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Vol. 46 **No. 1** **June 2020**

CONTENTS

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BIOEFFICACY OF THREE INDIGENOUS PLANT EXTRACTS AGAINST *CALLOSOBRUCHUS CHINENSIS* L. (BRUCHIDAE: COLEOPTERA)

S.M.S. AHMED, M.A. HOSSAIN, A.B. SIDDIQUE AND M. A.A. BACHCHU*
Department of Entomology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, Bangladesh

Abstract

Results of the evaluation on bioefficacy of three indigenous plant extracts for toxicity and residual effects against the pulse beetle, *Callosobruchus chinensis* L. (Bruchidae: Coleoptera) showed that the plant extracts had toxic and residual effects for controlling pulse beetle. Mortality and residual effects were statistically different among the plant extracts and doses applied. Neem extract showed the highest adult mortality (36.78%) whereas eucalyptus extract showed the lowest (22.75%). The order of the toxicity of three plant extracts was found as neem > custard apple > eucalyptus. Mortality was found directly proportional to the hour after treatments which increased with the progress of time. Between two solvents, acetone solvent possessed the highest toxicity (mortality 32.95%) but methanol showed the lowest toxicity (mortality 30.56%). The residual toxicity was evaluated on the basis of egg laid, adult emergence, seed infestation and weight loss caused by the insect. The highest residual toxicity was found in neem extract with acetone while the lowest in eucalyptus extract with acetone. Neem extract with acetone and custard apple extract with methanol solvent were found effective to toxic and residual effects against pulse beetle of three plant extracts applied.

Key words: Bioefficacy, *Callosobruchus chinensis*, Indigenous plant extracts, Toxicity, Residual effect

Introduction

Pulse is one of the best sources of plant protein and plays a pivotal role in the diet of common people of developing countries like Bangladesh (Darmadi-Blackberry *et al.* 2004). The cultivated area of pulse crops in Bangladesh is 10,11,000 acres with annual production of 7,26,000 metric tons (BBS 2016). This amount is not sufficient to meet up the demand. One of the major hindrance to increase the pulse production is the damage of pulse grain from insect infestation in storage. Among the storage insect pests, bruchids are known to cause both quantitative and qualitative losses to pulses. Several species of pulse beetle are reported to attack pulses in storage. Among them *Callosobruchus chinensis* L. is a major and destructive species which causes up to cent per cent losses of pulses in storage (Bhalla *et al*. 2008, Jat *et al*. 2013).

*Corresponding author: adnan_hstu@yahoo.com, adnan@hstu.ac.bd

The beetles breed rapidly in the storage of the tropical and subtropical environment. *C. chinensis* larvae can easily penetrate into the gram and feed the endosperms resulted the infested grains unsuitable for human consumption; deteriorate nutritional value and loss of seed viability (Deeba *et al.* 2006). This pest is a serious problem at small farmers' level, village traders and average households where storage conditions are poor and inadequate. The extent of damage varied with different kinds of legumes, duration of exposure time, storage facilities and other factors associated with seeds. Therefore, it is utmost necessary to control this pest. To protect the stored pest, fumigation with synthetic chemicals like methyl bromide and phosphine is an effective method being used only in the warehouses. This technique is expensive to rural farmers, and impractical in the primitive nature of storage in many of the villages (Kim *et al.* 2003). Synthetic insecticides have been used for a long time with serious drawbacks such as insecticide resistance in pest insects, hazards to human and the environment, destruction of non-target organism, outbreak of secondary pests and human health hazards (Lee *et al.* 2001).

Several researchers studied on the insecticidal properties of plant materials (Shukla *et al.* 2007, Kirubal *et al.* 2008). These botanical materials can be used as an alternative to synthetic pesticides. Due to several advantages of plant-derived pesticides, like biodegradable, less harmful to environment, non toxic to other animals etc., are becoming popular for the management of insect pests worldwide (Yuya *et al.* 2009). Although, a number of authors have conducted research on toxicity, repellency, antifeedant activity of botanical pesticides against field and stored grain insects (Bachchu *et al.* 2003, 2013, Cosimi *et al.* 2009, Saroukolai *et al.* 2010, Ghani *et al.* 2014, Hossain *et al.* 2014), more investigations are needed to explore the pesticidal properties of indigenous plant materials. Therefore, the present research was undertaken to determine the toxicity and residual effects of three indigenous plant extracts, namely neem, custard apple and eucalyptus on *Callosobruchus chinensis* in a laboratory condition.

Materials and Methods

The present experiments were conducted to evaluate the toxicity effects of three indigenous plants extracts against *Callosobruchus chinensis* in the ambient laboratory conditions (28 ± 5°C, and 75 ± 10% RH) of the Department of Entomology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, Bangladesh during May to August 2017.

Collection and preparation of botanical extracts: The fresh plant leaves of neem (*Azadirachta indica),* custard apple (*Annona reticulata*), and eucalyptus (*Eucalyptus*

camaldulensis) were collected from the HSTU campus, Dinajpur and surrounding areas. Collected leaves were kept for 7 days in the laboratory for air drying followed by one day sun drying before making powder. They were powdered separately by an electric grinder (Nova Blackberry Blender, AD 999, Bangladesh) in the laboratory and passed through a 60-mesh sieve to get fine powder. For preparation of botanical extracts, 100 gm of each plant powders were mixed separately with 300 ml of acetone and methanol solvents separately in a 500 ml conical flask. Then the mixture was stirred (600 rpm) for 30 minutes and then allowed to shaking in the shaker machine. The mixture was filtered through a filter paper (Whatman no. 1). The solvents were allowed to evaporate by vacuum rotary evaporator (Lab Tech EV311H Rotary Evaporator, China) and finally semi solid crude extracts were obtained. The crude extracts were then preserved in tightly corked vials (8 ml) and stored in a refrigerator (4^{o}C) for further experimental use.

The insect culture: Healthy gram seeds were collected from the local market of Dinajpur town to culture the insect. They were sterilized and cooled at 8 - 10% moisture content level and preserved in a big air tight plastic container for experimental use. Adults of *C. chinensis* were collected from naturally infested gram seeds of the local market of Dinajpur town. The beetles were cultured in separate glass jar (500 ml) with gram seed in ambient laboratory conditions (28 ± 5°C, and 75 ± 10% RH). Approximately, 200 adults were released in each jar (500 ml) containing 500 gm of seeds. Then the jars were closed with pieces of white muslin cloth and tightly fixed with the help of rubber bands to avoid skip out the beetles. The jars were then left undisturbed for a period of 7 days for oviposition. Then the beetles were separated carefully from the seeds by sieving and seeds along with eggs left undisturbed for emergence of adult. The newly emerged adults were collected and again introduced in new seeds allowed for oviposition in different jars for maintaining stock culture and the stock culture of the test insect was continued during the experimental period. Only 1 to 2 days old adult of *C. chinensis* were used for the experiments purposes.

Toxicity test: To evaluate the direct toxic effect of different botanical extracts against the pulse beetle, different concentrations (5.0, 2.50, 1.25, 0.625, and 0.3125%) along with control treatments were made. One ml liquid of each dose was dropped separately on Petri dishes (60 mm) with the help of pipette. Before conducting study, a pilot experiment was done to obtain the appropriate doses (data not shown). Then the plant extracts were covered uniformly the whole area of the Petri dishes. The Petri dishes were air dried for 30 minutes. Control Petri dishes were treated with acetone and methanol solvents only. Two-day-old 10 adult beetles of *C. chinensis* were released in each Petri dish. Three replications were made for each concentration of plant extracts including control

treatment. The Petri dishes were then kept without food and insect mortality was recorded at 24, 48, and 72 hours after treatments (HATs). The percentage of mortality was corrected using Abbott's formula (Abbott 1987).

$$P = \frac{p' - C}{100 - C} \times 100$$

where, P = Percentage of corrected mortality, P' = Observed mortality (%), C = Mortality (%) at control.

Residual toxicity test: For residual effect of plant extracts on insect mortality, three different concentrations (5, 2.5 and 1.25%) for each plant extract were mixed with gram seed separately (1 ml/50 g seed) followed by air dried for 30 minutes. Five pairs one-day-old adult beetles were released into the glass bottle (250 ml) containing plant extracts treated gram seed and bottle was covered with perforated lid. Three replications were maintained for each of the concentration of each plant extracts separately along with control. All treated bottles were kept at ambient room temperature (28 ± 5°C) in the laboratory for the oviposition. After 7 days, dead and alive beetles were removed from each container and number of eggs was counted. After emergence, adult beetles and seed holes were counted and recorded. Inhibition rate (% IR) of adult emergence was calculated by the following formula:

% IR = Cn – Tn/Cn × 100 (Shukla *et al.* 2007).

where, Cn = Number of insect on control treatment, Tn = Number of insect on treated treatment.

Statistical analysis: The collected data were statistically analyzed by completely randomized design (CRD) using MSTAT-C statistical software. Before analyzing, the percentage insect mortality was corrected by Abbott's formula. The treatment mean values were adjusted by Duncan's Multiple Range Test (DMRT). The insect mortality data were also subjected to probit analysis.

Results and Discussion

Direct toxicity of three plant extracts against Callosobruchus chinensis: Insect mortality of three plant extracts differed significantly ($p<0.05$, $F=603.25$, $df=2$) among the treatments (Table 1). The results indicated that the highest insect mortality was found in the neem extracts (28.89, 38.33 and 43.11%) while the lowest in eucalyptus extracts (15.28, 23.89 and 29.10%) at 24, 48 and 72 HATs, respectively. The insect mortality increased propor-tionally with the time interval. Average adult mortality revealed that the highest mortality was recorded in the neem extract (36.78%) but the lowest in eucalyptus

extract (22.75%). The insect mortality was significantly ($p<0.05$, $F=1319.05$, $df=5$) different among all the doses of plant extracts including the control (Table 1). The highest dose (5.0%) indicated the highest insects mortality (45.00, 60.00 and 65.32%) and the lowest dose (0.3125%) revealed the lowest mortality (12.78, 21.11 and 26.78%) at 24, 48, 72 HATs, respectively. The insect mortality was also increased proportionally within time intervals. Average insects mortality of different HATs also indicated that the highest insect mortality (56.77%) was found in the maximum dose (5.0%) while the lowest (20.22%) in the minimum (0.3125%) dose. The interaction effects of plant extracts, their doses at different HATs were deferred significantly ($p<0.05$, $F=26.05$, $df= 10$) among three plant extracts at different doses where the lowest mortality (0.16%) was recorded in untreated control (Table 1). The highest insect mortality (61.80%) was found in the neem plant extract followed by custard apple (61.76%) at the highest doses (5.00%) while the lowest (9.46%) mortality was found in eucalyptus plant extracts at the lowest dose (0.3125).

The interaction effects of three plant extracts, solvents, doses and times are presented in the Table 2. There was a significant ($p<0.05$, $F=45.53$, $df=10$) different among the toxicity of the plant extracts when applied against the adult pulse beetle. The percentages of insects mortality at 24, 48 and 72 hours after treatment indicated that the highest mortality (73.60) showed in the neem extracts with acetone solvent at highest dose but eucalyptus extract showed lowest mortality (7.810) at lowest dose. Conversely, custard apple extracts with methanol solvent recorded insect mortality 70.00% at highest dose while eucalyptus extract with methanol solvent recorded insect mortality 11.11% at the lowest dose.

The present results agree with other workers. The major active constituent of neem is azadirachtin, which is well known for its antifeedant, toxic and growth regulating effects on insects (Saxena *et al.* 2004). Azadirachtin ingredient is also active on insects, including stored grain pests, aphids, caterpillars and mealybugs (Morgan 2009). Reports showed that black pepper (*Piper nigrum*), ceylon cinnamon (*Cinnamonnum zealanicum*), black cardamom (*Amonum subulatum*), nutmeg (*Myristica fragnans*), black cumin (*Nigella sativa*), turmeric (*Curcuma longa*) and red pepper (*Capsicum frutescens*) caused highest mortality of *C. maculatus* (Hossain *et al*. 2008). Lawati *et al*. (2002) cited that among the extracts of eight local plants in Oman, seeds of *A. squamosa* caused the highest mortality of beetles within 24 hours of exposure in methanol extracts. The other extracts that caused high mortality were *A. nilotica, C. juncea, M. communis* and *S. aegyptica* in methanol and *B. saca, J. dhofarica, S. aegyptica* and *A. indica* in ethanol (Hossain *et al*. 2008).

Table 1. Mortality of pulse beetle at different HATs (Interaction of plant extracts, doses and times).

	Botanicals/ doses (%)	Mortality (%) at different HATs			Average mortality (%)
		24	48	72	
Plants effects	Neem	28.89 a	38.33 a	43.11 a	36.78 a
	Custard apple	27.78 b	37.50 b	41.92 b	35.73 b
	Eucalyptus	15.28 c	23.89 c	29.10 c	22.75 c
	LSD	1.108	0.783	0.639	0.722
	CV (%)	9.830	5.010	3.580	4.840
	p-value	0.000	0.000	0.000	0.000
Doses effects	Dose 1(5.000)	45.00 a	60.00 a	65.32 a	56.77 a
	Dose 2(2.500)	36.11 b	48.33 b	55.27 b	46.57 b
	Dose 3(1.250)	29.44 c	39.44 c	44.66 c	37.85 c
	Dose 4(0.625)	20.56 d	30.56 d	35.72 d	28.94 d
	Dose 5(0.3125)	12.78 e	21.11 e	26.78 e	20.22 e
	Control	0.00 f	0.00 f	0.50 f	0.16 f
	LSD	1.570	1.110	0.900	1.020
	CV (%)	9.830	5.010	3.580	4.840
	p-value	0.000	0.000	0.000	0.000
Interaction effects of plants and doses	Neem-dose 1	50.00 a	65.00 a	70.40 a	61.80 a
	Neem-dose 2	41.67 b	55.00 b	60.35 b	52.34 b
	Neem-dose 3	35.00 c	45.00 d	50.30 d	43.43 d
	Neem-dose 4	26.67 d	36.67 e	41.92 f	35.08 e
	Neem-dose 5	20.00 e	28.33 f	35.20 h	27.84 f
	Custard apple-dose 1	50.00 a	65.00 a	70.30 a	61.76 a
	Custard apple-dose 2	41.67 b	55.00 b	60.25 b	52.31 b
	Custard apple-dose 3	35.00 c	45.00 d	50.20 d	43.40 d
	Custard apple-dose 4	25.00 d	35.00 e	40.15 g	33.39 e
	Custard apple-dose 5	15.00 f	25.00 g	30.10 j	23.36 g
	Eucalyptus-dose 1	35.00 c	50.00 c	55.25 c	46.75 c
	Eucalyptus-dose 2	25.00 d	35.00 e	45.20 e	35.07 e
	Eucalyptus-dose 3	18.33 e	28.33 f	33.48 i	26.72 f
	Eucalyptus-dose 4	10.00 g	20.00 h	25.10 k	18.36 h
	Eucalyptus-dose 5	3.33 h	10.00 i	15.05 l	9.460 i
	Control	0.00 h	0.00 j	0.50 m	0.16 j
	LSD	2.713	1.918	1.566	1.770
	CV (%)	9.830	5.010	3.580	4.840
	p-value	0.000	0.000	0.000	0.000

HAT = Hour after treatment, within column values followed by different letter(s) are significantly different by DMRT at 5% level of probability.

Table 2. Interaction effects of plant extracts and solvents against pulse beetle mortality at different HATs.

Name of the plant extracts	Solvents	Doses (%)	Mortality (%) at different HATs			Average mortality (%)
			24	48	72	
Neem	Acetone	5.000	60.00 a	80.00 a	80.80 a	73.60 a
		2.500	53.33 b	70.00 b	70.70 b	64.68 c
		1.250	50.00 c	60.00 c	60.60 c	56.87 e
		0.625	40.00 d	50.00 d	50.50 d	46.83 h
		0.3125	30.00 f	40.00 e	40.40 e	36.80 j
	Methanol	5.000	40.00 d	50.00 d	60.00 c	50.00 g
		2.500	30.00 f	40.00 e	50.00 d	40.00 i
		1.250	20.00 g	30.00 f	40.00 e	30.00 l
		0.625	13.33 h	23.33 h	33.33 g	23.33 n
		0.3125	10.00 i	16.67 j	30.00 h	18.89 p
Custard apple	Acetone	5.000	40.00 d	60.00 c	60.60 c	53.53 f
		2.500	33.33 e	50.00 d	50.50 d	44.61 h
		1.250	30.00 f	40.00 e	40.40 e	36.80 j
		0.625	20.00 g	30.00 f	30.30 h	26.77 m
		0.3125	10.00 i	20.00 i	20.20 i	16.73 p
	Methanol	5.000	60.00 a	70.00 b	80.00 a	70.00 b
		2.500	50.00 c	60.00 c	70.00 b	60.00 d
		1.250	40.00 d	50.00 d	60.00 c	50.00 g
		0.625	30.00 f	40.00 e	50.00 d	40.00 i
		0.3125	20.00 g	30.00 f	40.00 e	30.00 l
Eucalyptus	Acetone	5.000	40.00 d	50.00 d	50.50 d	46.83 h
		2.500	30.00 f	40.00 e	40.40 e	36.80 j
		1.250	20.00 g	30.00 f	30.30 h	26.77 m
		0.625	10.00 i	20.00 i	20.20 i	16.73 p
		0.3125	3.33 h	10.00 k	10.10 j	7.810 r
	Methanol	5.000	30.00 f	50.00 d	60.00 c	46.67 h
		2.500	20.00 g	30.00 f	50.00 d	33.33 k
		1.250	16.67 g	26.67 g	36.67 f	26.67 m
		0.625	10.00 i	20.00 i	30.00 h	20.00 o
		0.3125	3.33 h	10.00 k	20.00 i	11.11 q
Control		0.000	0.001 j	0.001 l	0.001 k	0.001 s
LSD		-	3.837	2.713	2.215	2.503
CV (%)		-	9.830	5.010	3.580	4.840

HAT = Hour after treatment, within column values followed by different letter(s) are significantly different according to DMRT at 5% level of probability.

*Probit analysis of direct toxic effect***:** The LD_{50} values indicated that neem plant extract (1.72 mg/cm^2) with acetone showed the highest toxic effect followed by the custard (2.42 mg/cm^2) apple with methanol extracts at 24 HAT (Table 3). The LD_{50} values at 48 HAT indicated that neem plant with acetone extracts (0.621 mg/cm^2) showed the highest toxic effect followed by the custard apple with methanol solvent (1.248 mg/cm^2). Among the three extracts with two solvents, neem plant extract with acetone solvent extracts (0.37 mg) also performed the highest toxicity as compared with the LD_{50} values at 72 HAT. The Chi-square values were insignificant at 5% level of probability of different plant extracts at different HATs and mortality data did not show any heterogeneity.

Table 3. Probit mortality of different plant extracts with acetone and methanol solvent against *C. chinensis* after 24, 48 and 72 HATs.

Plant extracts used	No. of insects used	LD50 (mg/cm^2)		95% fiducial limits				χ^2 values with 3 df	
				Acetone		Methanol			
		Acetone	Methanol	Lower	Upper	Lower	Upper	Acetone	Metha-nol
				24 HAT					
Neem	30	1.717	10.036	0.812	3.631	2.647	38.043	0.220	0.089
Custard apple	30	8.679	2.470	2.187	34.435	1.319	4.626	0.712	0.016
Eucalyptus	30	6.943	17.760	3.062	15.741	3.153	100.036	0.419	0 .694
				48 HAT					
Neem	30	0.621	5.348	0.321	1.202	1.860	15.373	0.019	0.032
Custard apple	30	2.470	1.248	1.319	4.626	0.718	2.171	0.016	0.013
Eucalyptus	30	4.592	6.188	2.110	9.992	2.320	16.502	0.201	0 .768
				72 HAT					
Neem	30	0.372	2.343	0.177	0.7843	1.047	5.242	0 .242	0.277
Custard apple	30	1.397	0.621	0 .821	2.377	0.321	1.202	0.022	0.019
Eucalyptus	30	2.732	2.569	1.478	5.049	1.359	4.856	0.082	0.082

HAT = Hours after treatment, values were based on five concentrations, three replications of 10 insects each. χ^2 = Goodness of fit, Tabulated values of χ^2 = 12.838 with 3 df at 5% level of probability.

