to Cappellari *et al.* (2017), peppermint (*Mentha piperita*) plants with strains of helpful rhizobacteria infiltrated branches had volatile organic compound emissions that were around three times higher than in control. These findings indicate that both light and organic compounds would have a big impact on the growth and quality of mint. The present work was designed to study the effect of shading and nutrient management on mint.

MATERIALS AND METHODS

Experimental site

The research was conducted at the nursery of the Department of Agroforestry and Environmental Science, Sylhet Agricultural University, Sylhet during the summer season (April to July) of 2019. The location of the site is about 5 kilometers northeast of Sylhet city with 24^{o}54´N to 33^{o}67´N latitude and 91^{o}54´to 95^{o}88´E longitude and elevation of 27 m above the sea level (Das *et al.*, 2022). The soil at the experiment site is highly acidic (Rahman *et al.* (2003). From May to September, the area has a subtropical environment with high temperature, frequent rainfall, and high humidity. Mint seedlings were collected from a local commercial nursery (Shoukhin Nursery), Khadimnagar Union, Sadar Upazila, Sylhet. The soil, used for this experiment, was collected from near the Department of Agroforestry and Environmental Science, Sylhet.

Experimental Design and Layout

The experiment was set up on the cornice of a building in pots (top diameter 37 cm, bottom diameter 22 cm, depth 31 cm) filled with the experimental soil. . The pots were laid out in a randomized complete block design (RCBD, factorial) with 3 replications. The organic matter treatments were: O1= cow dung (CD), O2= vermicompost Vm) and O3= trichocompost (Tc), and the shading treatments were: S1= full shade (4×100 lux) and S2= semi-shade (125×100 lux) where intensity of full sunlight was 790×100 lux. Nine plants for each replication were transplanted after four weeks.

Data collection and analysis

Plant growth parameters such as, plant height, no. of leaves and stems per plant at 50, 80 and 110 DAT (days after transplanting) were recorded. The Statistics 10 software was used for statistical analysis of the data and tests of significance.

RESULTS AND DISCUSSION

Effect of organic substances on growth of mint plant

Organic substances significantly influenced plant growth parameters (Table 1). The maximum plant height (20.73 cm), no. of leaves (209.83/plant) and no. of stems (42.27/plant) were observed at 110 DAT with Tc while the shortest plants (14.40 cm) were found with CD which were similar in height as those produced with Vm (15.31). The lowest total no. of leaves were produced (151.17/plant) with CD at 50 DAT and 80 DAT and CD also gave the lowest no. of stems (25.99/plant). Thus, Tc gave the best

results in plant growth parameters which might have been due to the production of growth regulatory material and phytohormones such as, indole acetic acid (IAA) with the active chemical auxin produced by a number of microorganisms, including PGPR (plant growth promoting rhizobacteria) (Lynch, 1985). Roy *et al.* (2010) found that plant height and shoot weight were highest with vermicompost. Rahman and Asad (2013) observed that plant height, no. of branch/plant, no. of leaf/plant, leaf length, 1000- fresh leaf weight, and fresh yield were the highest with cow dung application and lowest without manure.

Table 1: Effect of organic substances on the growth performance of mint plant

Treatment	Plant height(cm)			No. of leaves/plants			No. of stems/plants		
Organic substances	50DAT	80DAT	110DAT	50DAT	80DAT	110DAT	50DAT	80DAT	110DAT
O_1	7.50b	10.57b	14.40b	68.33c	104.50b	151.17c	19.67c	23.58c	25.99c
O_2	7.02b	10.02b	15.13b	82.83b	138.17a	173.00b	24.78b	29.88b	34.72b
O_3	9.13a	14.82a	20.73a	95.33a	150.00a	209.83a	26.33a	33.92a	42.27a
Level of significance	**	**	**	**	**	**	**	**	**
$LSD_{(0.05)}$	0.91	1.22	1.24	12.02	16.85	8.70	1.12	1.82	3.49

Note: Figures similar to the letters do not differ significantly. * Significant at 5% level, ** Significant at 1% level, O_1 = cow dung, O_2 = vermicompost, O_3 = trichocompost; DAT = Days after transplanting, LSD = least significant difference.

Effect of shade level on the growth of mint plant

Plant height per pot was significantly influenced by shading level except at 50 DAT (Table 2). Taller plants (18.42 cm), and greater no. of leaves (217.33plantand stems (40.56/palnt) were produced at 110 DAT with the semi-shade condition. Full shading decreased plant height (15.09 cm), no. of leaves (138.67/plant) and no. of stems (28.09/plant).Thus, mint grew better with semi-shading than with full shading. The oil yield of Philippine mint (*Mentha cordfolia*) was 28% lower in plants grown under 2% of full sunlight plants than in plants grown under 25% of full sunlight (Cantoria and Cuveas, 1974).