It was observed that all the plant extracts were more or less effective for controlling the pulse beetle but neem extract with acetone solvent was the most effective followed by the custard apple and eucalyptus plant extracts with acetone solvent (Table 3). The custard apple plant extracts with methanol solvent showed the highest effect followed by the neem and eucalyptus plant extracts against the pulse beetle. The results agree with other workers. They reported that acetone extract of botanicals significantly reduced the adult population of *C. chinensis* (Dwivedi and Kumari 2000 and Dwivedi and Venugopalan 2001). From the above results it was concluded that acetone extract of neem and custard apple of methanol was effective for controlling the pulse beetle. The present result agree

with the findings of Mamun *et al*. (2009) who reported that acetone extract of neem seed showed highest toxicity against stored grain pest. Rahman and Talukder (2004), reported that the different plant/weed derivatives the development of the pulse beetles, *C. maculatus* (Coleoptera: Bruchidae) feed on black gram, *Vinga mungo* seeds. Plant extracts, powder, ash and oil from several plant materials inhibited oviposition of pulse

Table 4. Residual toxicity effect of plant extracts against pulse beetle egg laid, adult emergence, seeds infestation and weight loss.

Solvents	Plant extracts	Doses (%)	Inhibition of egg laid (%)	Inhibition rate of adult emergence (%)	Inhibition of seed infestation (%)	Weight loss (%)
Acetone	Neem	5.00	84.89 d	87.38 d	88.03 d	95.00 b
		2.50	82.10 e	84.88 e	86.55 e	94.40 d
		1.25	79.11 f	80.60 f	82.34 f	94.00 e
	Custard apple	5.00	75.88 h	75.95 g	76.42 h	92.60 f
		2.50	72.56 j	72.97 i	73.33 j	92.20 g
		1.25	68.11 l	69.52 j	69.88 l	92.00 h
	Eucalyptus	5.00	63.78 n	65.83 m	65.80 n	90.60 j
		2.50	58.89 q	58.81 p	58.27 q	90.20 k
		1.25	54.67 r	52.97 q	53.58 r	90.00 l
Methanol	Neem	5.00	76.67 g	76.24 g	78.61 g	91.00 i
		2.50	73.58 i	73.68 h	75.41 i	90.60 j
		1.25	69.17 k	68.80 k	70.56 k	90.20 k
	Custard apple	5.00	90.83 a	91.62 a	92.64 a	96.00 a
		2.50	88.67 b	90.26 b	91.26 b	95.00 b
		1.25	86.17 c	88.89 c	90.04 c	94.60 c
	Eucalyptus	5.00	66.75 m	66.71 l	66.75 m	89.20 m
		2.50	61.83 o	62.65 n	61.99 o	88.60 n
		1.25	59.42 p	59.92 o	59.74 p	88.20 o
LSD		0.700	0.505	0.016	0.471	0.700
CV %		0.570	0.410	0.000	0.390	0.570

IR = Inhibition rate, within column values followed by different letter(s) are significantly different by DMRT at 5% level of probability.

beetle. Other researcher reported that the oviposition of pulse beetle markedly reduced when stored seeds were treated with different botanical extracts like neem, jatropha, sweetsop and bishkatali (AL-Lawati *et al*. 2002 and Mollah and Islam 2002). It is reported that acetone extract of botanicals significantly reduced the adult population of *C. chinensis* (Dwivedi and Kumari 2000 and Dwivedi and Venugopalan 2001). Sathyaseelan

et al. (2008) also cited that some kinds of botanicals with ethanol extracts reduced the adult emergence of *C. chinensis* in green gram seeds.

Residual toxicity of three plant extracts against pulse beetle: The results of residual toxic effects of neem, custard apple and eucalyptus plant extracts on *C. chinensis* are presented in Table 4. The highest inhibition of egg laid (90.83%) was calculated at 5.0% in custard apple with methanol solvent while the lowest (54.67%) in eucalyptus with acetone extracts at 1.25%. The highest inhibition of adult emergence (91.62%) and seed infestation (92.64%) was also recorded in custard apple at highest dose but the lowest inhibition of adult emergence (52.97%) and seed infestation (53.58%) in eucalyptus with acetone solvent extracts at lowest doses (1.25%). The highest of weight loss (96%) was also found in custard apple with methanol extract.

From the present study it is clear that the botanical plant extracts of neem, custard apple and eucalyptus extracts used as pesticides have a great economic and environmental importance. Among the three botanical extracts with two solvents, neem extracts with acetone solvent and custard apple extracts with methanol solvent showed the highest toxic and residual effect against pulse beetle. The findings of the present investigation revealed the broad spectrum toxic properties of neem, custard apple and eucalyptus extracts against the adult of pulse beetle.

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OCCURRENCE OF PROTOZOAN PARASITES OF *CHANNA PUNCTATUS* IN BANGLADESH

FATIMA NAHAR KABITA[1], MD. AMINUL ISLAM BHUIYAN[1*], ZANNATUN NAHAR JHINU[1] AND RITA PARVEEN[2]
[1]*Department of Zoology, University of Dhaka, Dhaka-1000, Bangladesh*
[2]*BANBEIS, Nilkhet, Dhaka-1000, Bangladesh*

Abstract

Three species of myxozoa (*Henneguya chaudhuryi, Henneguya bengalensis* and *Myxobolus* sp.) and 3 species of ciliophora (*Trichodina pediculus, Epistylis lwoffi* and *Apisoma piscicolum*) and two actinosporean stages of myxoza were identified. Some 51.72% of total host fishes which were found infected with at least one of the above mentioned parasites with average load of 95.93±41.53 per infected host. High percentage (98.05) of *C. punctatus* possessed myxozoan infection and 1.95 had chiliophoran infection. The highest prevalence of parasitic infection was observed in host sample collected from Faridpur district and lowest (33.33%) in fish sample collected from Mymensingh. The association of parasitic infection of *H. bengalensis* and *Myxobolus* sp. with study areas was found statistically significant (p=0.024 and 0.049, respectively). Protozoan parasites were most abundant in gills of hosts. Shannon Diversity Index indicated that host fishes were not infested by more parasites and the parasite community was poorly diverged in all study sites. However, Simpson's Diversity showed that, parasites community was moderately diverged in host fishes collected from Mymensingh district and in rest of the areas parasite community was poorly diverged.

Key words: Protozoa parasites, Prevalence, Diversity, *Channa punctatus*

Introduction

Parasitic infections are the major issues causing low productivity in fish farming as well as in open water bodies (Dogiel 1961, Kayis *et al.* 2009). Because of its inherent difficulty in perusal compared to other larger parasitic fauna, protozoan parasite exploration has been neglected for a long time. Both ecto- and endoparasitic protozoa occupy a very important role in fish growth retardation and nutrition that most often results in multiple fish pathogenesis as being one of the hazardous threats to fish health (Enayat 2011). Most common pathogenesis can be detected as: (i) massive destruction of skin and gill epithelium of fish with internal damage and (ii) loss of appetite and ability to swim properly; sometimes it can be fatal even in the case of mild infection (Enayat 2011). Parasites generally increase in abundance and diversity in more polluted waters

*Corresponding author: aminul_zool@du.ac.bd

which indicates the quality of the water (Poulin 1992). Apart from mortality and treatment expenses, growth retardation during disease outbreak can cause economic loss that influences against expansion of aquaculture (Omeji *et al.* 2011).

A considerable number of studies have been conducted on the protozoan parasites of *C. punctatus* in Bangladesh (Sanaullah 1996, Arthur and Ahmed 2002, Miah *et al.* 2013, Deb *et al*. 2015, Akther *et al.* 2018). From these studies a limited knowledge about the species identification, taxonomy, distribution and prevalence of protozoan infection was found in the host. However, precise information regarding prevalence and intensity of the protozoan parasites of this host was not mentioned. Therefore, it seems to be essential to know the current status of protozoan infestation in the wild fish of Bangladesh. The present study was an attempt to assemble a base line data of protozoan parasites of one of the important fish species, namely of *Channa punctatus* in Bangladesh.

Materials and Methods

Collection of host sample: The host species *Channa punctatus* was sampled from the freshwater bodies of Kishoreganj (Kuliar char- 24°10′40″ N, 90°50′57″ E), Mymensingh (Ishawrganj-24°41′16″ N, 90°35′58″ E), Faridpur (Dumain union 23°32′50″ N, 89°31′22″ E) and Jashore (Purondorpur, Jhikorgacha Upazila- 23°5′51″ N, 89°5′53″ E). A total of 29 fish specimens were collected alive with the help of fishermen from mid-April, 2018 to end of the March, 2019. Sample sizes collected from each area were not equal. However, area wise sample size was: Kishoreganj-5, Mymensingh-9, Faridpur-5 and Jashore-10 fishes.

Sample preparation and examination: Immediately after collection, the external surfaces of the fish were observed carefully using a magnifying glass. Specimens were kept moist during examination by spraying them with a fine mist of water. The collected host fishes were examined as soon as possible after capture. Samples were collected from the body slime, gill slime and blood of the host fishes which are generally the best site to colonize for protozoan parasites. Smears of body slime, gill slime and blood were placed on glass slides on the spot of collection of fishes and fixed them in ethanol solution for the observation in the Parasitology laboratory, Department of Zoology, University of Dhaka.

Klein's dry silver impregnation method: It was applied to detect the presence of ciliates in body slime and gill slime. Mucus was scraped gently off gills and skin with a scalped, spread thinly on a grease-free slide, and dry rapidly. The slide was covered with a 2% aqueous solution of silver nitrate ($AgNO_3$) for 8 min. After that they were rinsed

thoroughly with distilled water and were placed facing up in a dish of distilled water and expose to bright sunlight for 1 - 2 hours. Finally, they were allowed to dry and mount with a neutral medium, Canada balsam.

Giemsa's stain after acid hydrolysis- To detect the parasites in blood sample, the slides were stained using Giemsa stain and cover slipped by DPX mountant. During this process smears were fixed in Schaudinn's fluid and rinsed well in distilled water. After that they were hydrolysed for 8 min in 1N HCl at >60^{o}C. Again, they were rinsed for several times in distilled water and stained with stocked Giemsa's stain (diluted 1:20 with water at pH 7.0 - 7.2) for about 20 min and rinsed with tap water. Then they were allowed to dry directly and finally mounted with a neutral medium, Canada balsam.

The slides were carefully examined under microscope (40x and 100x) to note the presence or absence of protozoan parasites. Counts of parasites found in selected organ were recorded. The numbers of examined parasites were counted for statistical analysis and microscopic photographs were made for identification of species with the help of 10-megapixel digital camera.

Protozoans were identified according to the description of Lom and Dyková (1992), Eiras (2002), Kalavati and Nandi (2007) and Bashě and Abdullah (2010). Some parasites could not be identified up to species level (*Myxobolus* sp.) because these were not seemed to be matched with any of the available published description. Moreover, it appeared reasonable to make detailed observation to come to a conclusion.

Calculation: Prevalence, mean intensity and abundance of infection were calculated according to Margoles *et al.* (1982). Simpson's Diversity index (Simpson 1949) was used to evaluate for both richness and abundance of parasites within the samples, which was counted by the formula: D =1/C where, C = $\sum P\,i^2$ ($Pi^2 = (Ni/N_T)^2$); here, Pi is the proportional abundance of the ith species. Shannon's Diversity index (Shannon and Weaver 1949), which measures the "information content" of a sample unit, was used to measure the diversity and it was calculated by the formula: $H = -\sum_{i=1}^{s} Pi \ln Pi$ where, Pi is the proportion of individuals found in the ith species and ln is the natural logarithm. A greater number of species and a more even distribution both increased diversity as measured by H.

The most commonly used index of evenness is that based on the Shannon - Wiener index (Pielou 1977) which was calculated by the formula: $E = \frac{H}{\ln s}$.

Margalef Index of species richness (Margalef 1958) was used to evaluate the richness of parasites species within the samples, was calculated by the formula: $R = (S-1))/\ln(n)$.

Data analysis: Statistical analyses were carried out using Microsoft Excel 2010 and IBM SPSS version 20. Fisher's Exact test (as the sample size was small fisher exact test was done instead of Chi square test) was performed and level of significance was set at $p \leq 0.05$.

Results and Discussion

During the investigation, the protozoan parasites were collected from body slime and gills but no parasites were found in blood samples of host fishes from the four study sites. A total of 1437 protozoan individuals were collected from different body parts of 15 infected *C. punctatus* (out of 29 fish examined). Among them 1409 (98.05%) were Myxozoan and 28 (1.95%) were Ciliophoran parasite. Six genera/species and two Actinosporean stages were encountered from *C. punctatus* during this study (Fig. 1). Of them there were 3 Myxozoan (*Henneguya chaudhuryi*, *Henneguya bengalensis* and *Myxobolus* sp.) and 3 Ciliophoran (*Trichodina pediculus*, *Epistylis lwoffi* and *Apisoma piscicolum*) and two Actinosporean stages of Myxozoan parasite were found (Table 1).

Among the parasites *Trichodina pediculus* was previously recorded in *C. punctatus* in Bangladesh (Deb *et al.* 2015, Akther *et al.* 2018)**.** *Henneguya chaudhuryi* (Bajpai and Halder 1982, Chaudhary *et al.* 2017) and *Henneguya bengalensis* **(**Raychaudhuri and Chakravarty 1970**)** collected from *C. punctatus* were previously recorded in India but recorded as a new locality in the present study for the first time in Bangladesh (Table 2). The rest two parasite species, namely *Epistylis lwoffi* and *Apiosoma piscicolum* were recorded first in the host fish in Bangladesh (Table 2). Additionally, two Actinosporean stages of Myxozoa were found in the present study. *Trichodina cyprinocola, T. pediculus, Trichodina* sp., *Chilodonella* sp., *Ichthyobodo* sp., *Actinophrys* sp., *Ichthyophthirius* sp. and *Myxobolus* sp. were recorded from *C. punctatus* in Bangladesh in various other studies (Deb *et al.* 2015, Miah *et al.* 2013, Akther *et al.* 2018) and *Trichodina cobitis* were found from *Channa striatus* in Bangladesh (Asmat *et al.* 2017).

The Actinosporean stage of Myxozoa had not been reported as yet in *C. punctatus* host in this locality. Wolf and Markiw (1984) discovered that an Actinosporean (triactinomyxon) is a required alternate life cycle stage of *M. cerebralis.* Similar life cycles have now been described for around 25 species assigned to the genera *Myxobolus, Henneguya, Sphaerospora, Ceratomyxa, Myxidium, Zschokkella, Theloha-nellus, Hoferellus,* and *Tetracapsula* (Kent *et al.* 2001).

Among the parasites found, *Trichodina pediculus* had the highest prevalence (24.14%) and *Apisoma piscicolum* showed the lowest prevalence (3.45%) (Table 3). Mean intensity of parasitic infection varied from 1.5±0.41 to 240±62.81 in *C. punctatus* (Table 3). Of

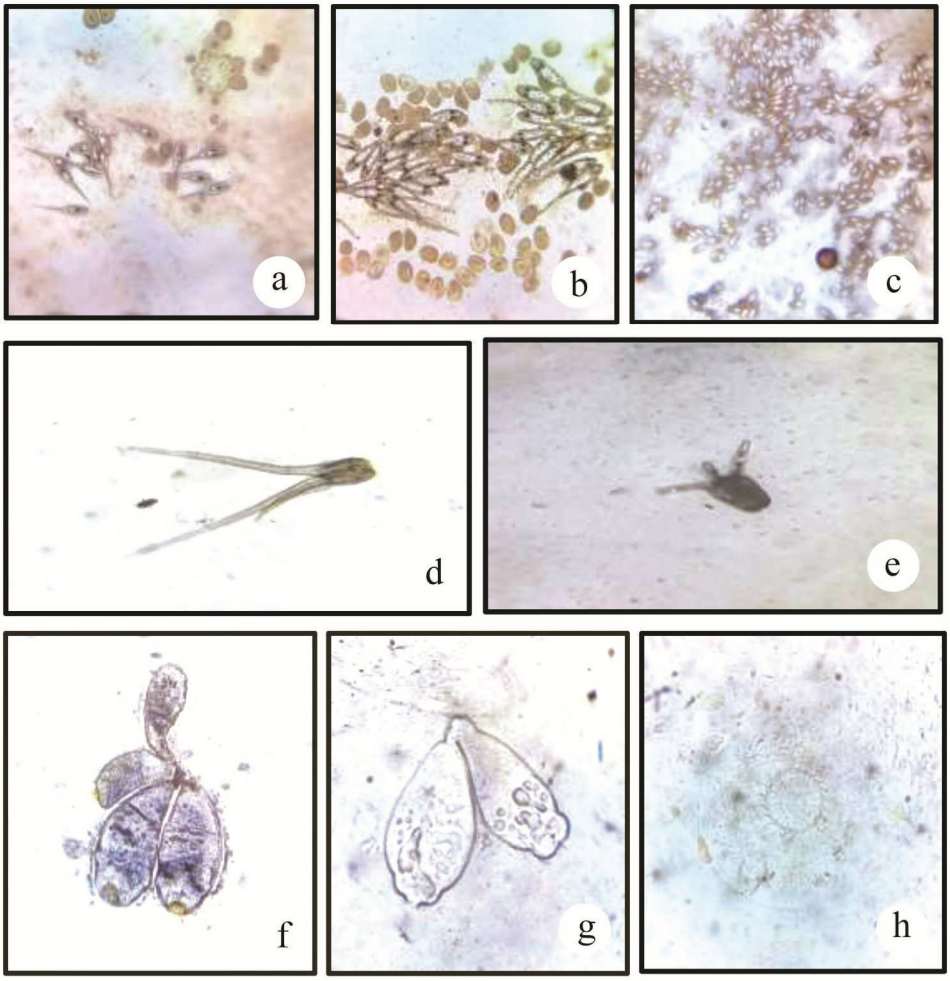

Fig. 1. Photomicrograph of different protozoan parasites. (a) *H. bengalensis* (100x), (b) *H. chaudhuryi* (100x), (c) *Myxobolus* sp. (100x), (d-e) Actinosporean stage of Myxozoa (observed in 100x), (f) *A. piscicolum* (100x), (g) *E. lwoffi* (40x), (h) *T. pediculus* (100x). Fig. a, b, c, f = Stained with silver nitrate impregnation.

the parasites found in the different body parts of *C. punctatus*, *Henneguya bengalensis* (55.05%) was the most abundant and Actinosporean stage showed the lowest abundance (0.21%) (Table 3).

Chaudhary *et al.* (2017) reported higher prevalence (59.3%) of *C. punctatus* infected by *Henneguya chaudhuryi* in India which was relatively higher in comparison with that of the present study. Deshpande and Verma (2015) reported that approximately 28.1% *Channa striatus* were found to be infected with *Myxobolus* sp. in India which was recorded higher than in this study. Deb *et al.* (2015) reported that almost 3.33% *C. punctatus* were infected by *Trichodina pediculus* and around 33.33% of host fish infected

Table 1. List of protozoan parasites recorded from *Channa punctatus*.

Group of the parasites	Name of parasites	Sampling area	Site of infection
Myxozoa	*Henneguya chaudhuryi*	Jashore	Gill
	Henneguya bengalensis	Faridpur, Jashore	"
	Myxobolus sp.	Kishoreganj	"
	Actinosporean stage	Mymensingh	Body slime
Ciliophora	*Trichodina pediculus*	Mymensingh, Faridpur, Jashore	Body slime, Gill
	Epistylis lwoffi	Faridpur, Jashore	Body slime
	Apiosoma piscicolum	Jashore	"

Table 2. Updated list of protozoan parasites from *Channa punctatus* in this region (Bangladesh, India and Pakistan).

Parasites	References*
Henneguya chaudhuryi Δ	Bajpai and Halder1982, Chaudhary *et al.* 2017, Present study
Henneguya bengalensis Δ	Raychaudhuri and Chakravarty 1970, Gupta and Khera 1987, Present study
Myxobolus sp.	*
Actinosporean stage Ω Δ	Present study
Trichodina pediculus §	Deb *et al.* 2015, Present study
Epistylis lwoffi Ω Δ	Present study
Apisoma piscicolum Ω Δ	"

*References of parasites identified up to genus level have not been included in this chart. Ω New host record; Δ New locality record in Bangladesh; § Previously recorded in Bangladesh.

by *Trichodina cyprinocola* in their study. Approximately 32.50% of *C. punctatus* were found to be infected with *Trichodina* sp. (Miah *et al.* 2013) in Bangladesh and interestingly Trichodinids were neither host specific nor site specific (Thilakaratine *et al.* 2003). Prevalence of *Apiosoma* sp. was recorded 8.33% (Mofasshalin *et al.* 2012)

previously in Bangladesh from skin of *Cirrhinus reba* but no infection status was recorded in *C. punctatus* in Bangladesh. *Apisoma piscicolum* showed a quite wide range of host variability such as Europe, Asia and South Africa (Li *et al.* 2007). Mixed infections of *Epistylis lwoffi* and *Apiosoma piscicola* were found in the fry of *Salvelinus fontinalisin* in Canada (Cone and Odense 1987). Similar findings were also recorded in the present study.