Table 2: Effect of shade level on the growth of mint plant

Treatment	Plant height(cm)			No. of leaves/plants			No. of stems/plants		
Shade level	50DAT	80DAT	110DAT	50DAT	80DAT	110DAT	50DAT	80DAT	110DAT
S_1	8.97a	11.49a	15.09b	77.33a	108.00b	138.67b	20.19b	24.37b	28.09b
S_2	6.80b	12.11a	18.42a	87.00a	153.78a	217.33a	27.00a	33.89a	40.56a
Level of significance	**	NS	**	NS	**	**	**	**	**
$LSD_{(0.05)}$	0.74	0.99	1.02	9.81	13.76	7.10	0.91	1.49	2.85

Note: Figures similar to the letters do not differ significantly. * Significant at 5% level, ** Significant at 1% level, S_1 = full shade, S_2 = semi-shade, DAT = days after transplanting, LSD = least significant difference.

Interaction effect of organics and shade

The combined effect of different organic substances and shade levels was significant except at 80 DAT. The tallest plants (24.33 cm) were produced at 110 DAT by the combination of trichocompost and semi-shade (O_3S_2) while the lowest plant height (14.00 cm) was found with the cowdung and full shade combination (O_1S_1) (Table 3). The highest no. of leaves (285.00/plant) was produced at 110 DAT by the combine effect of trichocompost and semi-shade (O_3S_2), where the lowest no. of leaves were produced (128.00/plant) by the cow dung-full shade (O_1S_2) combination which was similar as that (134.67) with trichocompost and full shade (O_3S_1) (Table 3). The maximum no. of stems were produced (53.33/plant) at 110 DAT by the combine effect of trichocompost and semi-shade (O_3S_2) and the lowest was observed (23.97/plant) by the cowdung-full shade combination (O_1S_1). (Table 3). Thus, the combination of trichoderma and semi-shade level gave the best results in terms of growth parameters of mint. *Trichoderma spp* mediated production of phytohormones was the primary driver of mint growth. By decreasing the canopy temperature by 2-4°C in comparison with exposure to full sunshine, it is possible to boost water retention capacity and encourage plant growth (Guyot *et al.*, 1996; Muschler, 2002; Vaast *et al.*, 2006; Geromel *et al.*, 2008). Similar results were observed for *Gynura procumbens* by Robi *et al.* (2023), who found good leaf and stem growth.

Table 3: The growth of the mint plant is influenced by the combine effect of organic substances and shade levels

Treatment Interaction	Plant height(cm)			No. of leaves/plants			No. of stems/plants		
	50DAT	80DAT	110DAT	50DAT	80DAT	110DAT	50DAT	80DAT	110DAT
O_1S_1	7.00bc	10.00b	14.00d	62.33c	85.00d	128.00e	19.67c	22.17d	23.97d
O_1S_2	8.00b	11.13b	14.80cd	74.33bc	124.00c	174.33c	19.67c	25.00c	28.00cd
O_2S_1	7.97b	10.17b	14.13d	80.00b	127.33bc	153.33d	20.23c	24.43d	29.10c
O_2S_2	6.07c	9.87b	16.13bc	85.67ab	149.00b	192.67b	29.33b	35.33b	40.33b
O_3S_1	11.93a	14.30a	17.13b	89.67ab	111.67c	134.67e	20.67c	26.50c	31.20c
O_3S_2	6.33c	15.33a	24.33a	101.00a	188.33a	285.00a	32.00a	41.33a	53.33a
Level of significance	**	NS	**	NS	*	**	**	**	**
$LSD_{(0.05)}$	1.29	1.72	1.76	16.99	23.83	12.30	1.58	2.57	4.94
CV (%)	8.97	8.02	5.77	82.167	10.01	3.80	3.68	4.86	7.91

Note: Figures similar to the letters do not differ significantly. * Significant at 5% level, ** Significant at 1% level, O_1 = cow dung, O_2 = vermicompost, O_3 = trichocompost; S_1 = full shade, S_2 = semi-shade, DAT = days after transplanting, CV = co-efficient of variation, LSD = least significant difference.

CONCLUSION

This study showed that a combination of trichocompost and semi-shading would be suitable for enhancing the vegetative growth of mint.

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CONFLICTS OF INTEREST

The authors declared there were no conflict of interests

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VALUE CHAIN AND PROFITABILITY ANALYSIS OF TEA IN SELECTED AREAS OF GREATER SYLHET DISTRICT

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ABSTRACT

The study was carried out to examine the problems in the value chain map of tea and profitability of tea production and suggest probable solutions. Primary data were collected from 35 tea estates of the Sylhet and Moulvibazar districts interviewing 70 tea garden workers, 5 blenders, 10 wholesalers, 10 retailers, 10 tong owners and 5 consumers to elucidate socio-economic status of tea garden workers and economic activities of other stakeholders involved in the tea value chain. The study identified seven actors in the tea value chain, i.e., tea producers, brokers, blenders, wholesalers, retailers, tong owners and consumers. It was observed that out of the sale price of Tk. 198/kg of tea, value addition by producers, brokers, blenders, wholesalers, retailers, and tong owners amounted to Tk. 82, 2, 57, 65, 45 and 35, respectively. Among the actors, producers added the highest value (28.7%) of total value addition followed by wholesalers (22.8%). The estimated price spread of Tk. 169 was very high indicating a low efficiency of the tea marketing system. Price and demand fluctuation, strong competition in storing tea in warehouses, lack of proper market information and lack of use of modern technologies were the main problems faced by the producers while price instability, lack of credit, lack of storage facility and high transportation costs were the main problems faced by tea traders. Tea production was observed to be profitable with a benefit-cost ratio of 1.21. Some policy recommendations were made to overcome the problems in the tea business and increase its profitability.

Keywords: Bangladesh, factors, profitability, tea, value chain

INTRODUCTION

Tea is one of the oldest beverage crops widely consumed all over the world. Of the three most popular morning drinks namely coffee, cocoa and tea that human has discovered tea is the most popular in the world and Bangladesh is no different. It is served as the morning drink for nearly two-thirds of the world population daily (Nasir, 2012). Bangladesh is the 10th largest tea producing country contributing 1.68% of the global tea production, ranks 15th in terms of export with only a 0.58% of the world tea export and 16th in terms of consumption of tea in the world (Ali et al., 2014; Hossain, 2015).

Tea production in Bangladesh hit a two-year high in 2018 due to the Government's recent move to lower the bank interest rate for the growers and increased replantation activities in the last seven years (The Daily Star, 2019). Despite the production gain, export of tea

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in recent years has declined. Moreover, Bangladesh had to import tea in recent times, e.g., in 2017, 6.98 million kg of tea was imported as a result of increasing domestic demands but decreasing rate of production growth.

In a small country like Bangladesh tea cultivation is one of the most important agro-based industries which plays a significant role in the national economy. It is a major cash crop of Bangladesh which has a significant contribution to employment generation, export earnings as well as import substitution and poverty reduction in rural areas. It is a very labour-intensive industry providing occupation to around 140,000 people, of whom 50% are women. More than half a million people are directly or indirectly involved in tea production and trade through forward and backward linkages (Bangladesh Tea Board-BTB, 2016). About 0.81% of the GDP of Bangladesh, equivalent to about Tk. 1775 million, is earned annually by exporting about 18 million kg of tea (Kamruzzaman et al, 2015). In 2019, Bangladesh exported only 163 thousand kg of tea to only six countries they are Bahrain, China, Kuwait, Pakistan, U.A.E and USA. In 2018 the export amount was 228 thousand kg (Statistical Bulletin of Bangladesh Tea Board, 2019).

Value chain analysis is a tool to assess the inter-linkage of different actors involved in the production, processing and distribution of a product (Furuholt and Matotay, 2011). According to Kaplinsky and Morris (2001), the value chain describes the full range of activities which are required to bring a product or service from conception, through the different phases of production (involving a combination of physical transformation and the input of various producer services), delivery to final consumers, and final disposal after use.

The Bangladesh Tea Research Institute (BTRI) at Srimangal conducted a number of studies on various aspects of tea cultivation such as diseases, yield, effects of climate change, adoption of clones etc. (BTRI, 2016). Sabur (2002) studied marketing systems and price behaviors in the tea industry of Bangladesh. Tea production is an agro-based export oriented industry in Bangladesh. The stages of tea manufacturing are: withering, rolling, fermenting, drying, sorting, grading, tea tasting, packaging etc. Reddy (1991) analyzed the trends of tea in the global market. UNICEF (2009) made an assessment of the situation of children and women in the tea gardens of Bangladesh which showed that the situation of children and women in some areas was considerably worse that of their peers in Bangladesh as a whole. Hossain (2012) found that each household of the tea plantation workers had more than one or two earning members. Hossain (2017) studied the changing face of global and local entrepreneurship related to the tea industry in the Sylhet region and observed that overseas entrepreneurships such as the James Finlay Company played a decisive role in the success of the tea industry whereas there were too many ups and downs in the local entrs for the tea industry and opined that massive investments were needed to increase tea production to meet domestic demands and retain the export market.

Latif et al. (2012) found that that in Pakistan BCR (benefit cost ratio) for tea was 0.94 indicating a no loss no profit situation for the tea growers. Prakash et al (2013) conducted a study on marketing efficiency of tea under different supply chains and found that supply of tea through auction center was predominant. Tran et al (2016) found that tea production can create value along the chain contributing to overall food security. Khoi et

al (2015) conducted a study on the value chain approach in the Vietnam tea industry and concluded that farmers should firstly change their cultivation technique with a view to increasing tea yield and improving the quality of tea.

Research on production, profitability and value chain issues of tea production in Bangladesh is rare and many policy level questions still are remain unanswered. This study was designed to help the policy makers better understand the issues of financial profitability as well as development of the value chain map of tea. The specific objectives of the study were i) to examine the value chain map and estimate the value addition by different actors; ii) to estimate profitability of tea production; and iii) to identify the problems in relation to production and value addition of tea and suggest some policy options for necessary improvement.