Table 3. Overall prevalence (%), mean intensity and abundance (%) of different species of protozoan parasites in *C. punctatus*.

Name of parasites	No. of fish		Prevalence (%)	Parasites collected	Mean Intensity (±Sd)	Abundance (%)
	Examined	Infected				
Henneguya chaudhuryi	29	2	06.90	480	240.0±62.81	33.40
Henneguya bengalensis	29	4	13.79	791	197.8±85.33	55.05
Myxobolus sp.	29	2	06.90	135	67.5±22.36	9.39
Actinosporean stage	29	2	06.90	03	1.5±0.41	0.21
Trichodina pediculus	29	7	24.14	14	2.0±1.02	0.97
Epistylis lwoffi	29	3	10.34	10	3.3 ±1.11	0.70
Apisoma piscicolum	29	1	03.45	04	4.0 ± 0.74	0.28

In *C. punctatus*, single species of parasitic infections was found to be higher than multiple species infection at a time. In fact, 48.28% host fish had no parasitic infection. None of the host harboured more than 3 parasitic species at a time. No previous record was available on multiple infections of protozoan parasites in the host fish. However, Kaur and Katoch (2016) reported 65.15% mixed infection of Myxozoan species at a time in native carp fish and that result was slightly similar to this study.

The occurrence of protozoan infestation also varied in different organs of fish body. During the study, the highest prevalence (44.83%) was found in gill slime of *C. punctatus* than that of the body slime (41.38%) (Fig. 2). This could be explained as the gills are the centre of filter feeding and the site of gaseous exchange. No parasites were found in blood sample of *C. punctatus* during this investigation. Emere and Egbe (2006) reported highest load of protozoan parasites in the gill of host species *Synodontis clarias* and Nyaku *et al.* (2007) reported highest load of protozoan parasites in the gills of

Auchenoglanis ocidentalis, *Oreochromis niloticus* and *Bagrus bajad* in River Benue. According to Roberts and Somerville (1982), the sieving ability of the gill rakers might help to trap some organisms and this could be attributed to the presence of the protozoan parasites on that site.

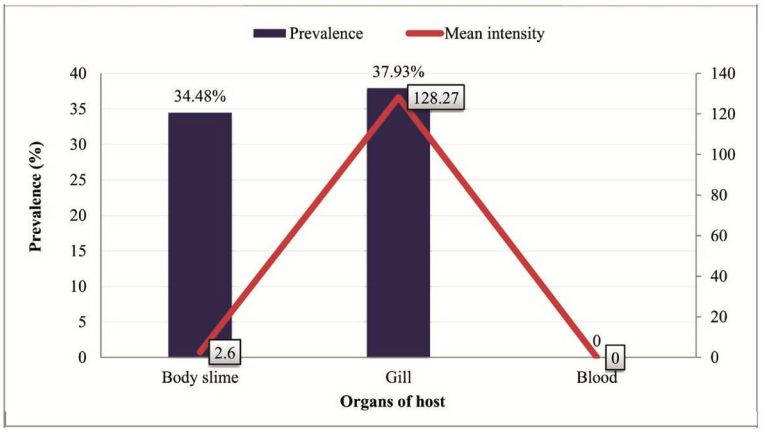

Fig. 2. Prevalence and mean intensity of parasitic infestation in organs of *C. punctatus*.

During the study, the highest prevalence (%) of parasitic infection of *C. punctatus* observed in Faridpur (100%) district which was followed by Jashore (50%), Kishoregonj (40%) and Mymensingh (33.33%) district, respectively (Fig. 3). Fisher's Exact test showed that the association of parasitic infestation with study areas was not statistically significant ($p=0.118$, since $p \leq 0.05$) in *C. puntatus*. The highest mean intensity was found in samples collected from Jashore district (173.8±60.44) and lowest mean intensity (2.67±0.58) was found in Mymensingh district (Fig. 3).

In the present investigation, *Trichodina pediculus* was recorded in three study areas with highest prevalence in Faridpur (60%) and lowest in Jashore (20%) (Table 4). *Henneguya bengalensis* and *Epistylis lwoffi* were recorded in two study areas where highest prevalence of both parasitic species were found in Faridpur and rest of the four species were found only in one study area (Table 4). Fisher's Exact test showed that the association of parasitic infestation of *Henneguya bengalensis* and *Myxobolus* sp. with study areas was statistically significant ($p=0.024$ and 0.049, whereas $p \leq 0.05$) in *C. punctatus*. Infections of rest of parasite species with samples sites were not statistically significant.

The site wise comparison of richness value showed that Jashore had the highest (0.550) species richness in *C. punctatus* (Table 5). The lowest value was observed in Mymensingh (0.138) district (Table 5). Evenness of parasitic distribution in Mymensingh showed moderately higher value (0.955) indicated that community structure was well constructed by evenly distribution of all parasite species that was well diverged (Table 5). In Jashore (0.465) and Faridpur (0.152) had poor evenness value which meant that parasite community was poorly constructed and low diverged (Table 5). In Kishoreganj area parasite community showed no evenness, richness and diversity in *C. punctatus* because there was found only one protozoan parasite (Table 5).

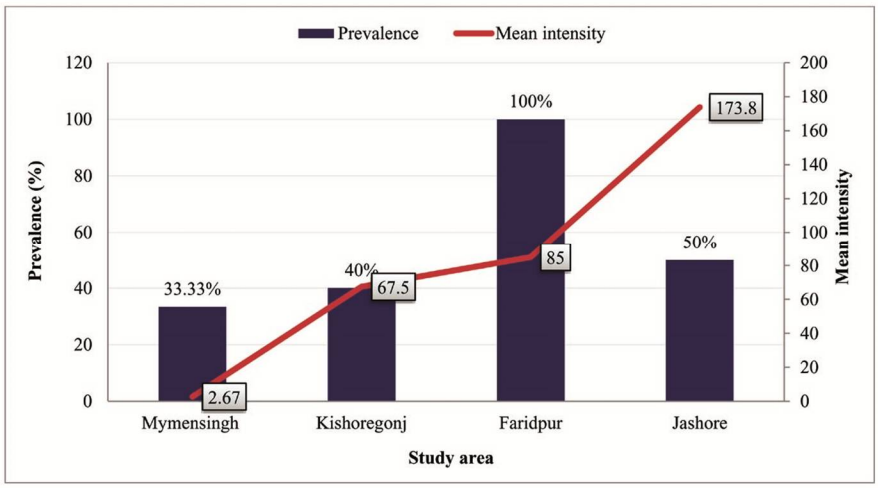

Fig. 3. Prevalence (%) and mean intensity of protozoan parasites of *C. punctatus* in study areas.

Shannon diversity index, H=0.662, 0.00, 0.167 and 0.748 in Mymensingh, Kishoreganj, Faridpur and Jashore site respectively, indicated that the sample fishes were not infested by more parasites and the parasite community were poorly diverged (Table 5). On the contrary, Simpson's diversity index, D=0.536, 0.00, 0.064 and 0.504 in Mymensingh, Kishoreganj, Faridpur and Jashore site respectively, indicated that parasite community were moderately diverse in sample fishes of Mymensingh and Jashore (Table 5). Whereas, fish sample collected from Kishoreganj district had no diversity and fish samples collected from Faridpur were not infected by more parasites and parasites community were poorly diverged (Table 5).

Table 4. Prevalence and mean intensity of different species of protozoan parasites in *C. punctatus* in study areas.

Name of parasites	Mymensingh		Kishoregonj		Faridpur		Jashore	
	Prevalence (%)	Mean intensity (± Sd)	Prevalence (%)	Mean intensity (± Sd)	Prevalence (%)	Mean intensity (± Sd)	Prevalence (%)	Mean intensity (±Sd)
Henneguya chaudhuryi	-	-	-	-	-	-	20	240 (± 102.93)
Henneguya bengalensis	-	-	-	-	60	137 (± 108.71)	10	380 (± 120.17)
Myxobolus sp.	-	-	40	67.5 (±52.39)	-	-	-	-
Actinospor-ean stage	22.22	1.5 (± 0.71)	-	-	-	-	-	-
Trichodina pediculus	22.22	2.5 (± 1.33)	-	-	60	2 (± 1.30)	20	1.5 (± 0.67)
Epistylis lwoffi	-	-	-	-	40	4 (± 2.30)	10	2 (± 0.63)
Apisoma piscicolum	-	-	-	-	-	-	10	4 (± 1.26)

Table 5. Comparison of the richness, evenness and diversity of the parasite communities of the different sampling areas in *C. punctatus.*

Characteristics	Mymansingh	Kishoreganj	Faridpur	Jashore
Number of fish examined	09	05	05	10
% of fish infected	55.56	40	100	50
No. of parasites collected	10	155	556	894
No. of parasite species	02	01	03	05
Species evenness	0.955	0.00	0.152	0.465
Species of richness 'R'	0.138	0.00	0.275	0.550
Shannon diversity index, H	0.662	0.00	0.167	0.748
Simpson's diversity index, D	0.536	0.00	0.064	0.504

During the study, protozoan parasites exhibited variation in composition, prevalence and mean intensity in host, which might be dependent upon the factors such as parasite biology, host size, feeding habits and habitat of the host, water quality, metabolic state and weak immune system of fish. There was no previous comparative data and the cause of diversity of protozoan parasites of *C. punctatus* in the study areas. So direct comparison of the present study was not possible. There was no available data on the water quality of sample collection areas. Banerjee and Bandyopadhyay (2010) reported that water quality has a great impact on the abundance of fish pathogens and their ability to survive on host. Therefore, the reasons caused the difference of distribution of parasites in sample collecting areas could not be exactly described.

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TEMPORAL DISTRIBUTION AND ABUNDANCE OF MOSQUITO VECTORS IN DHAKA CITY

M.G. SHAROWER[1]*, M.A. LATIF[2] AND S.M. UDDIN[3]
[1]*Department of Public Health, North South University, Bashundhara, Dhaka-1229, Bangladesh*
[2]*Department of Entomology, Sher-e-Bangla Agricultural University, Sher-e-Bangla nagar, Dhaka-1207, Bangladesh*
[3]*Dhaka North City Corporation, Gulshan-2, Dhaka-1212, Bangladesh*

Abstract

Species of *Anopheles*, *Aedes* and *Culex* mosquitoes showed that *Anopheles gambiae* s.s had the highest number (43.5%) out of the three malaria vectors (*viz. Anopheles gambiae, An. arabiensis* and *An. funestus).* For *Aedes* and *Culex* species, *Aedes aegypti* (37.6%) and *Culex fatigans* (37.1%) had the highest prevalence out of their sibling species. Temperature and rainfall were highly correlated with the abundance of mosquito vectors. It was observed that the rainy season (March to October) recorded the highest number (Total 11 specie) of mosquito vectors collected with the peak (*Aedes aegypty,* 140*)* in the months of July (932) and August (976) while the lowest (333) collection was in the dry season (November to February) with lowest (333) in the month of February when there was little or no rains.

Key words: Temporal distribution, Abundance, Mosquito vectors

Introduction

In warm and tropical climatic regions of the world, climatic factors have been associated with relative mosquito abundance and transmission of mosquito borne infections. Approximately half of the world's population is at the risk of malaria and an estimated 243 million infected cases resulted in nearly 86300 deaths in 2008 (WHO 2009). In Sub-Saharan Africa, 91% of malaria was detrimental and caused death. Malaria was estimated by calculating the result in an annual loss of 85% of the deaths amongst children below five years (WHO 2010). In addition 40% of all the public health spending is related to malaria (Haque *et al*. 2010). Out of all the diseases, such as malaria, filariasis, dengue and yellow fever caused by mosquito vectors, malaria is the most important tropical and parasitic disease in the world. Malaria alone accounts for up to 25% of hospital attendance, with young children under five years accounting for about 40% in Bangladesh (WHO 2012). Filariasis also has been shown to be a public health problem in Bangladesh, particularly in the Dhaka City Corporation (Ahmed *et al.* 2009). Studies

*Corresponding author: sharower_bau@yahoo.com

throughout the world have shown that incidence of malaria and other related diseases associated with mosquito vectors are linked to the pattern of rainfall, temperature and humidity (Briet *et al.* 2008). Rainfall is considered to be a major factor influencing malaria outbreak in Bangladesh (Haque *et al.* 2007) and a causal relationship between rainfall and malaria transmission is well recognized (Thomson *et al.* 2005). In the highland of Kenya, malaria cases increased by 1.4 to 10.7% per month for each 10 mm increase in monthly rainfall (with 2-3 months lag) (Hashizume *et al.* 2009). Natural climatic disasters such as floods and cyclones may also have significant relationship with malaria outbreaks (Lindsay and Birley 1996). Temperature, rainfall and humidity have been widely associated with the dynamics of malaria vector population and therefore with the spread of the disease.

However, at the local scale, there is lack of a systematic quantification of these factors of malaria transmission (Githeko and Ndgwa 2001). In East African Highland the research findings of Zhou *et al.* (2004) revealed that 1^{o}C temperature increase in minimum temperature having a lag of time of 1–2 months and 1^{o}C increase in maximum temperature with a lag time of 2-5 months led to an 80 - 95% increase in the number of malaria outpatients. Meteorological factors are important drives of malaria transmission. Ambient temperature plays a major role in the life cycle of the malaria vector. Temperature between 15 and 40ºC and humidity between 55 and 80% are suitable for the completion of the *Plasmodium falciparum* and *P. vivax* malaria parasites life cycle (Zhou *et al.* 2004). The development of the parasite within the mosquito (sporogonic) cycle is dependent on temperature. The sporogonic cycle takes about 9-10 days at 28ºC but stops at temperature below 16ºC (Lindsay and Birley 1996). The daily survival of vector is dependent on temperature as well. The suitable survivality of daily temperature is between 16 and 36^{0}C and the daily survival capability drops rapidly at temperature above 36ºC. The highest proportion of vector surviving incubation period is reported at temperature between 28 and 32ºC (Craig *et al.* 1999). The gonotrophic cycle which is the time between blood meals of the vector is short at higher temperatures because digestion speed increases (Haque *et al.* 2010). The mosquitoes survivality was prolonged at high temperature along with frequent rainfall and suitable relative humidity of at least 50 - 60% by providing breeding sites to lay eggs. Relative humidity below 60% shortens the life span of the mosquito vectors (Rogers and Randolph 2006). The goal of this work is to study the effects of these climatic factors on the distribution and abundance of mosquito vectors in the study area.

Materials and Methods

The study was carried out in some selected houses of all the 91 words of Dhaka City Corporation (both North and South) from July, 2014 to June, 2016 (two years). Adult mosquito samples were collected from the houses between 05.00 and 07.00 hrs in the morning and 19.00 and 21.00 hrs in the evening when the mosquitoes attracted to the light trap (CDC-LT) baited in the CO_2 and pyrethrum were collected with an aspirator. Human Landing Catches (HLC) were also performed by two trained collectors (adult male volunteers) working alternatively for one hour and resting for one hour. Medical nurses provided medical supervision of the collectors. Light trap per net catches was performed using a CDC mini light trap placed adjacently and above an occupied bed net. Pyrethrum Spray Catches were performed at 7 a.m. by spraying pyrethrum for 30 - 45 seconds in the room. After 10 minutes, dead and immobilized mosquitoes were collected. Two sites per location were randomly selected. In each site, three rooms were randomly chosen within a 15 m distance. Each night, a different sampling method was tested in each room. The houses used for mosquito collection were randomly selected close to the sites of larval habitats. Paper cups covered with netting materials, which contained cotton pad soaked in 10% glucose, were used for collection. The cups were placed in a cool box and transported to the laboratory where the mosquitoes were anaesthetized with ethyl acetate. They were sorted out and identified by morphological characteristics with the key aids of Strickland and Choudhury (1927), Giles (1968) and Koekemoer *et al.* (2002). Data were collected in dawn and just after sun set for maintaining lowest temperature and at noon for recording highest temperature. Aspirator was used to count the number of mosquitoes. They were later counted and recorded.

The data on minimum and maximum temperature, rainfall and relative humidity for these periods were collected from different parts of Dhaka city every month. The water from the sample sites was collected using rain barrels. A pH meter was used to test the pH level of the water.

Results and Discussion

A total number of 7,468 adult mosquito was collected in the whole year, out of which 1808 (24.2%) were *Anopheles* sp., 2900 (38.8%) *Culex* sp. and 2760 (36.9%) for *Aedes* sp. *Anopheles gambiae*, *An. funestus* and *An. arabiensis* were the malaria vectors found during the survey. *An. gambiae* had the highest prevalence of 787 individuals (43.5%) followed by *An. arabiensis* of 616 individuals (34.1%) and the least in number was found in case of *An. funestus* 405 (22.4%) (Table 1).

Table 1. Monthly mosquito species abundance in Dhaka city from July, 2014 to June, 2015.

Mosquito species	Monthly abundance of mosquitoes												
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
Anopheles gambiae	21	21	32	68	80	99	118	131	80	64	41	32	787
An. arabiensis	30	28	38	35	74	84	82	84	64	30	39	28	616
An. funestus	17	14	18	15	56	57	60	70	36	15	19	18	405
Total	68	63	88	118	210	250	260	285	180	109	99	78	1808
Aedes aegypti	58	54	55	87	83	100	140	142	95	98	96	84	1091
Ae. albopictus	35	40	41	70	64	80	101	98	70	75	77	64	816
Ae. vittatus	38	32	41	58	58	68	80	81	47	58	46	48	655
Ae. qalpalis	18	28	22	37	46	57	20	20	28	24	24	14	338
Total	149	154	159	252	251	305	341	341	240	255	243	210	2900
Culex fatigans	55	50	50	87	79	108	128	128	98	90	90	69	1025
Cu. pipens	36	29	47	64	66	71	110	110	75	70	71	50	792
Cu. quenquefascia	29	21	30	48	58	61	87	87	46	53	51	42	605
Cu. tigripes	20	16	24	17	44	51	33	33	24	22	28	29	338
Total	140	116	151	247	247	291	358	358	243	235	240	190	2760
Grand total	357	333	408	586	708	846	932	976	663	599	582	478	7468

The highest numbers of mosquito species were recorded in the month of August with *Aedes aegypti* and *Culex fatigans* was found to be the most common of all the species. The distribution and abundance of the mosquito with temperature and rainfall are shown in Fig. 1.

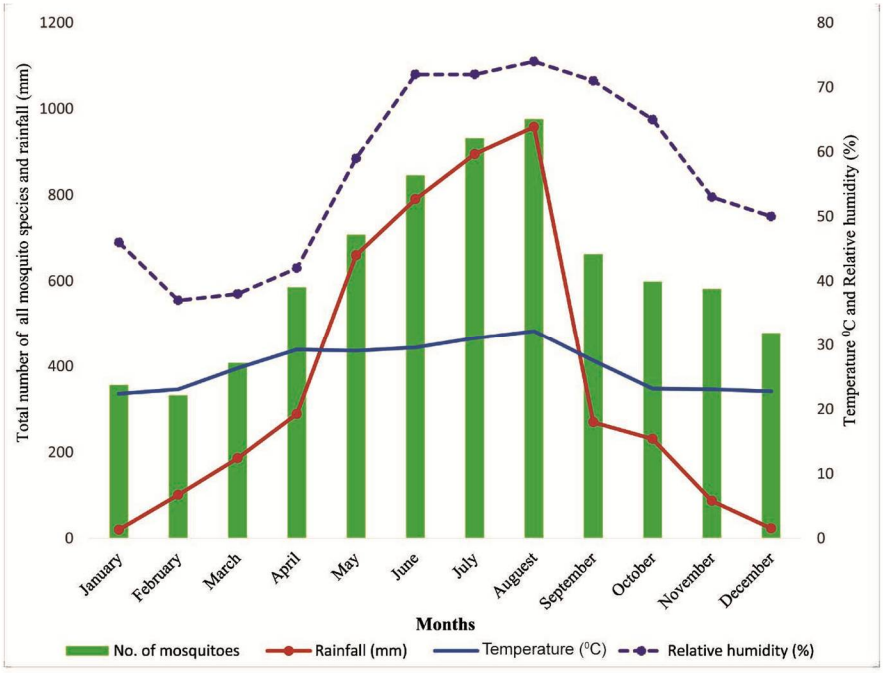

Fig. 1. Monthly prevalence of all mosquito species with temperature, rainfall and relative humidity in Dhaka city from July, 2014 to June, 2015.