MATERIALS AND METHODS

Study area and sample size

The study was conducted in the tea growing Sylhet and Moulvibazar districts of the Sylhet Division of northeastern Bangladesh. Primary data were collected from 35 tea estates where 110 stakeholders (i.e., workers, blenders, wholesalers, retailers, tong owners and consumers) were interviewed (Table 1). Field survey method was followed to collect primary data from the sample stakeholders using a structured questionnaire. Focus group discussions (FGD) were conducted to collect group information and cross-check the data and information. The questionnaire was constructed and pre-tested for necessary modifications before starting the data collection. Besides, secondary information sources in the form of handouts, reports, publications and notifications etc. having relevance and similarity with this study were also used. Primary data were collected from January to May 2021. The findings from these interviews were incorporated in profitability analysis and value chain mapping.

Table 1: Sample distribution for the study

Stakeholders	Sample size
Tea estates	35
Workers	70
Blenders	5
Wholesalers	10
Retailers	10
Tong owners	10
Consumers	5
Total	145

Analytical techniques

For analyzing the data, a combination of descriptive and statistical techniques was used as follows: To examine the socioeconomic characteristics of different stakeholders' descriptive statistics like sum, average, percentage, ratio etc. were used. Value addition

activities of different stakeholders involved in the value chain were also calculated using descriptive statistics.

In order to develop the value chain map of tea, descriptive statistics with the support of flowchart was used. Value additions at different stages of tea marketing by different stakeholders were estimated using the following equations (Acharya and Agarwal, 1998):

Gross margin = Sales price – Production cost/Purchase price; Value addition = Gross margin – Marketing cost; Price spread = Retailers' sales price – Farmers' sales price; Producers' share to consumers' Tk. = (Farmers' sales price/ Retailers' sales price) x 100.

Production price was considered for tea producers and purchase price was considered for other stakeholders. Production cost was calculated by summing up both variable and fixed costs.

To address the problems in relation to production, value addition and marketing of tea, the problem confrontation index (PCI) was used. For production related problems four selected items were computed. For problems related to value addition and marketing three items were calculated respectively using the following formula:

$PCI = (P_s \times 3) + (P_m \times 2) + (P_l \times 1) + (P_n \times 0)$

where, Ps = number of respondents with severe problems (weight assigned as 3); Pm = number of respondents with moderate problems (weight assigned as 2); Pl = number of respondents with low problems (weight assigned as 1); and P_n = number of respondents with no problems (weight assigned as 0).

Since tea is a perennial crop, cost and return was calculated using the budgeting procedure first, then long-term analysis approach was applied, namely discounting future streams of expenses and benefits to their present values (Bakhsh, *et al.*, 2006). Gross return, total cost, net present value and benefit cost ratio were calculated to evaluate profitability of tea production. Gross return was calculated by multiplying the total volume of output of an enterprise by the average price in the harvesting period (Dillon and Hardaker, 1993). Net present value (NPV) was calculated by taking the difference between the present value of cash inflows and the present value of cash outflows over a period of time. Benefit cost ratio (BCR) was estimated as a ratio of gross return to gross cost. The formulas used for calculating profitability are given below:

$GR = \sum\{(Q_n \times P_n)/(1+i)^n\}$; $TC = \sum\{(P_n \times X_n + TFC_n)/(1+i)^n\}$; NPV= GR-TC (discounted); $BCR = \sum\{GR_n/(1+i)^n\}/\sum\{TCn/(1+i)n\}$.

where, GR= goss return (Tk./ha); TC = total cost (Tk./ha); NPV= net present value (Tk./ha); BCR = benefit cost ratio; TVC = total variable cost (Tk./ha); TFC = total fixed cost(Tk./ha); Q = quantity produced (unit/ha); and P= per unit price (Tk./kg); X= quantity of input; n= number of years; i= interest.

Taking interest rate at i= 10 percent (bank rate) the value of denominator $(1+i)^n$ is calculated as follows:

$(1+i)^0 = (1+0.10)^0 = 1$

$(1+i)^1 = (1+0.10)^1 = 1.10$

$(1+i)^2 = (1+0.10)^2 = 1.21$

$(1+i)^n = (1+0.10)^n = N$

Since prices of year four are used the above figures are used for discounting of future values (Hussain, 2006). For Year- 1 to 3, the figures of gross return and total cost were compounded using the following formulas:

$GR = \{R_n (1+i)^n\}$

$TC = \{C_n (1+i)^n\}$

In order to assess the individual effect of different inputs on gross return of tea production the Cobb-Douglas production function was used due to theoretical and economic considerations. In this study the following model was used:

$Y = b_0 \times X_1 b_1 \times X_2 b_2 \times X_3 b_3 \times X_4 b_4 \times X_5 b_5 \times X_6 b_6 \times X_7 b_7 \times U$

The logarithmic form of the model is as follows:

$\ln Y = \ln b_0 + b_1 \ln X_1 + b_2 \ln X_2 + b_3 \ln X_3 + b_4 \ln X_4 + b_5 \ln X_5 + b_6 \ln X_6 + b_7 \ln X_7 + U$

where, Y = gross return (Tk./ha); X_1 = age of tea workers;X_2 = size of land (ha);X_3 = human labour (Tk./ha); X_4 = fertilizer cost (Tk./ha); X_5 = electricity cost (Tk./ha); X_6 = irrigation cost (Tk./ha); X_7 = pesticide cost (Tk./ha);a = intercept; b_0 = constant/intercept; b_0, b_1 …… b_7 = coefficients of the respective variables; and U = error term.