Correlation coefficient between the average temperature and the total number of mosquitoes per month showed that the average temperature exhibited high correlation (r^2=0.27) with the total number of mosquito ($p<0.05$) which indicated that temperature had a significant effect on the abundance of mosquito vectors (Table 2). The correlation analysis between monthly rainfall and the total number of mosquito collected revealed that there was a very high correlation (r^2=0.79). Rainfall had a significant effect on the distribution and abundance of the mosquito vectors ($p<0.05$, Table 2). The result of the t test analysis carried out on the effects of both temperature and rainfall on the monthly abundance of mosquito vectors showed that they had a significant effect on climatic factors (Table 2).

Table 2. Analysis of correlation between the meteorological parameter and abundance of mosquito vectors (n=12).

Variables	CC	p values	r2
Rainfall vs TNMVs	0.85	0.001	0.79
Temperature vs TNMVs	5.02	2.200	0.27
Rainfall and temperature vs TNMVs	4.77	2.080	

CC=Correlation coefficient, TNMVs=Total number of mosquito vectors, $p<0.05$.

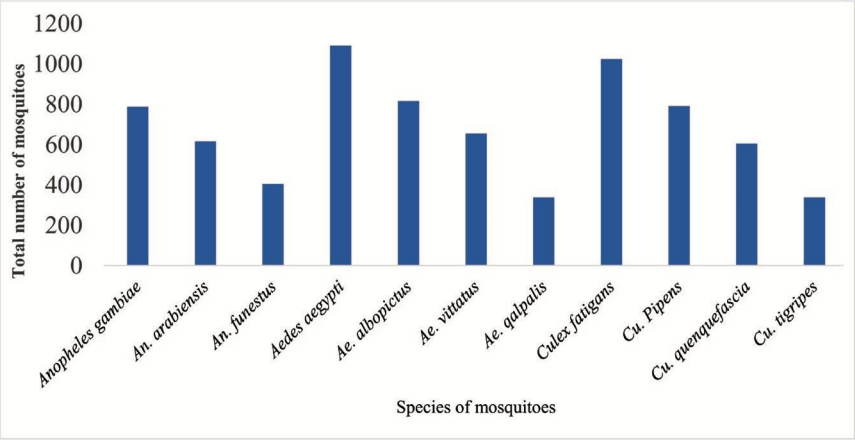

Fig. 2. Distribution pattern of all mosquito species in Dhaka city.

It is important to study the impact of weather on the transmission of malaria and other diseases associated with mosquito vectors, as global warming might change the pattern of temperature and rainfall which may directly or indirectly influence mosquito density or distribution (Wu *et al.* 2007). In this present study, the temperature was between 22.4 and 32.2^{o}C (Fig.1) with average humidity of 56.5% (Fig. 1) which might have facilitated the higher mosquito abundance. Similar kind of results has also been previously reported on the maximum survival rate of mosquito for the related temperature and humidity (Murty *et al.* 2010). Thu *et al.* (1998), also explained in their report that humidity was one of the vital factors affecting the population density of mosquitoes and it had been observed that the temperature at 28^{o}C with 55-55% relative humidity was the most appropriate condition for the elevation in mosquito density or abundance than the condition of lower temperature with higher humidity (22^{o}C/80-85% RH). Three main species of mosquito vectors were found, *viz.*, *Anopheles*, *Culex* and *Aedes.* Among the three malaria vectors species *Anopheles* found, *An. gambiae* had the highest number (Table 1) during the rainy

periods (May - August). For *Aedes* mosquitoes, four species (Table 1) were found out of which *Ae. aegypti* had the highest number of abundance (Table 1) while *Ae. palpalis* had the least number. *Cx. fatigans* had the highest number (Table 1) and *Cx. tigripes* had the least abundance (Table 1). This result may be as a result of the fact that their larvae can colonize and survive in almost all habitats, such as the water or barrels, drainages, tyres, pots, discarded plastics and bottles and tanks. The pattern of rainfall also affects larval habitat and vectors population size. On the basis of rainfall, increased rainfall may increase larval habitats and vector population by creating a new habitat. Excessive rain could also eliminate habitats through flooding, thus decreasing the vector population especially malaria vectors because they prefer sunlit pools of water. During the dry season, limited rainfall can also create new habitats of *Anopheles gambiae, Aedes aegypti* and *Culex fatigans* species when water in the rivers is drawn into pools, providing the perfect breeding site for a number of mosquito species, thus favouring disease transmission as also observed by Gubler *et al.* (2001).

The temporal change in mosquito abundance is mainly caused by rainfall. *An. gambiae* adults were more abundant during the rainy season than during the dry season which is consistent with the finding that the number of larval habitats was substantially higher in the rainy season than in the dry season as previously reported by Zhou *et al.* (2007). The lower abundance of *An. funestus* adults than *An. gambiae* was caused by the lack of suitable, long-lasting larval habitats for *An. funestus* because its larvae normally take three weeks to develop into adults, and *An. gambiae* s.s larvae require approximately 10 days in sunlit habitats. However, it was revealed that tree canopy coverage exhibited a significant effect on the mosquito abundance in houses because it reduces the water temperature of larval habitats surrounding the houses because canopy cover reduces the amount of solar radiation reaching the larval habitats. It was also observed that the air temperature inside a house is affected by tree canopy.

Apart from the importance of congenial environmental and ecological factors such as breeding sites, humidity, temperature and rainfall, human activities such as agricultural practices, lumbering etc. also contribute to the distribution and abundance of these mosquito vectors especially the availability of host for blood meal. In conclusion, there was a high correlation of temperature and rainfall on the distribution and abundance of malaria and other related diseases associated with mosquito vectors. A small temperature rise either through seasonal variability, local microclimatic changes due to modification in vegetation cover or to global warming can increase disease transmission. The meteorological parameters are good prediction of malaria and associated disease risk.

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MICROBIAL, PHYSICOCHEMICAL AND NUTRITIONAL QUALITY ASSESSMENT OF FRUIT JUICES IN TANGAIL MUNICIPALITY, BANGLADESH

SHAMIM ARA SHEAULI[1], PRITAM SAHA[1], MD. YOUNUS MIA[1*], REZUANA AFRIN[1] AND SHAHIN MAHMUD[2]

[1]*Department of Environmental Science and Resource Management, Mawlana Bhashani Science and Technology University, Tangail, Bangladesh*

[2]*Department of Biotechnology and Genetic Engineering, Mawlana Bhashani Science and Technology University, Tangail, Bangladesh*

Abstract

Analysis of the microbial, physicochemical and nutritional qualities of some commercially bottled and handmade fruit juices showed that total viable bacteria in bottle juices ranged from 9.6×10^7 to 2.0×10^{11} cfu/100 ml and handmade juices 1.3×10^5 to 9.6×10^7 cfu/100 ml. The log mean values of total bacteria count ranged from 9.14-10.19 cfu/100 ml (bottled) and 6.09-9.08 cfu/100 ml (handmade).Total coliform bacteria ranged from 0-7.6×10^9 cfu/100 ml (bottled) and 0 - 2.8×10^5 cfu /100 ml (handmade) with a range of log mean values of 3.18-6.95 cfu/100 ml (bottled) and 3.47-3.48 cfu/100 ml (handmade). The pH was acidic and mean value ranged from 3.14-4.03 for bottled juice and 3.72-3.73 (handmade). It was found that total soluble solids ranged from 10-11.33% for bottle and 11.33-12.33% for handmade juices. The concentration of vitamin C in bottled and handmade juices ranged from 0.74-2.22 mg/100 ml and 2.34-3.7 mg/100 ml, respectively, indicated that vitamin C content was very low. It was also revealed that quality of bottled and handmade juices was unsatisfactory and may not be useful for consumption. It is suggested that proper measure must be taken and manufacturing companies should develop the quality by maintaining hygiene and using good quality ingredients in preparing different types of juices.

Key words: Microbial, Physicochemical, Nutritional quality, Fruit juice

Introduction

Fruit juices are becoming an important part of the modern diet and considered to health and nutritional benefits in many communities. All over the world, in everyone's diet chart it is always included in different forms like as whole fruit, juice, beverage or still drink etc. Fruit juices are considered as a nutritious popular drink, but processed juice may not always be safe due to chemical hazards and microbial risks (Aneja *et al.* 2014). Nowadays, throughout the country people drink fruit juices without knowing actual safety and nutritional quality of these types of juices.

As a result they suffer from food borne and many other gastrointestinal diseases (Rashed *et al.* 2013). Microbial quality is very important in food since bacteria, fungi, viruses and

*Corresponding author: mdmia1998@gmail.com

protozoa which are potential pathogens of human known to cause food borne diseases while cause for spoilage (Acharjee *et al*. 2013). The quality of fruit juices is strictly maintained in developed countries under several laws and regulations. Most fruit juices contain sufficient nutrients that could support microbial growth. In recent years, the increasing consumer awareness has emphasized the need for chemically and micro-biologically safe food (Aneja *et al.* 2014). But in many developing and under-developed countries, the manufacturers are not conscious about the microbiological safety and hygiene of fruit juices because of lack of commitment and law enforcement system. Therefore, maintaining of the quality of processed fruit juices is an important concern. The chemical feature of juice considered in quality assessment include pH, total soluble solids, acidity, ash content, ascorbic acid etc (Tasnim *et al.* 2010). It should also be noted that changes in pH could transform a food into one which supports pathogens to grow (FDA 2001, ICMSF 1989). In Bangladesh, the manufacturers are not concerned about the microbiological safety and hygiene of fruit juices due to lack of proper knowledge. Juices are frequently consumed by most of the people, so the quality of these juices should be known. Considering the importance, this study has been undertaken to assessing the physicochemical, nutritional and microbiological quality of bottled and handmade juices in Tangail municipality, Bangladesh.

Materials and Methods

Study area, time and collection of samples: The study was conducted at Tangail municipality, Bangladesh. A total of 18 juice samples of different brands and handmade of different producers were collected from three different locations, namely New bus stand, Old bus stand and Santosh, Tangail (Fig. 1). Twelve bottles (brand- 1 and 2) of orange and litchi juice samples were collected from street side shops and the handmade juice samples were collected from different fast food during January - May, 2018. Samples were transferred aseptically into the ice box with sufficient ice blocks.

Microbial analysis: Spread plate technique was performed for bacterial total plate count with serial dilution by following the standard procedure (APHA 1976). Plate count agar, MacConkey agar, were used for the growth of total viable bacteria, total coliform, respectively (Alam 2013). Colonies formed in the plates were counted by using digital colony counter after incubation at 37ºC for 48 hours. The actual number of bacteria were

estimated as colony forming unit (cfu/100 ml). The bacteria plate counts per 100 ml per dilution were recorded using the following equation

$$\text{Total count} = \frac{\text{Total number of colonies}}{\text{Number of plates}} \times \frac{1}{\text{Dilution of actor}} \times \frac{1}{\text{Volume inoculated}}$$

cfu/100 ml (Bell *et al.* 2005).

Fig.1. Map of the study area.

Physicochemical analysis: The pH was determined using digital pH meter. Total soluble solids (TSS) content of fruit juices was determined using an refractometer whereby a drop of pulp solution was placed on its prism. The percentage of TSS was obtained from direct reading of the refractrometer. Vitamin C was estimated by 2,6-dichloro-phenolindophenol visual titration method according to AOAC (2004). The reagents used for the estimation of vitamin C were as follows: (i) Metaphosphoric acid (6%), (ii) standard ascorbic acid solutions and (iii) 2, 6-dichlorophenolindophenol dye. For estimation of vitamin-C, the following steps were followed: Standardization of dye solution, preparation of solution and titration (AOAC 2004).

Statistical analysis: MS Excel 2010 and SPSS 20 software were used for calculating average, standard deviation and preparation of graphs. Pearson's correlation coefficients were determined for analysis of correlation among the parameters.

Results and Discussion

Microbial analysis

Total viable count of bacteria: The range of total viable count was 2.0×10^{11} to 9.6×10^{7} cfu/100 ml in different brands of bottled juices at different locations. In bottled juices litchi (Pran) showed the highest value (2.0×10^{11} cfu/100 ml) at New bus stand and the lowest value (9.6×10^{7} cfu/100 ml) in Orange (Pran and BD Food) at both New and Old bus stand. The number of bacteria is very high because storage of products at refrigerator temperature or below is not always best for the maintenance of desirable quality of some fruits. Total viable count is shown in the graph as logarithm value.

The highest log of total viable count value was 11.505 cfu/100 ml in litchi (BD food) at Old bus stand and the lowest Log total viable count value was 7.98 cfu/100 ml in litchi (Pran) at New bus stand (Fig. 2). For handmade juices, the range of total viable count was 1.3×10^{5} to 9.6×10^{9} cfu/100 ml at Tangail fast food and Santosh. In case of orange juice the highest value of total viable count was found at Santosh, and the lowest value was found in litchi juices at New bus stand.

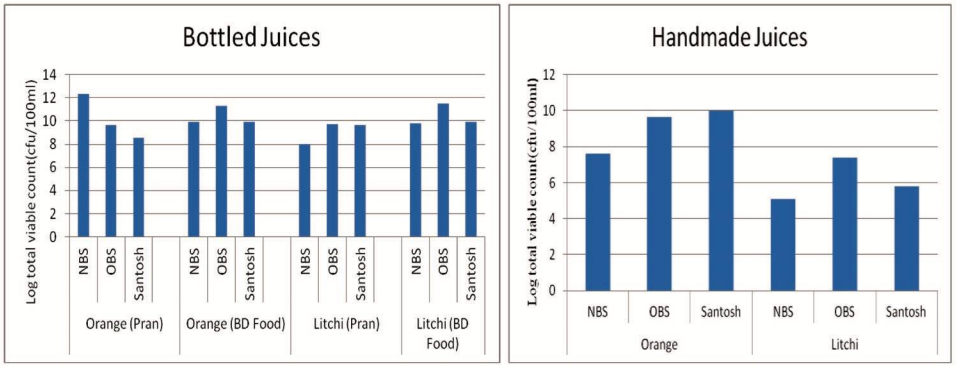

Fig. 2. Variation of total viable count of bacteria in different samples. NBS = New bus stand, OBS = Old bus stand.

The range of total viable count was 1×10^{4} cfu/ml (Gulf Standard 2000). In other study it was reported that the highest total bacterial load (2.66×10^{6} cfu/ml) for packed fruit juice sample was found in an orange juice (sample P-12), collected from Farmgate, lemon (3.94×10^{5} cfu/100 ml) and papaya (1.98×10^{6} cfu/100 ml) from Mouchack, Dhaka,

respectively (Rashed *et al.* 2013). Total viable bacterial count in most of the fresh juice samples was higher than the commercially packed juice, as the highest count was found as 2.4×10^4 cfu/ml and 3.2×10^3 cfu/ml in fresh and packed juice, respectively which was found to be lower than this study (Rahman *et al.* 2010). The load of viable bacteria in processed juice samples within the standard limit in the average of 10^3 cfu/ml (Tasnim *et al.* 2010). Total viable count of commercially produced litchi juices in a range of 5×10^5 (cfu/ml) and total vible count for handmade orange juices in a range of 1×10^5 cfu/ml (Mortuza 2016). In a similar study, Fatema *et al*. (2016) estimated the total viable count of minerals in *Allo vera*, grapes and papaya. The highest total viable count ranged from 1.4×10^6 - 1.2×10^6 cfu/ml was present in alovera and mango juice sample and lowest total viable count was present in papaya 9.0×10^5 cfu/ml and Malta 5.5×10^5 cfu/ml.

All brands of processed juices exceed the standard limit of Gulf Standard (2000) in the present study. Total viable bacteria was found in high amount in these juices may be due to the unhygienic maintenance during preparation of the juices.

Total coliforms count (TCC): In this study, the range of total coliform bacteria was 0-7.6 $\times10^9$ cfu/100 ml in different brands of bottled juices at different locations. In bottled juices, the highest value 7.6×10^9 cfu/100 ml was found in litchi (Pran) juices collected from New bus stand. The coliform count was nil in orange (Pran and BD Food) of Old bus stand and Santosh. The highest log total coliform count 11.38 cfu/100 ml was found in litchi (BD Food) juices collected from Santosh (Fig. 3). On the other hand, for handmade juices, the range of total coliform count was 0 - 2.8×10^5 cfu/100 ml at different locations. The highest value of total coliform count was found (2.8×10^5 cfu/100 ml) in litchi juices of Santosh and the total coliform count was nil in orange and litchi juices at different points.

The range of total coliform count is 1×10^2 cfu/ml (Gulf standard 2000). The presence of coliform in fruit juice is not allowed by safe food consumption standard (Andres *et al.* 2004). In another study, total coliform count for handmade lemon juices in a range of lemon 2.8×10^4 cfu/ml (Oranusi *et al*. 2012). Total coliform count found in litchi juices in a range of 4×10^2 cfu/ml and total coliform count for handmade orange juices in a range of 5×10^3 cfu/ml (Mortuza 2016).

In the present study it is showed that the total coliform was nil in some samples of bottled and handmade juices whereas some bottled and handmade juices exceeded the Gulf standard limit. This contamination could also be occurred due to lacking of proper quality control system for juice preparation, lacking of right storage conditions and bad packaging system (Kader *et al.* 2014). It is also found that the total coliform bacteria

were found in high concentration of some juices due to unhygienic maintenance during prepraration of handmade juices.

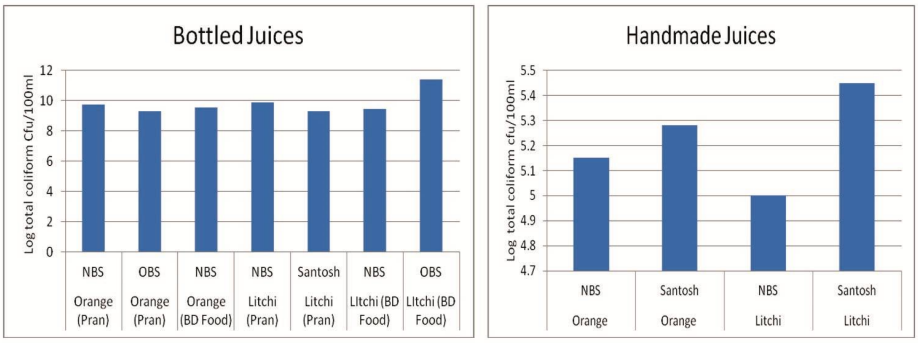

Fig. 3. Variation of total coliform count in different samples. NBS = New bus stand, OBS = Old bus stand.

Physicochemical analysis

pH: The highest pH (4.05) was found in litchi juice (Brand-1) in 200 ml bottled and the lowest pH (3.03) was found in litchi juice (Brand-2), Fig. 4. On the other hand, for handmade juices the highest pH (3.8) was found in orange juices and the lowest pH (3.68) was found in litchi juices (Fig. 4). The highest pH was 5.45±0.09 in papaya juices and the lowest of pH 2.40±0.07 was in lemon juices.

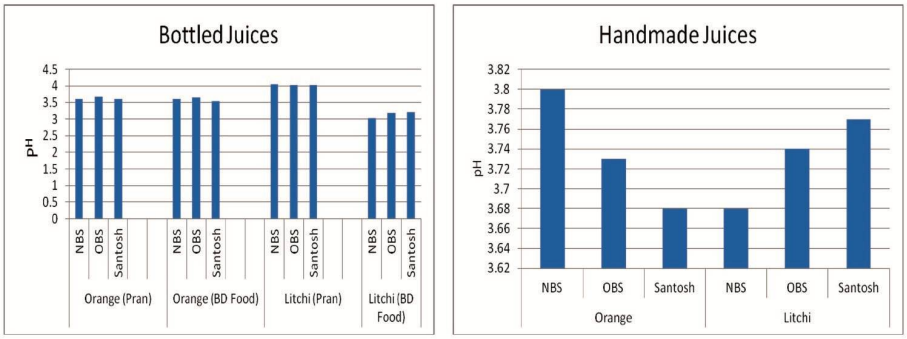

Fig. 4. Variation in concentrations of pH in different samples. NBS = New bus stand, OBS = Old bus stand.

In other study, the pH for ripe mango juice was 3.4-4.8 (Anon. 1962). The mean pH value of total fruit juices was 4.9 with range of 3.88-5.71. The pH of both mango and papaya was 3.8 and 4.9, respectively and more acidic. FAO (2005) recommended value of pH for mango juice is 3.5-4.0. In present study authors have found that commercially

bottled litchi juices of Brand-1 are slightly acidic than litchi juices of Brand-2 because Brand-1 (litchi) exceeded the standard limit (FAO 2005). This present study noted that pH values were within the standard limit.