Contributions of the included factors in the model could be explained through the value of these coefficients.ould explain the A recommendation matrix was used to point out the facts of intervention for enhancing tea production in the study area.

RESULTS AND DISCUSSION

Socioeconomic characteristics of value chain actors

To get a complete picture of the tea value chain, socioeconomic characteristics of tea garden workers and related traders is a must to understand. Majority of the sampled workers belonged to the age group of 20-49 years and the highest number of traders was in the age group of 36 to 50 years age group. The average family of tea garden workers consisted of 4.76 members and that of tea traders was 4.69. Most of the tea garden workers were illiterate while 80% tea traders were literate (Table 2). Homestead area for the tea garden workers had an average size of 3.36 decimal. Among the tea traders blenders and wholesalers had the highest average daily income. Most of the workers had a monthly income of Tk. 8001-13000 which is about 40 percent of total sampled workers and the workers expend mostly on food (rice) among all the other items which about 64.2% of total expenditure.

Table 2: Socioeconomic characteristics of value chain actors in tea production and trade in Bangladesh

Particulars	Workers	Traders
Age group	20-49	36-50
Family Size	4.76	4.69
Literacy rate	54.3%	80%

Source: Author's estimation based on field survey, 2021

Value chain analysis of tea production

Actors involved in tea value chain

The actors involved in tea value chain were found to be the tea estates, brokers, bidders, blenders, wholesalers, retailers, tong owners and consumers (Figure 1). Unlike for any other agricultural product where the value chain may begin from primary stakeholders, for tea the value chain starts from the tea estates. Tea estates produce and process tea. They sell tea in two different ways, either through the auction market or through direct sale from the respective tea estates. Almost 90% of tea in Bangladesh is sold through the auction market.

Value addition by different actors

The sales price of tea producers was Tk. 196/kg while the production cost was Tk. 114/kg indicating that the value addition by tea producers was Tk. 82/kg. The marketing cost of producers was Tk. 36/kg, so the net margin was Tk. 46/kg. Value addition by brokers was Tk. 2/kg. The purchase price of the blenders was Tk. 196/kg of tea, the marketing cost was Tk. 29/kg and sales price was Tk. 255/kg. So, the value addition by blenders was Tk. 57/kg and the net margin was Tk. 28/kg of tea. The purchase prices of wholesalers and retailers were Tk. 255 and 320/kg of tea and their marketing costs were Tk. 28 and Tk. 18 per kg, respectively. Value additions by wholesalers and retailers were Tk. 65 and Tk. 45 and their net margins were Tk. 37 and Tk. 27, respectively, per kg of tea. The purchase price of the tong owners was Tk. 365/kg of tea, the marketing cost was Tk. 9/kg and sales price was Tk. 400/kg. So, the value addition by tong owners was Tk. 35/kg and the net margin was Tk. 26/kg.

The total value addition by all the value chain actors was Tk. 286/kg of tea. Value addition was the highest by producers (28.7% of the total value addition) followed by those by wholesalers, blenders, retailers and tong owners (Fig. 1). Table 3 showed that the tea producers' share in consumer's Tk. was 53.7% which indicated that there is scope for improvement in the efficiency of the marketing system. It is also shown in the table that price spread (Tk. 169 per kg) was very high which indicate the lower efficiency of the marketing system. More efficient marketing system for tea is required to improve the condition.

Product flow

The product flow of tea was generally simple because of the auction market. Tea estates have their own nursery for producing tea saplings, as a result there is no outside supplier. Product flow of tea starts from the tea estates. Most of the tea produced in the study area was sent to the Chattogram auction market and from there tea was distributed throughout the country by different bidders and manufacturers. The major demand centers for tea are Sylhet, Moulvibazar, Srimangal, Chattogram, Dhaka, Panchagar, Bogura, Khulna etc.

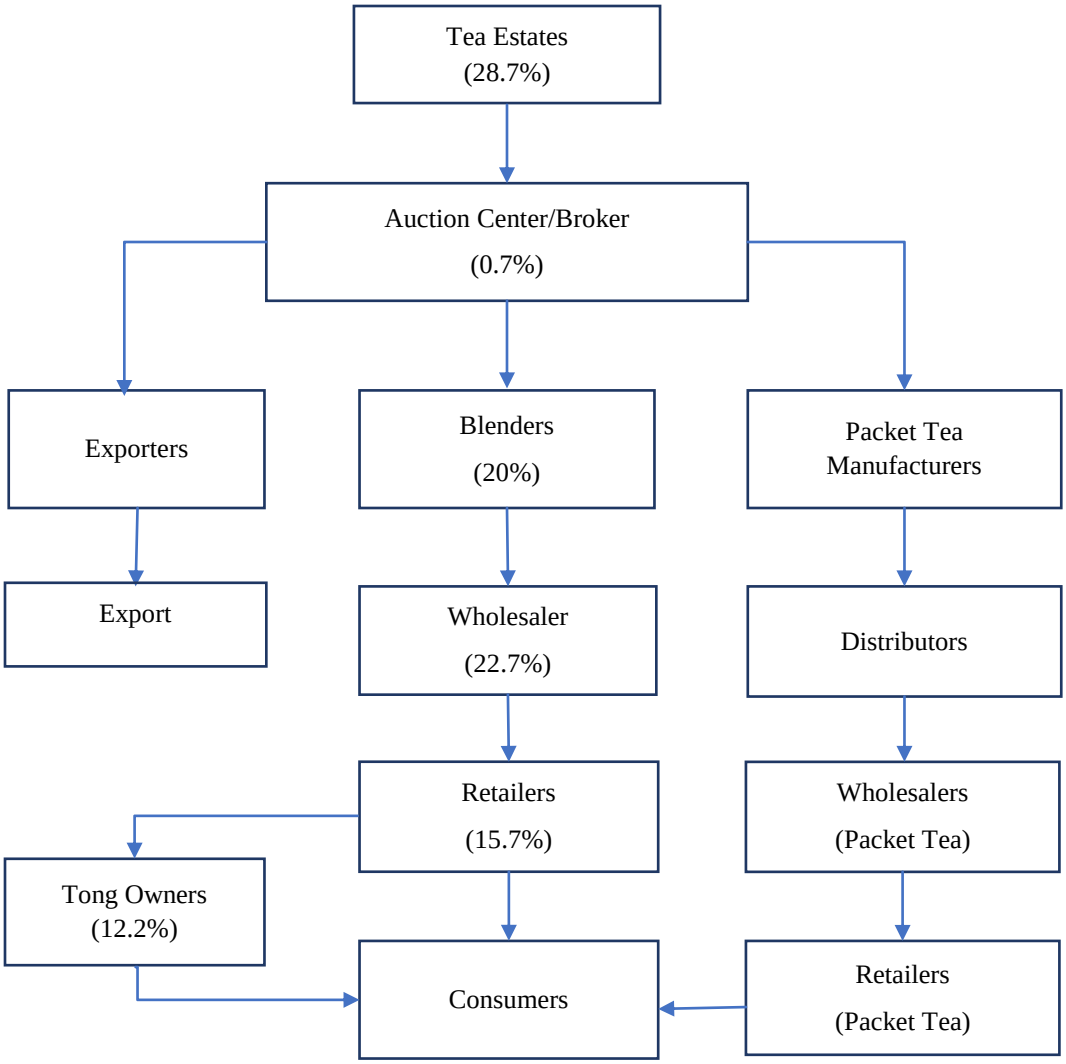

Fig. 1: Value chain map of tea production and trade in Bangladesh

Note: Figures within the parentheses indicate percentage of the total value addition

Table 3: Producers' share to consumers' price and price spread

Items	Value(Tk/kg)
a. Producer's price	196
b. Consumer's price	365
c. Producer's share (percent)	53.7
d. Price spread (b-a)	169

Source: Author's estimation based on field survey, 2021

Information flow

The marketing channel of tea is generally better structured than that for other agricultural products in Bangladesh. Information about different variables related to tea can be easily obtained. Unlike in case of other agricultural products where information flows from farmer to other stakeholders, for tea the information flow does not start from tea producers. In case of tea the main issues are grades of tea, prices for different grades, quantity of sale, present market demand, future market demand, etc. Tea estates use this information for planning future production. The bidders or blenders or manufacturers provide information about price, volume etc. to the wholesalers. The wholesalers provide information about quality, availability, price, volume etc. to the retailers. Retailers provide the same information to the tong owners and consumers.

Profitability analysis

The profitability of tea production was estimated in terms of gross return, net present value and BCR. The fixed cost item for tea production was land use cost (details in Appendix 1). The cost of production of tea and gross return were Tk. 15,38,104/ha (details in Appendix 1) and 18,57,948/ha (Table 4), respectively. Net present value was 319844 which is positive and benefit cost ratio (BCR) was 1.21which indicates that tea production was profitable.

Table 4: Profitability of tea production and trade in Bangladesh

Serial No.	Items	Amount (Tk/ha)
i.	Total present value of cost (Appendix 2)	1538104
ii.	Total Present value of gross return (Appendix 3)	1857948
iii.	Net Present Value (ii-i)	319844
iv.	BCR (discounted) (ii/i)	1.21

Source: Author's estimation based on field survey, 2021.

Factor contributions

Functional analysis was used to reveal the quantitative relationship between dependent variables and set of explanatory variables (Gujarati, 2003). The ordinary least square multiple regression was estimated to determine farm profitability and also to find out the effects of explanatory variables on the dependent variable. The multiple regression function was better in terms of expected signs and magnitudes of the coefficients, R^2 and F-values. For this study Cobb-Douglas production function has been chosen to analyze the effects of selected factors on gross return. Seven explanatory factors were included in the model to analyze the effect of explanatory variables on net return of tea production.