Total soluble solids (TSS): The range of TSS was found from 10 to 12% for bottled juices and from 10 to 13% for handmade juices at different samples (Fig. 5). The highest TSS value was found in bottled (11.33%) orange Brand-2 and litchi Brand-2 juices. On the other hand, highest TSS was 12.33% in orange handmade juices. The lowest concentration (10%) was found in Brand-1 of orange and litchi bottled juices whereas, for handmade juices, lowest concentration of TSS (11.33%) was found in litchi juices. The overall concentration of TSS of bottled juices was 11.33±0.58 and for handmade juices was 12.33±0.58. FAO (2005) estimated that TSS of bottled juices was 9.00±0.02.

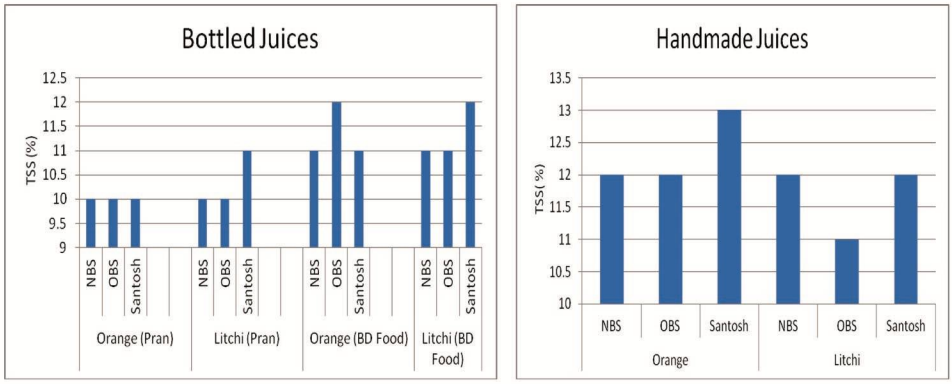

Fig. 5. TSS of different juice samples. NBS = New bus stand, OBS = Old bus stand.

The recommended value of TSS is 11.8% for orange (bottled) and 11.5% for papaya in handmade juices (Tasnim *et al*. 2010). According to Bangladesh standards specification for fruit or vegetables juice, Brix the TSS in fruit or vegetables juice is minimum (12%). It may be said that TSS content in orange and litchi juices of Brand-1 in this study was comparatively lower than Brand-2 (bottled) and for handmade juices, TSS content in orange and papaya juices was comparatively higher than lemon and litchi juices. In 2001, FDA estimated that TSS of mango juices in a range of 11-13% which are almost similar findings of FAO and FDA. The present study also found that TSS of orange and litchi juices (bottled) is similar to other studies. But TSS ranges of handmade juices such as orange, litchi, papaya are slightly higher than the standard level. It may be concluded that these fruits are mature and ripe that's why TSS crossed the standard limit.

Nutritional analysis

Vitamin C: The range of concentration of vitamin C both for the bottled and handmade juices was from 0.74 to 2.22 mg/100 ml and 1.1 to 4.1 mg/100 ml, respectively. In bottled juices, highest concentration of vitamin C (2.22 mg/100 ml) in Brand-1 and 2 (orange juice) and the lowest concentration of vitamin C (0.74 mg/100 ml) was found in Brand-1 and 2 (litchi juice) (Fig. 6). In case of handmade juices highest concentration of vitamin C (4.1 mg/100 ml) was found in orange juice and the lowest concentration of vitamin C (1.1 mg/100 ml) was found in litchi juices (Fig. 6). In other study, Tasnim *et al*. (2010) estimated that vitamin C concentration of processed orange juice is 5.64±0.08. Comparing between the nutritional analysis of vitamin C of bottled and handmade juices, it is assessed that highest concentrations of vitamin C are found in both for brand 1 (bottled) (orange, 2.22 mg/100 ml) and for handmade (4.1 mg/100 ml) juces. In the present study, it is estimated that the amount of vitamin C in bottled juices is less than handmade juices.

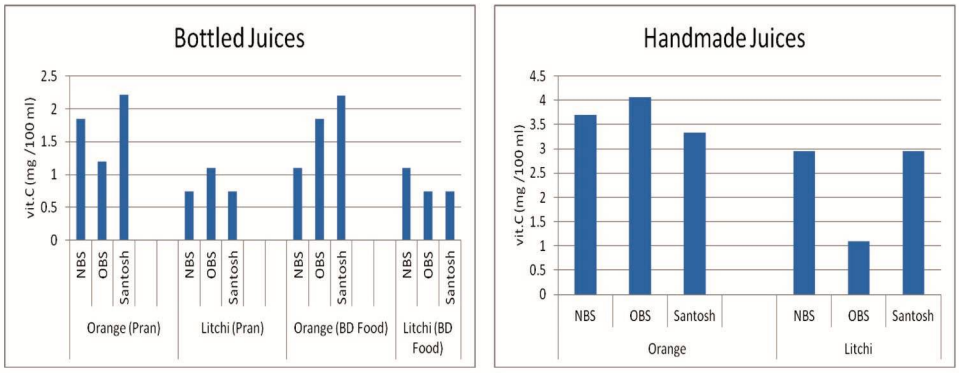

Fig. 6. Variation in concentrations of vitamin C in different samples. NBS = New bus stand, OBS = Old bus stand.

Correlation matrix among different microbial, physicochemical and nutritional parameters of fruit juices: Correlation matrix among the parameters was determined by Pearson's correlation coefficient along with their significant test (Table 1). Total viable count showed a negative corelation with pH and vitamin C and insignificant positive correlation with TSS and TCC. Total viable count, total coliform count, TSS had negative relation with pH.

Correlation matrix among the parameters was determined by Pearson's correlation coefficient along with their significant test are given in Table 2. From the table there found that vitamin C showed positive relation with pH, TSS, TVC and TCC but the

relation is not significant ($p>0.05$). pH has positive correlation with vitamin C but negative relation with TSS, TVC and TCC. Total coliform count showed a positive relation with TSS and Vit. C and negative relation with pH and TVC.

Table 1. Correlation matrix among the microbial, physicochemical and nutritional parameters of bottle juices.

	pH	TSS	VIT-C	TVC	TCC
pH	1				
TSS	–0.521	1			
Vit. C	0.492	–0.521	1		
TVC	–0.257	0.303	–0.127	1	
TCC	–0.258	0.038	–0.252	0.073	1

TVC = Total viable count, TCC = Total coliform count, TSS = Total soluble solids.

Table 2. Correlation matrix among the microbial, physicochemical and nutritional parameters of handmade juices.

	pH	TSS	Vit C	TVC	TCC
pH	1				
TSS	–0.395	1			
Vit. C	0.054	0.681	1		-
TVC	–0.142	0.418	0.351	1	
TCC	–0.008	0.618	0.323	–0.352	1

The results of the present study showed that, the juices were prepared and served in unhygienic environments, where a number of pathogenic microorganisms were found. However, while collecting juice samples, it was found that chopping board, knives, spoons, glass and jugs were also not frequently washed and a chance of cross contamination was also possible. Since juice samples, collected from different sampling sites of Tangail municipality were not satisfactory as total viable count, total coliform count were detected in large amount, so it could be hardly recommended that, consumption of commercially and handmade processed juices was safe. Most of the brands (bottled) and handmade juices exceeded the standard permissible in this study. Though the chemical parameters (pH, TSS) were within the recommended range, in some cases it exceeded the standard. From nutritional analysis, vitamin C was lower in bottled juices than the handmade juices. In respect to microbial, chemical and nutritional quality,

these juices were not safe for human consumption. In the long run, people may suffer from different diseases such as diarrhea, vomiting, cholera, botulism, nausea etc. However, government health agencies must adopt measures to educate the producers on food safety and hygienic practices. Regular monitoring of the quality of fruit juices for human consumption must also be enforced by authority/law enforcement agencies.

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ESTIMATION OF CARBON STOCK IN THE SYLHET BASIN SOILS OF BANGLADESH

M.J. UDDIN*, ARAFAT RAHMAN, A.H.M. ZULFIQUAR ALI
AND MD. KHALILUR RAHMAN
Department of Soil, Water and Environment, University of Dhaka, Dhaka-1000, Bangladesh

Abstract

Wetland basin soils are the major store houses of organic carbon where there is a scope to use this carbon in mitigating the climate change. A study was conducted in these basin soils at 100 cm depth regarding their carbon stock. The study showed that total soil organic carbon (SOC) stock in the Sylhet basin soils of Bangladesh is 0.094 Pg where the SOC stock was 0.044 Pg in medium low land sites and it was about 0.050 Pg in lowland sites. There was no previous study on SOC stock in the Sylhet basin soils of Bangladesh. These may act as benchmark SOC stock datasets for the future agricultural planning. The soil organic carbon stock is higher in the lowland than medium lowland sites. The contents of SOC are low is compared to its threshold levels. Moreover, it is apprehended that basin soils may lose their carbon due to the decrease of inundation level by climate change, and other eco-environmental changes. So, it is very much urgent to take steps in preserving the organic carbon of lowland basin soils.

Key words: Estimation, Carbon stock, Sylhet basin soils

Introduction

Soil organic carbon (SOC) is one of the main factors affecting soil quality and agricultural productivity. Being a source as well as storage of plant nutrients, SOC plays an important role in terrestrial C cycle (Freixo *et al.* 2002). Landuse has a significant effect on SOC storage, since it affects the amount and quality of litter input, litter decomposition rate, and stabilization of SOC (Bronson *et al.* 2004). Information on global and regional SOC pool in topsoil is generally available for a variety of landuse and climatic conditions (Batjes 1996). However, study on SOC storage in soils as affected by inundation of land is very scanty, particularly in Bangladesh. It is widely accepted that SOC is largely concentrated in the top 30 cm of the soil, but there is a growing evidence that deeper soil horizons have the capacity to sequester high amounts of SOC (Jobbagy and Jackson 2000, Swift 2001) and that this should be considered for SOC emission-storage analysis. The importance of SOC sequestration in sub-soils mitigating the greenhouse effect is related to the fact that subsoil SOC occurs in fairly stable and highly

*Corresponding author: mjuddin66@yahoo.com

recalcitrant forms to biodegradation (Batjes 1996, Kogel-Knabner 2002, Nierop and Verstraten 2003). SOC surveys usually consider a fixed soil depth, typically 1 meter. Global surveys based on vegetation units (Post *et al.* 1982) and soil taxonomic units (Eswaran *et al.* 1993, Batjes 1996) indicated that soil stores 1500-1600 Pg C in the top one meter depth. However, soil carbon can be underestimated in its global budgets by fixing a lower boundary at 1 m depending on the vertical distribution of SOC. A very important aspect of such studies would be in the area of mitigating the effects of global climate change, which has a direct relationship to agriculture and specifically to organic carbon management in soils (Johnson and Kerns 1991).

There are a few studies on the estimation of carbon stocks in the wetland basin soils of Bangladesh. Wetland basin soils are the major storehouse of organic carbon where there is a scope to use this carbon and energy in mitigating climate change. In Bangladesh, exploration of this research area may provide valuable information regarding their usage by estimating their storage. On the other hand, possibly due to the decrease of inundation level in Bangladesh and consequent intensive agricultural usage (Brammer 2002), the basin soils are losing their carbon contents, and thus lowland basin ecosystems are degrading. So, exploring the carbon storage as well as their sustainable usage is very much important in the recent days. In this connection, an attempt was undertaken to estimate carbon stock in the Sylhet basin soils of Bangladesh under the present situation of climate change.

Materials and Methods

The Sylhet basin is located in the extreme north-eastern part of the main basin, surrounded by the Shillong plateau, Tripura hills, and the Madhupur terrace Pleistocene uplands. The south boundary is a major fault scarp. The basin has an average altitude of about 4.5 m above mean sea level (MSL) at its center. It was earlier considered as a part of the Ganges-Brahmaputra delta. The Old Brahmaputra River (the original channel of the Brahmaputra) passes through the westernmost part. The present Brahmaputra course has an elevation of about 15 m above mean sea level, which shows that the Sylhet basin has subsided about 10.5-12 m during the last couple of hundred years (Mukherjee *et al.* 2009). The cause is certainly tectonic, associated with movements of the fault systems. Holocene fossil wood fragments have been found at depths of 15-18 m below surface. The surface is inundated every year during monsoon season. Geomorphologically, the basin possesses natural, continuously meandering levees with dendritic drainage (Umitsu 1985). The sediment composition of the basin grades from sandy or silty near the surface to fine sand at a depth of about 12 m.

Forty soil samples from the eight profiles at different soil depths (0-20, 20-40, 40-60, 60-80 and 80-100 cm) were collected. The sampling covers the north-eastern *Haor* basin regions of Bangladesh covering the 4 districts of Sylhet, Moulvibazar, Habiganj and Brahmanbaria. These samplings at different soil depths were used for the purpose of SOC storage and distribution as affected by soil depths and inundation land types. The inundation land type is a unique feature in Bangladesh and has taken into account in land management. In Bangladesh, five categories of inundation land types were identified by FAO-UNDP (1988). These were highland (HL), medium highland (MHL), medium lowland (MLL), lowland (LL), and very lowland (VLL), as outlined in Table 1.

Table 1. Classification of land types of Bangladesh and Sylhet basin based on inundation flood level.

Land types	Flooding depth	Bangladesh (%)	Sylhet basins (%)
Highland (HL)	Land which is above normal flood level	18	6
Medium highland (MHL)	Land which normally is flooded up to about 90 cm deep during the flood season	32	20
Medium lowland (MLL)	Land which normally is flooded up to between 90 and 180 cm deep during the flood season	12	19
Lowland (LL)	Land which normally is flooded up to between 180 and 300 cm deep during the flood season	6	20
Very lowland (VLL)	Land which is normally flooded deeper than 300 cm during the flood season	2	11

Source: (FAO-UNDP 1988, SRDI 2010).

These inundated land types are regarded as the biophysical units of a landscape. The inundated land types, MLL and LL were considered here only. Because these land types bear the lowland basin properties. The samplings were done in a topo sequence arrangement e.g. 2 profile samples from Zakigang border of Barak river where Surma and Kushiyara coincide, 2 profile samples from the Hakaluki *Haor* of Moulvibazar district, 2 profile samples from the Hobigang *Haor* sites and the other 2 profile samples from the *Haor* sites of Sarail, Brahmanbaria. Thus, these samples reflect a whole scenario of the basin or *Haor* ecosystems.

Soil samples from each site up to 1 m depth were collected in thick polythene bags. The soil samples were air dried under shade. The samples were then gently ground with rolling wooden roller and also with a wooden hammer and passed through 0.5 mm sieve

and mixed thoroughly. The samples were then preserved in plastic bags for soil organic carbon analysis.

Soil organic carbon (SOC) was determined by following the method of Walkley and Black (1934). Soil bulk density was determined by using the core method as described by Blake and Hartge (1986). Soil particle size analysis was determined by hydrometer method after pretreatments as described by Gee and Bauder (1986). It may be noted that the bulk density and SOC concentration (%) are the two prerequisites for estimating SOC stock or storage. Thus, the total soil organic carbon and total soil nitrogen storage were calculated using the following equations (Batjes 1996, Chen *et al.* 2007, Zhang *et al.* 2013).

Total soil organic carbon (TSOC) = $SOC_i \times B_i \times D_i$ Eq. (i)

Total soil nitrogen (TSN) = $TN_i \times B_i \times D_i$ Eq. (ii)

where, equation (i) represents TSOC; SOCi is the SOC content on the i^{th} layer (g/kg);

Equation (ii) represents TSN; TN_i is the total nitrogen content on the i^{th} layer (g/kg);

B_i is the bulk density of the i^{th} layer (g/cc), and D_i is the depth of the i^{th} layer (cm).

Results and Discussion

Guidelines for estimating greenhouse gas (GHG's) emissions from agriculture, forestry, and other land uses are provided by the Intergovernmental Panel on Climate Change (IPCC). The IPCC guidance includes specific details for estimating C stocks of upland forest ecosystems, however, specific provisions for tropical wetland soils and peat lands are seriously lacking (IPCC 2007). The close relationship between C density and bulk density allows for a reasonably accurate estimation of C stocks for tropical wetland soils. Eswaran *et al.* (1993) estimated soil organic carbon contents in the soil orders at global level as well as tropical regions. From the above datasets, Hussain (2002) reported that the soils of Bangladesh have a total of 2.2 Pg organic carbons. The above report revealed that in Bangladesh, Inceptisols and Entisols orders contains about 1.73 Pg organic carbons (Table 2). Possibly, this is the base line datasets of organic carbon mass in the soils of Bangladesh. Idris and Uddin (2013) showed that Sylhet basin soils occupy the soil orders of Inceptisols and Entisols. There is a serious lacking of SOC stock or storage data sets in Bangladesh even in the Sylhet basin soils of Bangladesh. So, it is expected that the soil organic carbon contents in the study site may be within this range.

The bulk density distribution of medium lowland (MLL) site in the Zakiganj Upazila under Sylhet district varied from 1.37 to 1.59 g/cc and the mean bulk density is 1.47 g/cc. The particle size analysis showed that silt is the dominant size fraction that varied from 60 to 85 per cent and its mean value is 69 per cent. The bulk density value in the Hakaluki *Haor* under Moulvibazar district ranged from 1.61 to 1.82 g/cc and the mean bulk density is 1.71 g/cc. The particle size analysis showed that clay is the dominant size fraction that varied from 53 to 68 per cent and the mean value is 59 per cent. The bulk

Table 2. Organic carbon mass in the soils of the world and of Bangladesh.

Soil orders	Area (10^3 Km^2)			Organic C (Pg)*		
	Global	Tropical	Bangladesh	Global	Tropical	Bangladesh**
Entisols	14921	3256	14.20	148	19	0.14
Inceptisols	21580	4565	97.50	352	60	1.59
Ultisols	11330	9018	0.89	105	85	0.08
Histosols	1745	286	1.20	357	100	0.25
Alfisols	18283	6411	1.20	127	30	0.03
Misc. land	7644	1358	24.00	18	02	0.05
Total	-	-	147.00	-	-	2.20

*Pg = Petagram = 1×10^{15} (Source: Eswaran *et al.* 1993), Hussain 2002**

density distribution in the Hobiganj site varied from 1.31 to 1.41 g/cc and the mean bulk density is 1.36 g/cc. The particle size analysis showed that silt is the dominant size fraction that varied from 68 to 83 per cent and the mean value is 75 per cent. The bulk density distribution in the Sarail site under the Brahmanbaria district varied from 1.44 to 1.67 g/cc and the mean bulk density is 1.56 g/cc. The particle size analysis showed that silt is the dominant size fraction that varied from 75 to 88 per cent and the mean value is 83 per cent. The higher bulk density has been observed in the Hakaluki *Haor* site of Moulvibazar district than that of other sites (Table 3).

The bulk density distribution of lowland (LL) site in the Zakiganj site under Sylhet district varied from 1.56 to 1.74 g/cc and the mean bulk density is 1.66 g/cc. The particle size analysis showed that silt is the dominant size fraction that varied from 45 to 60 per cent and the mean value is 51 per cent. The bulk density in the Hakaluki *Haor* under Moulvibazar district ranged from 1.59 to 1.92 g/cc and the mean bulk density is 1.70 g/cc. The particle size analysis showed that clay is the dominant size fraction that varied from 45 to 65 per cent and the mean value is 56 per cent. The bulk density distribution in

the Hobiganj site varied from 1.32 to 1.53 g/cc and the mean bulk density is 1.46 g/cc. The particle size analysis showed that silt is the dominant size fraction that varied from 75 to 88 per cent and the mean value is 81 per cent. The bulk density distribution in the Sarail site under the Brahmanbaria district varied from 1.43 to 1.75 g/cc and the mean bulk density is 1.56 g/cc. The particle size analysis showed that silt is the dominant size fraction that varied from 75 to 88 per cent and the mean value is 80 per cent. The higher mean bulk density has also been observed in the Hakaluki *Haor* site of Moulvibazar district than that of other sites (Table 4).

Table 3. Bulk density distribution (g/cc) at different soil depths in medium lowland sites across the Sylhet basin soils.

Depths (cm)	Sylhet Sadar site (Zakigonj site)	Moulvibazar site	Hobiganj site	Brahmanbaria site
0 - 20	1.49	1.65	1.37	1.49
20 - 40	1.59	1.77	1.36	1.52
40 - 60	1.37	1.70	1.32	1.67
60 - 80	1.44	1.82	1.31	1.44
80 - 100	1.50	1.61	1.41	1.65
Mean	1.47	1.71	1.36	1.56

Table 4. Bulk density distribution (g/cc) at different soil depths in lowland sites across the Sylhet basin soils.

Depths (cm)	Sylhet Sadar site (Zakigonj site)	Moulvibazar site	Hobiganj site	Brahmanbaria site
0 - 20	1.67	1.70	1.46	1.43
20 - 40	1.64	1.59	1.32	1.58
40 - 60	1.74	1.61	1.49	1.47
60 - 80	1.70	1.92	1.51	1.75
80 - 100	1.56	1.67	1.53	1.58
Mean	1.66	1.70	1.46	1.56

Soil organic carbon (SOC) distribution in medium lowland (MLL) soils in the Zakiganj site under Sylhet district varied from 0.81 to 0.96 per cent from surface to 100 cm depth and the mean organic carbon was 0.92 per cent (Table 5). SOC distribution in the Hakaluki *Haor* soils under Moulvibazar district ranged from 0.73 to 1.38 per cent from surface to 100 cm depth and the mean organic carbon was 0.97 per cent. SOC distribution in the Hobiganj site ranged from 0.73 to 0.88 per cent from surface to 100 cm depth and

the mean organic carbon was 0.80 per cent. SOC distribution in the Sarail site under Brahmanbaria district varied from 0.49 to 0.77 per cent from surface to 100 cm depth and the mean organic carbon was 0.58 per cent. On the other hand, SOC distribution of lowland (LL) soils in the Zakiganj sites under Sylhet district varied from 0.81 to 1.28 per cent from surface to 100 cm depth and the mean organic carbon was 1.11 per cent (Table 6). SOC distribution in the Hakaluki *Haor* soils under Moulvibazar district ranged from 0.53 to 0.98 per cent from surface to 100 cm depth and the mean organic carbon was 0.76

Table 5. Soil organic carbon distribution (%) at different soil depths in medium lowland sites across the Sylhet basin soils.

Depths (cm)	Sylhet site	Moulvibazar site	Hobiganj site	Brahmanbaria site
0 - 20	0.96	1.38	0.88	0.77
20 - 40	0.90	1.02	0.77	0.61
40 - 60	0.81	0.81	0.73	0.53
60 - 80	0.94	0.90	0.81	0.49
80 - 100	0.98	0.73	0.85	0.49
Mean	0.92	0.97	0.80	0.58

Table 6. Soil organic carbon distribution (%) at different soil depths in lowland sites across the Sylhet basin soils.

Depths (cm)	Sylhet site	Moulvibazar site	Hobiganj site	Brahmanbaria site
0 - 20	1.28	0.98	0.85	0.89
20 - 40	1.10	0.81	0.77	0.61
40 - 60	0.81	0.77	0.73	0.49
60 - 80	1.22	0.73	0.65	0.40
80 - 100	1.14	0.53	0.61	0.40
Mean	1.11	0.76	0.72	0.55

per cent. SOC distribution in the Hobiganj site ranged from 0.61 to 0.98 per cent from surface to 100 cm depth and the mean organic carbon was 0.72 per cent. SOC distribution in the Sarail site under Brahmanbaria district varied from 0.40 to 0.89 per cent from surface to 100 cm depth and the mean organic carbon was 0.55 per cent. The highest SOC concentration was found in the topsoil (0 - 20 cm) across the eight land types (Tables 5-6).

Soil organic carbon concentration depends on the balance between organic carbon input and loss from soils (Zhuang *et al.* 2007). Topsoil layer (0 - 20 cm) is tilled and receives greater residue inputs which are subsequently mineralized. Thus this layer possesses higher SOC than the other soil layers. Chaplot *et al.* (2010) reported that the topsoil layer may be able to sequester atmospheric CO_2 and thus mitigate climate change effect where more biophysical activities take place. Xiao-Wei *et al.* (2012) noted that surface soils are rich in SOC due to being covered by highly productive vegetation or subject to long-term use of organic fertilizers or flooding conditions. SOC concentration showed a decreasing trend from the top soil layer to the bottom layer for all land types of the study sites. It is important to note that lowland (LL) sites contain higher SOC than the medium lowland (MLL) sites due to the nature of inundation depths. Roose and Barthes (2001) noted that SOC is lost from soils of higher topography level, through erosion, runoff and leaching where erosion and runoff contribute a large portion of carbon losses and these are highly accelerated in cultivated land compared to undisturbed land. Uddin *et al.* (2019) mentioned that land inundation influences the organic carbon levels in soils. SOC threshold for sustaining soil quality is widely suggested to be about 2% (20 g/kg) below which deterioration in soil quality occurs (Patrick *et al.* 2013, BARC 2018). Krull *et al.* (2004) discussed some of the minimum and maximum thresholds of SOC, above or below which the effects of SOC on soil functions are noticeable. However, Sparling and Schipper (2002) argued that other than defining such maximum values, it is reasonable if minimum SOC levels (e.g. 2% i.e. 20 g/kg) are established to inform the farming community on levels below which there would be a loss of important soil characteristics. Thus, it is found that the study sites belong to minimum threshold of SOC level.

Soil organic carbon storage of medium lowland (MLL) soils in the Zakiganj sites under Sylhet district varied from 2.21 to 2.94 kg/m^2 from surface to 100 cm depth and the total organic carbon storage was 13.57 kg/m^2 (Table 7). The dominant landuse types were transplanted Aman and Boro rice. SOC storage in the Hakaluki *Haor* soils under Moulvibazar district ranged from 2.35 to 4.55 kg/m^2 from surface to 100 cm depth and the total organic carbon storage was 16.54 kg/m^2. Hakaluki *Haor* soils are used for the cultivation of Boro rice and in the dry season these are used as grazing grassland. SOC storage in the Hobiganj site ranged from 1.92 to 2.41 kg/m^2 from surface to 100 cm depth and the total organic carbon storage was 10.93 kg/m^2. The dominant landuse of this study site is deep transplanted Aman rice and it remains waterlogged for most of the year. SOC storage in the Sarail site under Brahmanbaria district varied from 1.41 to 2.29 kg/m^2 from surface to 100 cm depth and the total organic carbon storage was 8.93 kg/m^2.

Soil organic carbon storage of lowland (LL) soils in the Zakiganj sites under Sylhet district varied from 2.81 to 4.27 kg/m^2 from surface to 100 cm depth and the total organic carbon storage was 18.38 kg/m^2 (Table 8). The dominant landuse type is Boro rice. SOC storage in the Hakaluki *Haor* soils under Moulvibazar district ranged from 1.77 to 3.33 kg/m^2 from surface to 100 cm depth and the total organic carbon storage was 12.94 kg/m^2. Hakaluki *Haor* soils are used for the cultivation of Boro rice only and in the dry

Table 7. Soil organic carbon storage (kg/m^2) at different soil depths in medium lowland sites across the Sylhet basin.

Depths (cm)	Sylhet site	Moulvibazar site	Hobiganj site	Brahmanbaria site
0 - 20	2.86	4.55	2.41	2.29
20 - 40	2.86	3.61	2.09	1.85
40 - 60	2.21	2.75	1.92	1.77
60 - 80	2.70	3.28	2.12	1.41
80 - 100	2.94	2.35	2.39	1.61
Total SOC kg/m^2	13.57	16.54	10.93	8.93

Table 8. Soil organic carbon storage (kg/m^2) at different soil depths in lowland sites across the Sylhet basin.

Depths (cm)	Sylhet site	Moulvibazar site	Hobiganj site	Brahmanbaria site
0 - 20	4.27	3.33	2.48	2.55
20 - 40	3.60	2.57	2.03	1.92
40 - 60	2.81	2.47	2.17	1.44
60 - 80	4.14	2.80	1.97	1.40
80 - 100	3.56	1.77	1.86	1.26
Total SOC kg/m^2	18.38	12.94	10.51	8.57

season these are used as grazing grassland. SOC storage in the Hobiganj site ranged from 1.86 to 2.48 kg/m^2 from surface to 100 cm depth and the total organic carbon storage was 10.51 kg/m^2. The dominant landuse of this study site is Boro rice and it remains waterlogged for most of the year. SOC storage in the Sarail site under Brahmanbaria district varied from 1.26 to 2.55 kg/m^2 from surface to 100 cm depth and the total organic carbon storage was 8.57 kg/m^2. The variation in the soil organic carbon storage possibly due to their landuse, inundation level and land cover variations.

Soil organic carbon stock across the study sites up to 100 cm depth was estimated for the medium lowland and lowland sites was about 0.024 Pg and 0.022 Pg, respectively. The total SOC stock in the study sites was about 0.046 Pg (Table 9). Similarly SOC stock calculation was done in other sites of Sylhet basin e.g. Netrokona, Kishoregang and Sunamganj districts using the mean SOC values. The result showed that in medium lowland (MLL) sites, the SOC stock was 0.020 Pg and in the lowland (LL) sites, the SOC stock was 0.028 Pg. So, the total SOC stock in the above two sites was 0.048 Pg. So, it is found that the total SOC stock in the Sylhet basin of Bangladesh was 0.094 Pg. There is no previous study on SOC stock in the Sylhet basin soils of Bangladesh. Lal (2004) estimated that SOC pool in India was 21.0 Pg up to 30 cm depth and 63.0 Pg up to 150 cm depth, respectively. He also reported that SOC concentration in most cultivated soils is less than 10g/kg, which is consistent with the present study. The prevalent low levels of SOC concentrations are attributed to excessive tillage, imbalanced fertilizer use, and little or no return of crop residues to the soil.

Table 9. Carbon stock (Pg) across the study sites at 100 cm depth.

Study sites	*Areas (ha) in MLL sites	SOC stock in MLL sites (Pg)	*Areas (ha) in LL sites	SOC stock in LL sites (Pg)	SOC stock in both MLL & LL sites (Pg)
Sylhet Sadar	74,315	0.010	68,688	0.012	0.022
Moulvibazar	29,019	0.004	16,149	0.002	0.006
Hobiganj	48,214	0.005	59,530	0.006	0.011
Brahmanbaria	58,844	0.005	28,614	0.002	0.007
Total SOC (Pg)		0.024		0.022	0.046

*Soil Resource Development Institute (SRDI) 2010.

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STOCK SEPARATION OF *TENUALOSA ILISHA* IN BANGLADESH WATERS USING PARASITES AS BIOLOGICAL TAG

MD. AMINUL ISLAM BHUIYAN* AND ZANNATUN NAHAR JHINU
Department of Zoology, University of Dhaka, Dhaka-1000, Bangladesh

Abstract

Fourteen species under eleven genus of endohelminths were identified from alimentary canal and associated organs of host, only two of them had satisfied the criteria of being as biological tag. These are one species of cestode parasite named *Ilisha parthenogenetica* and two acanthocephalan parasite species named *Acanthocentis indica* and *Acanthocentis hilsai,* which are in together termed as *Acanthosentis* spp. The presence of these parasites in all the habitats indicates host's anadromous nature as well as higher prevalence of these tag parasites at respective sites and the trend of prevalence of infection in size class of host fish reinforced the belief that *T. ilisha* population in Bangladesh are largely anadromous in nature that cannot currently be divided into a group of discrete stocks and as a whole, a single stock of *T. ilisha* migrates from the sea to the rivers through the estuaries and vice versa.

Key words: *Tenualosa ilisha,* Stock separation, Parasite, Biological tag

Introduction

The 'Hilsa shad', *Tenualosa ilisha* belonging to the order Clupeiformes occurs in foreshore areas, estuaries, brackish-water lakes and freshwater rivers of south and south East Asia especially in Bangladesh (Pillay and Rosa 1963). Hilsa shad is the largest single fishable species in Bangladesh, present in almost all the major river systems, estuaries and marine environments (Bay of Bengal).

The Hilsa shad is largely an anadromous species, but two other ecotypes - a fluvial potamodromous type and a marine type - have also been recognized (Raja 1985). The anadromous stocks, whose normal habitat is the lower region of the estuaries and the foreshore areas, ascend the rivers during the breeding season and return to the original habitat after spawning (Raja 1985). The potamodromous stocks (river stocks) appear to remain in the middle reaches of the rivers throughout the year and breed there in. Marine stocks live all their life in the foreshore of the sea and not migrating to the river system (Amin *et al.* 2004). These stocks may differ in various aspects of their life history such as having different spawning grounds or feeding areas.

*Corresponding author: aminul_zool@du.ac.bd

The commercial value of this species has led to the use of parasites as biological marker for precise identification and definition of fish stocks as well as knowledge of the parasitic fauna and the spatial distribution of both parasite and fish population (Mosquera *et al*. 2000). Many different groups of parasites make suitable biological tags for stock separation, mainly because it is not usually necessary to select for parasites with long life spans in the subject host as well as being a natural process which avoid the stress associated with capture, handling and possible abnormal behavior of mechanically tagged species (Mackenzie 1987).

The purpose of the present study was to determine if parasites could be used to distinguish between different stocks of *Tenualosa ilisha* occurring in Bangladesh waters and, if so, to determine the stock composition and structure to identify intraspecific groups which are distinguished by different patterns of behaviors at certain stages of their life history within 'hilsa' population in Bangladesh waters.

Materials and Methods

The study was aimed at analyzing the stock composition and discriminating the stock of adolescent *Tenualosa ilisha* in Bangladesh waters using data on metazoan endoparasites, more specifically gut endohelminths as biological tags. Primary selection of parasites as biological tags was executed by using established criteria and then inferences were drawn on stock composition and stock discrimination.

Selection criteria of tag parasite to study fish stock composition: The sets of criteria for the study of anadromous fish stock (hilsa) in Bangladesh might be best fitted with the Margolis *et al.* (1982) selection criteria for salmonids. The criteria are mentioned as follows:

1. The parasite must be present in one stock or group of stocks and absent or rare in others.
2. The parasite infection should occur within a limited area and time before the stock become mixed in the ocean.
3. The parasite should have significantly different levels of infection in the target host in different parts of the study area.
4. The parasite might have long life span, preferably as long as that of its fish host, or at least as long as that period of the host's life over which observation on stock was being made.

5. The parasite should have no marked effects on survival and behavior of the fish if the parasite is to be used to estimate proportions of stock in a mixed stock sample.
6. The parasite should be easily detected and identified.

Selection of variable: Prevalence is considered to be a most useful parameter as it is less variable and represents the entire data set (both infested and non-infested fish) that is used as an indicatory variable for tagging in this study.

Sample collection: A total of 2667 host fish, *Tenualosa ilisha* were collected from three different ecological habitats of Bangladesh waters, to be more specific 1565 host fishes from the rivers in Chandpur (23°14.40 N 90°40.73 E), Aricha (23°46.10 N 89°46.83 E), Paksey (24°04.46 N 98°02.15 E), Sherpur 24°37.65 N 91°40.82 E); 609 host fishes from estuaries in Patuakhali (22°21.42 N 90°21.10 E), Barguna (22°09.45 N 90°07.61 E), Bhola (22°43.10 N 90°40.53 E), Barishal (22°42.23 N 90°22.49 E) and Sandwip (22°30.56 N 91°42.78 E); 493 host fishes from the sea in Cox′s Bazar (21°25.09 N 91°59.97 E) and Kuakata (21°48.97 N 90°07.32 E).

Sample processing: Both morphometric and meristic characteristics of the host fishes were analyzed and the weight, length and sex of each fish were recorded. The viscera and organs (cardiac stomach, pyloric caeca, stomach, intestine and mesenteries) were removed individually through simple dissection and kept in 10% formalin in polyethylene bags with a label inside. Laboratorial analysis was performed at Parasitology laboratory, Department of Zoology, University of Dhaka, Dhaka, Bangladesh. Extensive search was made for helminth parasites infecting the fish using microscope. Parasites from each organ were sorted, cleaned and counted which was followed by preservation in 70% alcohol. Berland's methods were used for staining and mounting.

Data analysis: As parasites counts were not normally distributed, nonparametric ANOVA using ranked scores (SAS PROC GLM procedure) was performed to accomplish multivariate analysis among samples of different habitat. Square root transformation of parasite numbers was done to bring frequency distribution close to normal for ANOVA. All statistical tests were done by SAS version-6. Only the data on component parasites were used in calculation.

Results and Discussion

The biological tag studies are usually based on differences in prevalence of infection between samples of different ecological habitats. A rich and versatile parasitic fauna have

been found in host fish, *Tenualosa ilisha* in the study. Overall 14 species under 11 genus of endohelminths have been collected and identified from alimentary canal and associated organs (mesenteries and caeca). These are five trematode species: *Aphanurus stossichi, Faustula brevichrus, F. gangetica, F. ilishii, Lecithaster indicus;* two cestode species: *Ilisha parthenogenetica* (plerocercoid) and *Otobothrium ilisha* (plerocercoid); five nematode species: *Goezia bangladeshi* (adult and larvae), *Camallanus* sp. (larvae), *Porrocaecum* sp. (larvae), *Capillaria* sp. (larvae) and *Hysterothylacium* sp. (larvae) and two acanthocephalan species: *Acanthosentis indica* and *Acanthosentis hilsai.* Three species of *Faustula* were counted together and referred as *Faustula* spp. and two species of *Acanthocentis* were counted together and referred as *Acanthosentis* spp.

All of them were present in freshwater, brackish and salt water stock indicating a remarkable spatial stability. Prevalence of different parasites in *Tenualosa ilisha* from three different habitats such as freshwater, brackish water and marine water bodies are shown in Table 1.

Table 1. Prevalence (%) of different parasite species in different habitats.

Group	Parasites	Fresh water	Brackish water	Marine	p
Trematoda	*Aphanurus stossichi*	77.45	94.80	91.70	> 0.05 NS
	Faustula spp.	49.13	77.89	67.17	> 0.05 NS
	Lecithaster indicus	17.16	58.72	29.43	< 0.05*
Cestoda	*Otobothrium ilisha*	1.92	0.00	1.51	> 0.05 NS
	Ilisha parthenogenetica	4.02	38.23	11.32	< 0.01**
Nematoda	*Goezia bangladeshi*	34.76	31.80	34.34	> 0.05 NS
	Camallanus sp.	1.00	0.00	0.38	> 0.05 NS
	Capillaria sp.	1.40	0.61	0.75	> 0.05 NS
	Porroceacum sp.	1.00	0.00	0.75	> 0.05 NS
	Hysterothylacium sp.	10.20	2.75	1.51	> 0.05 NS
Acanthocephala	*Acanthosentis* spp.	8.40	3.36	4.91	< 0.01**

NS, not significant; *significant at 5% level and **significant at 1% level. Three species of *Faustula* were counted together and referred as *Faustula* spp. And two species of *Acanthocentis* were counted together and referred as *Acanthosentis* spp.

Of the parasites only 9 had the status of component parasite (prevalence above 10%) (Bush *et al.* 1990). These are *Aphanurus stossichi, Lecithaster indicus, Faustula brevichrus, F. gangetica, F. ilishii, Ilisha parthenogenetica, Goezia bangladeshi,*

Acanthosentis indica and *Acanthosentis hilsai*. Prevalence of only three species of them named *Lecithaster indicus*, *Ilisha parthenogenetica* and *Acanthosentis* spp. were distinctly different which were statistically significant. However, short life span (<1 year) of *L. indicus* (adult digenean) in the alimentary canal of fish limited their use as biological tags. Therefore *Ilisha parthenogenetica* and *Acanthosentis* spp. were appeared to be satisfied with the selection criteria due to their long survival (several years) often for the life of the fish for the use of parasites as tags to study stock composition. It can be compared with the work of Stanley *et al.* (1992), in which study only 2 parasites were found out of 25 and Boje *et al.* (1997), in which study 6 parasites were found out of 21 to satisfy the criteria of tag parasite.

A number of researchers (Khan *et al.* 1980, Gaevkaya and Shapiro 1981, MacKenzie and Mehl 1984, Bamber and Henderson 1985) concluded that for fish which spend their entire lives in either marine or freshwater such parasites are often found throughout the host range and stocks are separated by differences in prevalence or mean intensity of infection. The dominant component of parasite faunas, however, does not necessarily make the best tags. Species which occur less commonly or only as incidental parasites of the host being studied are probably the most convincing. Therefore the role of incidental parasites as tags is also discussed. These parasites can only be acquired by the host in the restricted areas within its range. Outside these enzootic areas the life cycle of the parasite cannot be completed because conditions are unfavorable.

An obvious requirement of tag parasites are significantly different levels of infection in the subject host in different ecological sites of study area. This is based on the premise that each site has its own characteristic parasite community which a migrant host may acquire or lose as it travels. In this study among the component parasites the prevalence of *Faustula* spp., *A. stossichi*, *L. indicus* and *Goezia bangladeshi* did not vary considerably between sites but that of *I. parthenogenetica* and *Acanthosentis* spp. varied. Detection of encysted or free *Acanthosentis* spp., *Goezia bangladeshi* and *I. parthenogenetica* or free adult *L. indicus* in different sites requires little dissection. They are clearly visible to the naked eye or dissection microscope and are identified easily. In their fish intermediate or paratenic host they are present as encysted or free during which no feeding take place; therefore no pathological impact is likely to be observed. Also, they are likely to have long life spans. Prevalence of helminthic infection in each sampling sites were observed (Table 2).