The constant represents the value of the composite impact of all other influencing variables that are excluded from the model. Estimated value of the regression coefficient of land size for net return of tea production was 0.256 (Table 5) which was positive and statistically significant at 5% level of probability. If land size is increased by 1% on an average net return of tea production would increase by 0.256% holding other factors constant. The regression coefficients for human labor cost, electricity cost, human labour

cost and irrigation cost were 0.407, 0.087, 0.047 and 0.264, respectively, which were positive and significant at 1%, 5% and 10% probability levels, indicating that if these variables were increased by 1%, production of tea would increase by 0.407%, 0.087%, 0.047% and 0.264%, respectively. The derived value of F for tea producers was 14.263 (Table 5) which was significant at the 1% probability level which implies that all the included explanatory variables in the model were important for explaining the variations in net returns from tea production. The R^2 value of 0.794 indicates that, about 79.4% of the total variation in net return from tea production could be explained by the explanatory variables. The returns to scale is 1.018 which, being greater than 1, indicates increasing returns to scale.

Table 5: Estimated values of coefficients and related statistics of Cobb-Douglas production function model for tea production in Bangladesh

Variables	Dependent variable = net return (Tk.)	
	Coefficient	p-value
Intercept	1.163	0.164
Age (X_1)	-0.117	0.274
Land size (X_2)	0.256**	0.037
Human labor cost (X_3)	0.407***	0.003
Fertilizer cost (X_4)	-0.028	0.259
Electricity cost (X_5)	0.087**	0.024
Irrigation cost (X_6)	0.264*	0.061
Pesticide cost (X_7)	0.047**	0.046
R^2	0.794	
F-value	14.263	
Returns to scale	1.018	

Source: Author's estimation based on field survey, 2021.

Note: ***, ** and * indicate significant at 1%, 5% and 10% probability level, respectively.

Problems of value addition, production and marketing

In case of value addition related problems lack of storage facilities was ranked number 1 with a problem facing index (PFI) value of 116 (Table 6). Other value addition related problems included inadequate skills in grading and packaging and lack of initiatives from the concerned Government organizations (ranked 2nd and 3rd with PFI scores of 108 and 96, respectively). In case of production related problems, the highest ranked problem was high price of inputs with a PFI score of 298. Pest and disease attacks presented as the least severe problem faced by the producers, with a PFI score of 229. Lack of use of modern technology and institutional credits had PFI scores of 272 and 248, respectively. Price and demand fluctuations came up as the number one marketing related problem with a PFI score of 90 while lack of proper market information, inadequate transportation and communication facilities had PFI scores of 78 and 71 ranking 2nd and 3rd, respectively (Table 6).

Table 6: Problem facing index of individual issues in tea production and marketing in Bangladesh

Issue	Extent of the problem				PFI	Rank order
	Severe (3)	Moderate (2)	Low (1)	Not at all (0)		
Production related problems						
High price of inputs	94	6	4	1	298	1
Lack of use of modern technology	82	9	8	6	272	2
Lack of institutional credits	69	16	9	11	248	3
Attack of pest and disease	60	17	15	13	229	4
Value addition related problems						
Lack of storage facility	34	6	2	3	116	1
Inadequate skills in grading and packaging	29	8	5	3	108	2
Lack of initiatives from the govt. organizations in value addition	24	9	6	6	96	3
Marketing related problems						
Price and demand fluctuation	26	5	2	2	90	1
Lack of proper market information	21	6	3	5	78	2
Inadequate transportation and communication facilities	18	7	3	7	71	3

Source: Author's estimation based on field survey, 2021

Policy recommendations

The tea producers of respective tea estates were asked about probable solutions for the problems they faced to improve conditions for tea production and making the tea business more profitable. It is also important that government and other relative organizations should take necessary steps immediately to overcome the problems faced by tea producers. Probable suggestions to solve the problems faced by tea producers are presented in Table 7 below.

Table 7: Probable solutions suggested by the actors in the tea production value chain in Bangladesh

Problem	Problems	Suggested Solutions
Input related	High price of inputs	Efforts should be taken to reduce the price of inputs
	Lack of use of modern technology	Training programs should be updated and extended by GO and NGO and Bangladesh Tea Board
	Lack of credit providing institutions	Credit should be made available under easy terms
	Attack of pest and disease	More attentions should be drawn and steps should be taken accordingly
Value addition related	Lack of storage facility	It has been proposed by the bidders, blenders and wholesalers that new storages with modern facilities should be established
	Inadequate skills in grading and packaging	Efforts should be taken to improve the quality of grading and packaging
	Lack of initiatives from the govt. organizations in value addition	Government should step in to find new ways to add more values to tea
Marketing related	Price and demand fluctuation	Provide adequate information about price and demand for different types and qualities of tea.
	Lack of proper market information	Tea market should be monitored throughout the year so that the stakeholders can sell tea with higher price when the demand is high
	Inadequate transportation and communication facilities	Better infrastructure and transportation facilities should be ensured

Source: Field survey, 2021.