Table 2. Prevalence of parasites of hilsa in all the eleven sampling sites.

Parasite	Chandpur	Aricha	Sherpur	Paksey	Bhola	Patua-khali	Barguna	Barisal	Sandwip	Kuakata	Cox's Bazar
Faustula spp.	72.01	82.39	76.27	86.79	93.98	100	98.95	95.59	88.57	94.34	89.94
A. stossichi	75.57	82.09	77.97	54.72	67.47	81.82	90.53	79.41	71.43	82.08	57.23
L. indicus	43.51	47.46	44.07	41.51	32.53	100	62.11	80.88	57.14	57.55	10.69
O. ilisha	01.53	01.79	0	0	0	0	0	0	0	0	02.52
I. parthenogenetica	14.25	09.55	20.34	22.64	21.69	0	54.74	42.65	37.14	27.36	0.63
Goezia bangladeshi	28.24	40.90	44.07	33.96	31.33	18.18	40.00	14.71	40.00	33.96	34.59
Camallanus sp.	0	0.30	0	0	0	0	0	0	0	0.94	0
Capillaria sp.	0.51	0.90	0	0	0	0	02.11	0	0	01.89	0
Porroceacum sp.	0	0.60	0	0	0	0	0	0	0	0	01.26
Hysterothylacium sp.	01.27	0.30	06.78	0	0	9.09	07.37	01.47	0	0	02.52
Acanthosentis spp.	04.83	08.36	22.03	15.90	3.61	9.09	02.11	01.47	07.14	05.66	04.40

It is evident (Table 2) that among the component parasites, *I. parthenogenetica* had higher prevalence in brackish water stock, respectively in Barguna, (54.74%), Barishal (42.65%) and Sandwip (37.14%). On the contrary, *Acanthosentis* spp. had higher prevalence in freshwater stock, respectively in Sherpur (22.03%), Paksey (15.90%), Aricha (8.36%) and Chandpur (4.83%). Though the prevalence of infection by nematodes was very low, they occurred more or less in almost all the sites.

To exemplify, the prevalence of *I. parthenogenetica* and *Acanthosentis* spp. was measured in respect of the body length of host fishes that can be resembled with their age or life stages. Host fishes were divided into nine arbitrary groups according to their length which ranges between 17.2 and 58 cm.

The results on distribution of parasites in different size groups indicate that *I. parthenogenetica* had highest prevalence (22.73%) in the smallest size group of hilsa in freshwater. In brackish water the prevalence of *I. parthenogenetica* was relatively higher than the freshwater and it showed conformity in Group-2 to Group-5 size classes (33.33 - 41.9%) that was followed by fluctuations in the larger groups (Group-6 to Group-9) ranging 14 - 60.61%. The higher prevalence of *I. parthenogenetica* in brackish water sites especially in middle size class indicated that the cestode parasite was recruited in brackish water sites after or before the host fish, 'hilsa shad' migrated to the other sites and the host fish spent there much time for feeding. In marine habitats, prevalence of *I. parthenogenetica* did not show any pattern with regard to the size groups of host fish and no *I. parthenogenetica* could be found in the smallest and the largest groups (Table 3).

Similarly, *Acanthosentis* spp. showed the highest prevalence in the smallest size group and infected all groups except largest size group (group-9) in the freshwater habitat while in brackish water and marine samples no fish were found infected in smaller size classes (1^{st} and 2^{nd} groups). In brackish water, *Acanthosentis* spp. infection was found only in the middle groups (Group-3 to Group-5). In marine sample, *Acanthosentis* spp. was found in the middle and larger groups (4^{th} to 8^{th} groups) (Table 3). The higher prevalence of *Acanthosentis* spp. in freshwater sites indicated that this parasite was recruited in the freshwater stock before or after migrating to sea or estuary.

Therefore, both the prevalence in different size groups and in different sites supported the assumption that *Acanthosentis* spp. was recruited in freshwater and *I. parthenogenetica* was recruited in brackish water habitats in host, *Tenualosa ilisha* in Bangladesh. The results on distribution of parasites in respect of size specify that the hilsa acquired infection of *Acanthosentis* spp. in the freshwater stock at a very tender age whereas, infection of *Acanthosentis* spp. was found in middle aged in brackish water stocks and in

Table 3. Prevalence of infection by tag parasites in different length groups of *T. ilisha*.

Class No.	Size classes (cm)	Freshwater		Brackish water		Marine	
		Ilisha parthenogenetica	*Acanthosentis* spp.	*Ilisha parthenogenetica*	*Acanthosentis* spp.	*Ilisha .parthenogenetica*	*Acanthosentis* spp.
Group-1	15 - 20	22.73	22.73	-	-	-	-
Group-2	20 - 25	08.52	05.68	33.33	0	20.00	0
Group-3	25 - 30	16.46	06.10	39.62	4.76	17.65	0
Group-4	30 - 35	11.56	04.76	41.90	7.46	05.00	03.33
Group-5	35 - 40	13.91	11.30	34.33	3.03	13.86	06.93
Group-6	40 - 45	15.46	13.40	60.61	0	08.93	03.57
Group-7	45 - 50	12.50	06.25	14.29	0	21.43	07.14
Group-8	50 - 55	16.67	13.89	22.73	0	11.11	11.11
Group-9	55 - 60	0	0	50.00	0	0	0

The fish were divided into 9 class sizes. No fish fell in the 1st length class in brackish water and marine samples.

middle and older age in marine stocks. This trend of *Acanthosentis* spp. infection supported the postulation that as the *T. ilisha* fishes become larger they move to the estuarine and marine stock.

Prevalence of *Goezia bangladeshi* did not vary widely in size classes of freshwater hilsa. It ranged from 29.88 - 50%. In brackish water prevalence in hilsa of *Goezia bangladeshi* was almost similar in 2^{nd} to 6^{th} class. Prevalence varied greatly in the larger size classes (7^{th} - 9^{th} groups). In marine hilsa higher prevalence of the parasite was observed in intermediate to larger size class.

Except for *Acanthosentis* spp. and *I. parthenogenetica* infection, there was little difference in the rest of the parasite fauna of *T. ilisha* captured in freshwater, brackish water and salt water. Several salt water parasites (notably all the trematodes and nematodes) were common in fish captured in freshwater and brackish water, illustrating the ability of these parasites to survive in their host during movement into estuarine and freshwater conditions. The scarcity of freshwater parasites in the *T. ilisha* suggested that although they do enter freshwater, most probably they do not spend extended periods of time feeding there. Additionally, the presence of some larval nematodes (incidental parasites) that have sea mammals (cetaceans) as their definitive hosts and which were found in all the habitats indicated that *T. ilisha* serves as an intermediate host of those parasites and travel from sea to freshwater via the estuary.

So it can be inferred that, the consistent prevalence of parasites which are not site specific (trematodes) and higher prevalence of *Acanthosentis* spp. and *I. parthenogenetica* parasites at respective sites which are site specific reinforced the belief that the hilsa, as a whole, migrates from the sea to the rivers via the estuaries and vice versa. Moreover, the presence of all the parasites in all the habitats and the trend of prevalence of infection in size class of hilsa (Table 3) indicated that *T. ilisha* population in Bangladesh are largely anadromous in nature that cannot currently be divided into a group of discrete stocks. Moreover, no other published information is available on stock composition or stock discrimination based on parasitological data, which limits the scope of direct comparison. Even results of earlier works using different method on stock composition based on different techniques (other than biological tags) are inconclusive.

Blaber *et al.* (2003) supported the inference as they failed to find any evidence of more than one stock of *T. ilisha* in Bangladesh waters. This study was based on genetic (allozymes), morphometrics and otolith microchemistry and concluded that *T. ilisha* mix and move through the country in different direction without any pattern. Conversely, Rahman *et al.* (1997) found that the riverine stocks of hilsa shad are significantly

different in genetic structure from those found in the marine environment and on the basis of morphometric and meristic characters. Rahman *et al.* (1998) predicted the possibility of having at least four stocks of hilsa in Bangladesh waters. However, extensive study over longer period on life cycle of the parasites and biology of hilsa will reveal more accurate conclusion on *T. ilisha* movements and stock composition in Bangladesh.

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GROWTH AND DEVELOPMENT OF NESTLINGS OF WHITE-THROATED KINGFISHER, *HALCYON SMYRNENSIS* (LINNAEUS, 1758)

HABIBON NAHER*[1] AND NOOR JAHAN SARKER[2]
[1]*Department of Zoology, Jagannath University, Dhaka-1100, Bangladesh*
[2]*Department of Zoology, University of Dhaka, Dhaka-1000, Bangladesh*

Abstract

The growth and development of the nestlings of white-throated kingfisher (*Halcyon smyrnensis*) showed that at the hatching day, the mean body weight was 14.6±1.0 g which gained up to 69.9±3.0 g during fledging. The mean length of the body, wing, beak, head, tarsus and feet were 59.4±12.4, 19.4±5.7, 3.1±1.2, 11.7±1.4, 8.5±1.8 and 17.3±3.6 mm, respectively at the hatching day and 203.5±14.1, 105.7±5.8, 40.4±1.3, 29.1±1.1, 14.9±0.4 and 29.8±0.7 mm, respectively during fledging day. The primaries, rectrices and the claw was started to grow from 3rd day hatching and grew up to 67.8±5.6, 27.7±3.4 and 5.2±0.2 mm, respectively during fledging time.

Key words: Nestlings, White-throated, Kingfisher, Development, Hatching, Fledging day

Introduction

White-breasted or white-throated kingfisher (*Halcyon smyrnensis*) is a very common resident bird of various habitats, mostly in the plains of open country with trees, electric wires and other perches (Ali *et al.* 2010). It ranges throughout much of the Indian subcontinent, except parts of the north-west (Grimmett *et al*. 1998). It nests in holes in sandy or loamy embankments which are considered as providing protection from changes in weather (Hoogland and Sherman 1976) and from predators (Lack 1968). But the Kingfisher's mortality increases as a result of human anthropogenic activities (Animal Diversity 2019). Water pollution, bioaccumulation of pollution and toxins in fishes affect the mortality rates of kingfishers or changes in water habitat reduce the number of nesting sites for kingfishers. Nestlings also die from flooding of the nest (Animal Diversity 2019). Kingfishers have relatively high reproductive rates, compensating for increased mortality in some areas (Fioratti 1992, Rayner *et al.* 1991).

In addition to some casual information on status and distribution of kingfishers in Bangladesh (Islam and Kamruzzaman 2008, Reza *et al.* 2003, Islam *et al.* 1999, Husain 1979, Khan 1986), some information are available on food habits, preying frequency, preying techniques, nesting behaviour (Naher and Sarker 2014, 2015, 2016, 2018). Some

*Corresponding author: likhi.habibon@gmail.com

information are available on the growth of white-throated kingfisher in Cauvery Delta of Tamil Nadu in Southern India (Ali *et al.* 2010). Information on growth and development of nestlings of kingfishers in Bangladesh is scanty. Therefore, an attempt has been taken to study the growth and development of the nestlings of white-throated kingfisher during their nestling periods. The main objective of this study was to establish the weight of the body and length of different body parts of the nestlings of white-throated kingfisher during nesting period.

Materials and Methods

The study was carried out from September, 2008 to August, 2011. Growth changes in the nestlings were measured from hatching to fledging day. All the nests were visited at every third day, taking both photographs of the young and morphometric measurements of body parts. In total, 167 observations were taken. Altogether, 27 nestlings were examined, 20 from Dhaka district and seven from Chittagong University Campus (CUC). A total of 17 nestlings (12 from Dhaka and 5 from CUC) were able to fly due to loss of nestlings for various reasons. Disturbances were minimized by handling the nestlings very carefully during taking the measurements. All the nestlings were allotted individual identification marks with permanent ink pen. The measurements of different parameters were taken followed by Ali *et al.* (2010): (i) body weight, using a spring balance (graduated between 5 and 1000 g) and pan balance (graduated between 0 and 2000 g), (ii) body length, from the tip of the beak to the tip of the longest rectrix, using a slide calipers (graduated up to 0 to 150 mm) and steel scale (0 to 300 mm), (iii) wing length, as the straight length from the bend of the wing to the tip of the wing, using a tape scale, (iv) length of the primaries, from the tip of the wing to the tip of the longest primary, using a tape scale, (v) tail feather or rectrices length, the distance from the tip of the longest rectrix to the base of the middle rectrices, using a tape scale, (vi) tarsus length, measurement from the base of the tarso-metatarsus to the base of the middle toe, using a slide calipers, (vii) head length, from the culmen to the nape, using slide calipers, (viii) bill/beak length, from the tip of the mandible to the base of the culmen, (ix) claw length, from the base to the tip of the claw, using a slide calipers and (x) feet length, from the middle toe to the posterior toe or hallux, using a slide calipers.

Study area: The study was carried out at Dhaka and Chattagram. In Dhaka, the nests in Madhabchala and Boro-Walia villages of Savar Upazila and in Chattagram, Chittagong University Campus (CUC), Bangladesh were selected for collecting data on growth of the nestlings.

The Madhabchala of Savar Upazila in Dhaka district is located at the western side of Jahangirnagar University of Savar. The Barawala was located at the western side of the village Madhabchala. The vegetation of the study sites was several fruiting (mango, *Mangifera indica;* jackfruit, *Artocarpus heterophyllus;* jam, *Syzygium cumini;* coconut, *Cocos nucifera;* jambura, *Citrus grandis;* guava, *Psidium guajava*) and wood trees (koroi, *Albizia procera;* segun, *Tectona grandis)* and bamboo (*Bambusa* sp.)

The CUC is located at the village Fatehpur under Hathazari Upazila of Chattagong district. The CUC stretches over 512.2 ha landscape of green hills, undulating valleys, moulds, plain grassland, bush and forests (Islam *et al.* 1979). Seventy-two per cent of the campus area is hilly and comprising small hills and the remaining areas are either plains or valleys (Islam *et al.* 1979). There are some creeks and streams within the hill area. Vegetation type is mixed-evergreen (Champion 1936). The natural vegetation of the campus is affected by biotic and abiotic factors especially due to human habitations and earth erosions. Consequently the primary vegetation of this area is totally lost. Hence, the vegetation of this area is now secondary one. The secondary forest grew with weeds environment such as thickest with a few scattered trees, thatching grasses and some bamboos (Chowdhury 2002).

Results and Discussion

The measurements of the growth and development of different body parts of the nestlings of the white-throated kingfisher are described under the following headings.

Body weight (in g): At hatching day, the average weight of the nestlings was 14.6±1.0 g (range 12.0-16.46, n=27). The nestling gained weight gradually and reached maximum of 70.8±4.6 g at 15 days (range 63.1-79.9, n=27) from the first hatching. The weight (63.5±5.9 g, range 54.9-75.9 g, n=17) then started to decline (13.2%). After 18 days, the nestling gained weight again (69.9±3 g, range 63.3-73.9 g, n=17) up to fledging time (21st days) (Fig. 1). Ali *et al.* (2010) reported that nestlings of the white-throated kingfisher grew from 3.7 to peak weight of 70.5 g, then slowly declined and reached to the weight of 61.8 g at the time of fledging. Many observers have noted a decrease in rate-of-gain in weight (Naher and Sarker 2011, Welty 1982) as feathers were being produced or as temperature control was being established (Ali *et al.* 2010) or due to the utilization of fat deposits and skeletal muscles for the energy to leave the nest (Welty 1982). This body weight reduction at fledging day is advantageous for moving out from the nest (Kumar and Rao 1984, Haggerty 1994, McCarty 2001, Penteriani *et al.* 2005, Greeny 2008, Asokan *et al.* 2009a,b, Asokan *et al.* 2010).

The average growth rate of the nestling of the white-throated kingfisher was 2.6 g per day during nesting period. The weight of the nestlings found significantly increasing (χ^2= 84.5, df=7, p<0.001) and the increasing trend was significantly correlated with the age of the nestlings (r=0.963, df=7, p<0.001).

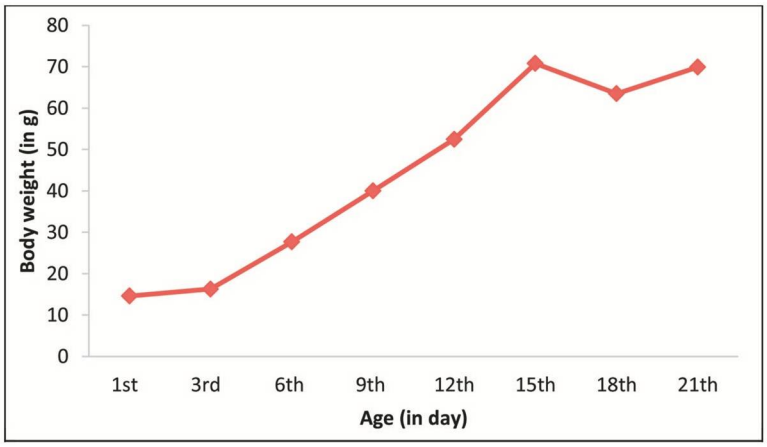

Fig. 1. Growth of the body weight (g) of white-throated kingfisher.

Body length (mm): The average body length of the nestlings was 59.4±12.4 mm (range 50-68.2 mm, n=27) at hatching day and 203.5±14.1 mm (range 182-230 mm, n=17) at the fledging day. Thus the average body length increased per day was 6.8 mm. The growth rate increased rather steadily from 3 to 6th day (Fig. 2). The body length of nestlings reached from 3.7±0.21 cm at hatching to 21.9±0.16 cm by the end of day 27 at Tamil Nadu in India (Ali *et al.* 2010) which is very close to present study.

The length of total body of the nestlings was found significantly increasing (χ^2=84.5, df=7, p<0.001) and the increasing trend was significantly correlated with the age of the nestlings (r=0.992, df=7, p<0.001).

Wing length (mm): At hatching day, the average wing length of the nestlings was 19.4± 5.7 mm (range 14-26.02 mm, n=27). It was increasing up to 105.7±5.8 mm (range 94-110 mm, n=17) during fledging day. Thus the average wing length increased per day was 4.1 mm. The growth rate increased rather sharply during 6-9th day.

The length of wing of the nestlings was found significantly increasing (χ^2=93.2, df=7, p<0.001) and the increasing trend was significantly correlated with the age of the nestlings (r=0.992, df=7, p<0.001).

Head length (in mm): The average head length of the nestlings' was 11.7±1.4 mm (range 8.1-18 mm, n=27) at hatching day and 29.1±1.1 mm (range 28-31 mm, n=17) at fledging day. Thus the average head length increased was 0.8 mm per day.

The length of head of the nestlings was found significantly increasing (χ^2=14.9, df=7, p<0.05) and the increasing trend was significantly correlated with the age of the nestlings (r=0.978, df=7, p<0.001).

Beak length (in mm): The average beak length of the nestlings' was 3.1±1.2 mm (range 2.1-5.12 mm, n=27) at hatching day and 40.4±1.3 mm (range 36.7-42.5 mm, n=17) at fledging day. Thus the average growth rate of beak was 1.8 mm per day. The bill length was 0.6±0.14 cm at hatching and it reached 4.5±0.05 cm on day 27 which is almost the same to the finding of Ali *et al.* (2010) at Tamil Nadu in India.

The length of beak of the nestlings was found significantly increasing (χ^2=47.8, df=7, p<0.001) and the increasing trend was significantly correlated with the age of the nestlings (r=0.973, df=7, p<0.001).

Length of primaries (mm): During hatching, the hatchling was naked, there was no feather over the body. From the 4th day of hatching, the primary feather of the wing started to grow slowly and it was increased up to 67.8±5.6 mm (range 57.1-74.2 mm, n=17) during fledging (Fig. 2). Thus, the average growth rate of primaries was 3.8 mm per day. The growth was highly occurred from 15 to 18 days. In Tamil Nadu in India the length of wing of white-throated kingfisher was 1.4±0.09 cm at the time of hatching, and it gradually increased and attained maximum length of 16.4±0.10 cm on day 27 (Ali *et al.* 2010). Cramp *et al.* (1988) reported that the feathers of pied kingfisher started to grow by the 4th day and flight feathers by the 11 to 13th days and fully developed in six weeks after leaving the nest.

The length of the primaries of the nestlings was found significantly increasing (χ^2=94.9, df=5, p<0.001) and the increasing trend was significantly correlated with the age of the nestlings (r=0.987, df=5, p<0.001).