CONCLUSION

Sylhet is the largest tea producing region in Bangladesh. The tea market is very competitive and special in comparison with other agricultural products of the country. The present study found that the primary actors of the tea value chain were tea producers, brokers, blenders, wholesalers and retailers, tong owners and consumers. The value chain actors in tea production and trade were prominent in their activities and tea production and trade were profitable enterprises. Among the value chain actors, tea producers added the highest amount of value and the highest net margin was also achieved by them. The marketing channel of tea in Bangladesh is dominated by brokers who play a major role in distributing tea to the local market by holding auctions and to the international buyers. High prices of inputs, lack of storage facility and price and demand fluctuation are the

major problems of production, value addition and marketing of tea. Formulation of adequate long-term plans and strategies in the fields of tea production, value addition and marketing will boost the production of tea in the country and enhance profits for the tea traders.

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Appendix 1: Cost of land for tea production in Bangladesh

Year	Particulars	Cost (Tk/ha)	% of total cost
1st Year	Cost of seedlings	98000	60.5
	Land preparation	3466	2.1
	Labor cost	43614	26.9
	Irrigation cost	574	0.4
	Organic manure	2120	1.3
	Electricity	1506	0.9
	Land rent	12608	7.8
	Total	161888	
2nd Year	Cost of seedlings	58960	50.9
	Labor cost	31140	26.9
	Irrigation cost	670	0.6
	Fertilizer	9841	8.5
	Electricity	1400	1.2
	Pesticide	931	0.8
	Land rent	12608	10.9
	Total	115740	
3rd Year	Cost of seedlings	38324	40.8
	Labor cost	30226	32.2
	Irrigation cost	580	0.6
	Fertilizer	9841	10.5
	Electricity	1500	1.6
	Pesticide	931	1.0
	Land rent	12608	13.4
	Total	94010	
4th Year	Cost of seedlings	24910	23.5
	Labor cost	56422	53.2
	Irrigation cost	600	0.6
	Fertilizer	8841	8.3
	Electricity	1700	1.6
	Pesticide	931	0.9
	Land rent	12608	11.9
	Total	106012	

Source: Author's estimation based on field survey, 2021.

Appendix 2: Cost of tea production in the last 30 years in Bangladesh

	Values in 2019 prices (of 4th year)	Present Value	
Years	Cost (Tk)	Weight for discounting	Cost (Tk)
Year 1	161888	1.3	210454
Year 2	115740	1.2	138888
Year 3	94010	1.1	103411
Year 4	106103	1.0	106103
Year 5	107245	1.1	97495
Year 6	106398	1.2	88665
Year 7	106946	1.3	82266
Year 8	108564	1.4	77546
Year 9	98878	1.6	61799
Year 10	107810	1.8	59894
Year 11	106018	1.9	55799
Year 12	106637	2.1	50780
Year 13	108564	2.3	47202
Year 14	98878	2.6	38030
Year 15	106715	2.8	38113
Year 16	106484	3.1	34350
Year 17	107326	3.5	30665
Year 18	108564	3.9	27837
Year 19	98878	4.2	23542
Year 20	106483	4.6	23148
Year 21	106449	5.0	21290
Year 22	106105	5.5	19292
Year 23	108564	6.1	17797
Year 24	98878	6.7	14758
Year 25	107274	7.4	14496
Year 26	106856	8.1	13192
Year 27	107734	8.9	12105
Year 28	108564	9.8	11078
Year 29	98878	10.8	9155
Year 30	106548	11.9	8954
Total	3223979		1538104

Source: Author's estimation based on field survey, 2021.

Appendix 3: Gross return from tea production for 30 years (Per hectare)

	Values in 2019 prices (of 4th year)		Present Value	
Years	Production (Kg)	Gross return	Weight for discounting	Gross return
Year 1	-	-	-	-
Year 2	-	-	-	-
Year 3	-	-	-	-
Year 4	757	149886	1.0	149886
Year 5	812	160730	1.1	146118
Year 6	820	162455	1.2	135379
Year 7	861	170469	1.3	131130
Year 8	896	177325	1.4	126661
Year 9	819	162092	1.6	101308
Year 10	1071	212023	1.8	117791
Year 11	1071	212023	1.9	111591
Year 12	1024	202729	2.1	96538
Year 13	1024	202729	2.3	88143
Year 14	575	113784	2.6	43763
Year 15	1238	245171	2.8	87561
Year 16	1071	212023	3.1	68395
Year 17	1024	202729	3.5	57923
Year 18	1024	202729	3.9	51982
Year 19	575	113784	4.2	27091
Year 20	1238	245171	4.6	53298
Year 21	1071	212023	5.0	42405
Year 22	1024	202729	5.5	36860
Year 23	1024	202729	6.1	33234
Year 24	575	113784	6.7	16983
Year 25	1238	245171	7.4	33131
Year 26	1071	212023	8.1	26176
Year 27	1024	202729	8.9	22779
Year 28	1024	202729	9.8	20687
Year 29	575	113784	10.8	10536
Year 30	1238	245171	11.9	20603
Total	24282			1857948

Source: Author's estimation based on field survey, 2021.

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