Length of tail feather or rectrices (mm): It is mentioned earlier that the hatchlings were naked during hatching. Rectrices started growing after the 7th day of hatching. During fledging it reached upto 27.7±3.4 mm (range 19.1-32.1 mm, n=17) (Fig. 2). Thus, the average growth rate of rectrices was 1.2 mm. In Tamil Nadu, India the length of the tail of white-throated kingfisher was 0.2±0.05 cm at hatching and it increased to 4.3±0.04 cm during day 27 (Ali *et al.* 2010).

The length of the tail feather (rectrices) of nestlings was found significantly increasing (χ^2=32, df=4, p<0.001) and the increasing trend was significantly correlated with the age of the nestlings (r=0.981, df=4, p<0.001).

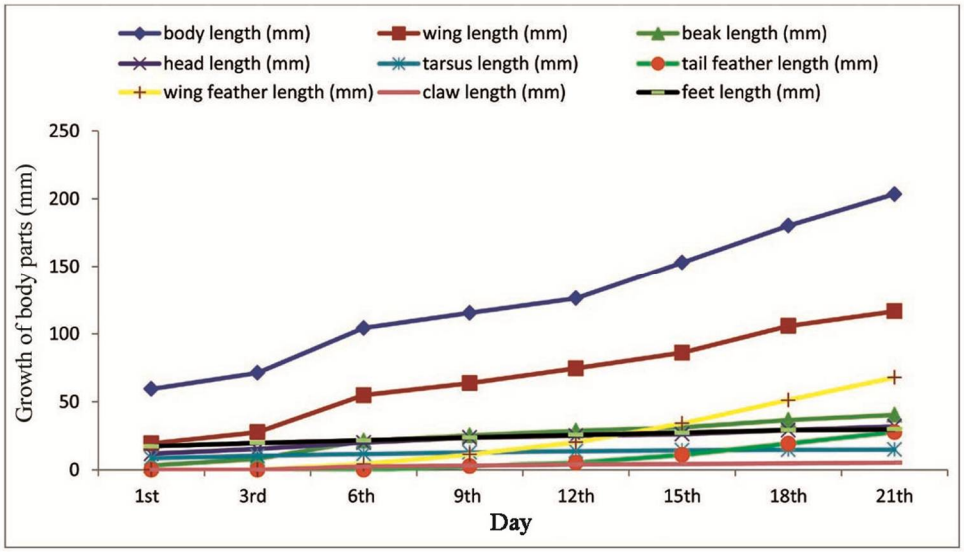

Fig. 2. Growth of different body parts (mm) of white-throated kingfisher.

Tarsus length (in mm): The average tarsus length of the nestlings' was 8.5±1.8 mm (range 8-9.9 mm, n=27) at hatching day and 14.9±0.4 mm (range 14.4-15.8 mm, n=17) at fledging day (Fig. 2). Thus, the average tarsus length increased was 0.3 mm per day. The tarsus length during hatching was 0.6±0.14 cm and attained the maximum size of 3.5±0.04 cm during fledging at Tamil Nadu in India (Ali *et al.* 2010).

The length of the tarsus of the nestlings was found significantly increasing (χ^2=2.9, df=7, p>0.05) and the increasing trend was significantly correlated with the age of the nestlings (r=0.964, df=7, p<0.001).

Claw length (in mm): There was no sign of claw of the nestlings at hatching day to the 3rd day of their life. The average claw length of the nestlings at fledging day was 5.2±0.2 mm (range 4.6-5.6 mm, n=17). Thus the average growth rate of claw was 0.2 mm per day.

The length of claw of the nestlings was found significantly increasing (χ^2=1.4, df=5, p>0.05) and the increasing trend was significantly correlated with the age of the nestlings (r=0.989, df=5, p<0.001).

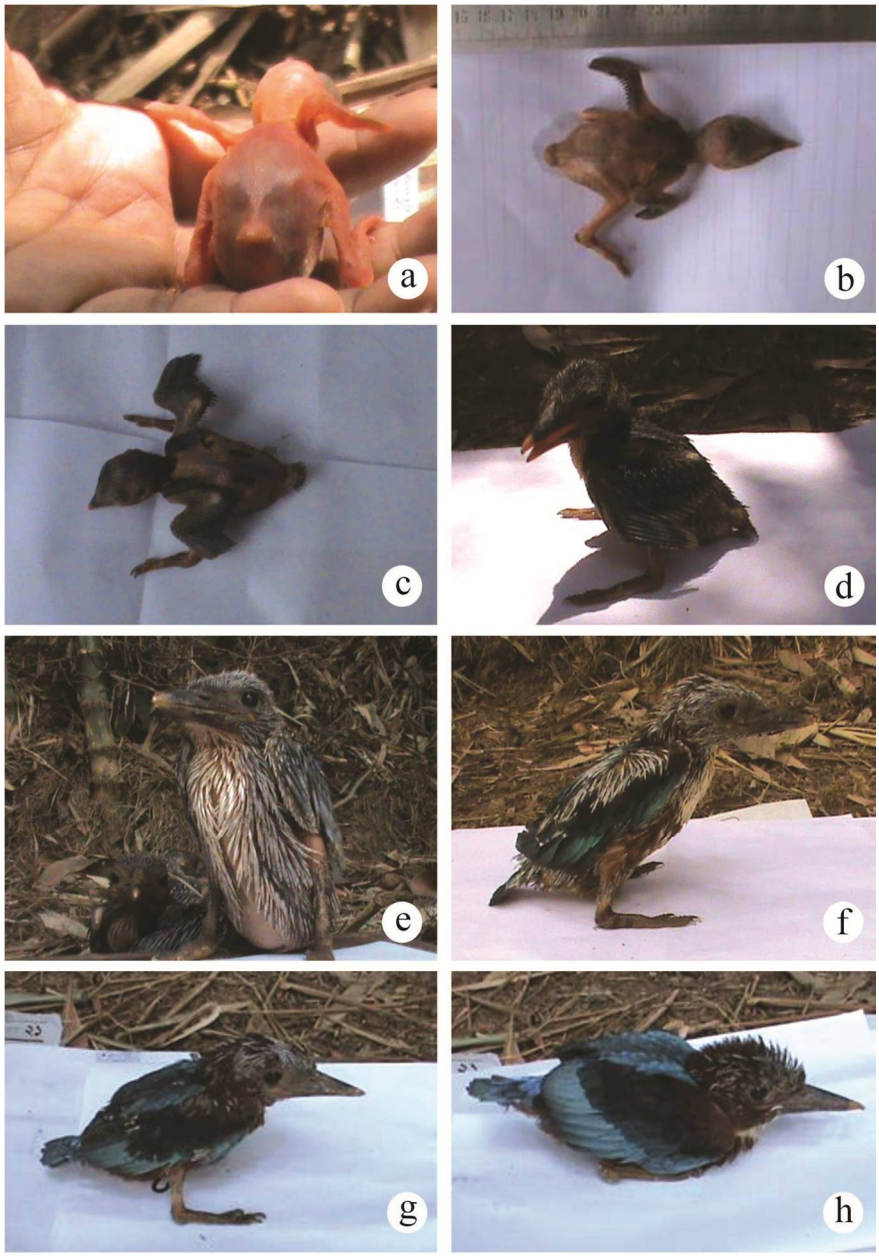

Fig. 3. Nestlings of white-throated kingfisher at different ages from hatching day to fledging day, (a) New born hatchling, (b) 3 days old, (c) 6 days old, (d) 9 days old, (e) 12 days old, (f) 15 days old, (g)18 days old, and (h) 21 days old.

Feet length (mm): The average feet length of the nestlings was 17.3±3.6 mm (range 16.3-19.8 mm, n=27) at hatching day and 29.8±0.7 mm (range 29.1-30.7 mm, n=17) at fledging day. Thus the average growth rate was 3.03 mm per day.

The length of feet of the nestlings was found significantly increasing (χ^2=5.84, df=7, p>0.05) and the increasing trend was significantly correlated with the age of the nestlings (r=0.994, df =7, p<0.001).

The growth and development of different body parts was gradually increasing from the hatching day to fledging day (Fig. 3). The length of all body parts attained the maximum maturity at the time of fledging stage (Fig. 2). The present observations support the views of Ali *et al.* (2010), Naher and Sarker (2011). The white-throated kingfisher used different body parts immediately after fledging for successful survival. This kind of growth in the adaptive parts was observed in several avian species by many workers (Zach and Mayoh 1982, Kumar 1983, Teather 1996, Aparicio 2001, Pereyra and Morton 2001, Asokan *et al.* 2009a,b, 2010).

During hatching, the hatchling was naked with pinkish colour. It could not stand and there was no primaries, rectrices and claws. It could stand at the 9th days of its age. During 21st days, it could glide from nest to nearest branch of the tree. Within 24 to 26 days after hatching, the total clutch would able to leave the nest.

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EVALUATION OF FUNGICIDES AND PLANT EXTRACTS AGAINST PATHOGENIC FUNGI ASSOCIATED WITH *BASELLA* SPP.

NUSRAT JAHAN SHOVA, SAROWAR HOSEN AND M.A. BASHAR*
Department of Botany, University of Dhaka, Dhaka-1000, Bangladesh

Abstract

Five fungicides *viz*., CM 75, Dithane M 45, Knowin 50 WP, Nativo 75 WG and Rovral 50 WP were evaluated against *Colletotrichum lindemuthianum, Drechslera sacchari* and *Fusarium semitectum* following poisoned food technique. Out of five fungicides complete inhibition of radial growth of *C. lindemuthianum* was observed in Nativo 75 WG at 100 ppm. On the other hand, the complete inhibition of the growth of *D. sacchari* was observed with Rovral 50 WP at 400 ppm, whereas Nativo 50 WP showed complete growth inhibition at 500 ppm. CM 75 WP, Knowin 50 WP and Nativo 75 WG showed complete growth inhibition of *F. semitectum* at 100 ppm. Five different plant leaf extracts *viz*., *Azadirachta indica* A. Juss., *Heliotropium indicum* L., *Lippia alba* L., *Michelia champaca* L. and *Thuja occidentalis* L. were tested against the test pathogens. Of the five plant leaf extracts, *Lippia alba* showed the highest growth inhibition in *C. lindemuthianum, D. sacchari* and *F. semitectum* at 20% concentration.

Key words: Fungicides, Plant extracts, Pathogenic fungi, *Basella* spp.

Introduction

Basella is a popular tropical leafy-green vegetable, belongs to *Basellaceae* (Sushila *et al.* 2010) and has two chief cultivars namely, *Basella alba* and *B. rubra* (Cook 2010). A vast amount of yield is lost in terms of quantity and quality due to various constraints (Hasan *et al.* 2016). Proper management strategy of leaf spot of *Basella* spp. is very essential for the economical point of view. Various workers in different countries of the world evaluated the efficacy of various fungicides against *Colletotrichum* spp., *Macrophomina phaseolina*, *Fusarium* spp., *C. gloeosporioides* and *Alternaria* spp. under laboratory and field conditions (Hossain and Bashar 2011, Ahmed *et al.* 2014, Chowdhury *et al.* 2015, Mamun *et al.* 2016, Hosen *et al*. 2016, Islam *et al.* 2017). Some researchers have used different chemical fungicides to control leaf spot disease of *Basella* and have achieved various degree of success (Khan and Smith 2005). The residue of chemical fungicides poses potential health hazard (Alemu *et al*. 2014). The alternate approaches like using plant extracts were found to be effective against the pathogen (Uddin *et al.* 2013, Maketon *et al.* 2008). Plant extracts are considered as new rays of hope because they are

*Corresponding author: botanybashar@yahoo.com

eco-friendly and can be used as an alternative measure to control plant diseases. Recently, some researchers have indicated the possibility of their exploitation as natural fungitoxicants for controlling plant diseases (Sharmin and Shamsi 2013, Mamun *et al.* 2016, Hosen *et al.* 2016, Islam *et al.* 2017). The aim of this study was to evaluate the common and easily available fungicides as well as selected plants to determine minimum inhibitory concentration (MIC) values of various levels to find out the most suitable one to reduce yield and post-harvest losses caused by leaf spot of *Basella* spp.

Materials and Methods

The samples were collected from three markets of Dhaka city *viz.,* Karwan Bazar, Anando Bazar and Polashi Bazar during April to November, 2017. Fungi associated with the leaf spot of *Basella* spp. were isolated following Tissue planting method (CAB 1968). Pathogenicity test has been done according 'detached leaf assay' followed by Azad and Shamsi (2011) with slight modification. Five fungicides with different active ingredients *viz*., CM 75 WP (Mancozeb 63% + carbendazim 12%), dithane M 45(80% Mancozeb), Knowin 50 WP [50% carbendazim (methyl benimidazol-2-ylcarbamate)], Nativo 75 WG (500 g tebuconazol, 250 g trifloxy-strobin) and Rovral 50 WP (Iprodione) were collected from the Siddique Bazar, Fulbariya, Gulistan, Dhaka. These fungicides were evaluated for their *in vitro* efficacy at different concentrations (100, 200, 300, 400 and 500 ppm) against pathogenic fungi associated with *Basella* spp.

A total of five different plant leaf extracts *viz., Azadirachta indica* A. Juss., *Heliotropium indicum* L., *Lippia alba* L., *Michelia champaca* L. and *Thuja occidentalis* L. were screened against the selected test pathogens. Leaves of *Lippia alba* L. were collected from Bhairab, Narsingdi and the rest of the plant's leaves were collected from the Botanical garden of Curzon Hall campus, University of Dhaka. *In vitro* efficacy of selected plant leaf extracts at 5, 10, 15 and 20% concentrations were evaluated against the test pathogens following the method described by Helal and Shamsi (2018). The desired leaves of each plant were thoroughly washed in tap water, air dried and were prepared by crushing to known weight of fresh materials with distilled water in ratio of 1:1 (w/v). The pulverized mass of a plant part was squeezed through four-folds of fine cloth and the extracts were centrifuged at 3000 rpm for 20 min to remove particles. The supernatants were filtered through Whatman filter paper No. 1 and the filtrate was collected in 250 ml Erlenmeyer flask. The requisite amount of the filtrate of each plant extract was mixed with PDA medium to get 5, 10, 15 and 20% concentrations. In control, required amount of water was used instead of plant extract. All the Petri plates were incubated at 25±2^{o}C.

The radial growth of the test pathogen colonies was measured after 7 days. The per cent growth inhibition of each test pathogen was calculated using the following formula:

$$I = \frac{C - T}{C} \times 100$$

where, I = Per cent growth inhibition, C = Growth in control, T = Growth in treatment.

The data were collected as per cent inhibition of the radial growth of the test pathogens in mm and evaluated by ANOVA by using STAR statistical program and means were compared using DMRT.

Results and Discussion

Ten fungi *viz., Alternaria alternata, Aspergillus flavus, A. fumigatus, A. niger, Colletotrichum dematium, C. lindemuthianum, Curvularia lunata, Drechslera sacchari, Fusarium semitectum* and *Penicillium* sp. were isolated from the leaf spot of *Basella* spp. Among the isolated fungi, *C. lindemuthianum, D. sacchari* and *F. semitectum* were selected as test pathogens owing to their pathogenic potentiality.

Amongst the five fungicides, complete inhibition of the radial growth of *Colletotrichum lindemuthianum* was observed with Nativo 75 WG at 100 ppm concentration. Out of rest four fungicides, the highest growth inhibition of *C. lindemuthianum* was observed with CM 75 WP (68.84%) which was followed by Knowin 50 WP (63.08%), Rovral 50 WP (60.25%) and Dithane M 45 (59.10%) at 500 ppm concentration (Table 1).

Ann *et al.* (2017) reported the application of Nativo which significantly suppressed the development of leaf anthracnose and black berries disease caused by *Colletotrichum gloeosporioides.* Rajesha *et al.* (2010) reported that mancozeb completely inhibited the radial mycelial growth of *C. lindemuthianum* at 400 ppm concentration. Suresh and Ekbote (2005) also observed carbendazim as most effective in inhibiting the growth of *C. lindemuthianum.*

On the other hand, the complete inhibition of growth of *D. sacchari* was observed with Rovral 50 WP at 400 ppm concentration, whereas Nativo 75 WG showed complete growth inhibition at 500 ppm concentration and the highest growth inhibition (84.41%) at 400 ppm concentration (Table 2). Jadon and Shah (2012) observed that CM 75 WP at 500 ppm concentration completely inhibited the mycelial growth of *Drechslera bicolor.* Wahid *et al.* (1992) found thiophanate methyl as the best fungicide followed by captan against *Drechslera sacchari.* CM 75 WP, Knowin 50 WP and Nativo 75 WP showed

complete growth inhibition of *F. semitectum* at all the treated concentrations whereas Rovral 50 WP

Table 1. Inhibition of radial growth (%) of *Colletotrichum lindemuthianum* at different concentrations of fungicides.

Name of fungicides	% inhibition of radial growth at different concentrations (ppm)				
	100	200	300	400	500
CM 75 WP	50.14^{b}	56.58^{b}	59.05^{b}	61.42^{b}	68.84^{b}
Dithane M 45	14.18^{c}	46.73^{c}	49.17^{c}	52.01^{cd}	59.10^{d}
Knowin 50 WP	57.05^{b}	58.05^{b}	59.06^{b}	60.73^{bc}	63.08^{c}
Nativo 75 WG	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
Rovral 50 WP	15.40^{c}	21.38^{d}	36.78^{d}	50.57^{d}	60.23^{d}
CV (%)	8.62	3.70	5.82	5.50	2.60

Means followed by the same letter within a column did not differ significantly at 5% level by DMRT.

Table 2. Inhibition of radial growth (%) of *Drechslera sacchari* at different concentrations of fungicides.

Name of fungicides	% inhibition of radial growth at different concentrations (ppm)				
	100	200	300	400	500
CM 75 WP	36.59^{b}	52.51^{b}	61.45^{b}	64.24^{b}	65.64^{c}
Dithane M 45	22.11^{c}	57.19^{b}	60.35^{b}	60.70^{b}	73.68^{b}
Knowin 50 WP	11.08^{d}	18.18^{c}	22.72^{c}	26.14^{c}	38.35^{d}
Nativo 75 WG	74.03^{a}	79.74^{a}	80.00^{a}	84.41^{a}	100^{a}
Rovral 50 WP	71.80^{a}	72.58^{a}	84.33^{a}	100^{a}	100^{a}
CV (%)	6.56	7.03	4.41	3.11	3.57

Means followed by the same letter within a column did not differ significantly at 5% level by DMRT.

showed complete growth inhibition at 500 ppm concentration. Rovral 50 WP showed 62.46 and 75.44% growth inhibition at 300 and 400 ppm concentrations, respectively. Dithane M 45 showed 16.91, 20.69, 53.93, 57.72 and 69.39% growth inhibition at 100, 200, 300, 400 and 500 ppm concentrations, respectively (Table 3). It is also noticed from the results that the per cent growth inhibition of the test pathogens gradually increased with the increase of concentration of the fungicides in culture media.

Hoque *et al.* (2016) reported that radial colony diameter of *F. semitectum* was significantly reduced over control due to amendment of PDA with Bavistin (carbendazin). The aforesaid fungicide showed 100% radial growth inhibition of *F. semitectum*. Pramesh *et al.* (2016) also found effective against blast and sheath blight diseases of rice by using Nativo 75 WG.

In vitro efficacy of various fungicides against the test pathogens indicated that CM 75 WP, Nativo 75 WG and Rovral 50 WP showed promising results as compared to others (Tables 1-3). The same fungicides also showed different effects on tested fungi in the present investigation. This variation might be due to selection of different strains of test pathogens.

Table 3. Inhibition of radial growth (%) of *Fusarium semitectum* at different concentrations of fungicides.

Name of fungicides	% inhibition of radial growth at different concentrations (ppm)				
	100	200	300	400	500
CM 75 WP	100[a]	100[a]	100[a]	100[a]	100[a]
Dithane M 45	16.91[b]	20.69[c]	53.93[b]	57.72[c]	69.39[b]
Knowin 50 WP	100[a]	100[a]	100[a]	100[a]	100[a]
Nativo 75 WG	100[a]	100[a]	100[a]	100[a]	100[a]
Rovral 50 WP	21.05[b]	28.77[b]	62.46[b]	75.44[b]	100[a]
CV (%)	7.00	6.71	3.03	2.80	0.69

Means followed by the same letter within a column did not differ significantly at 5% level by DMRT.

Results of leaf extracts on the radial growth of *Colletotrichum lindemuthianum, Drechslera sacchari* and *Fusarium semitectum* are presented in Tables 4-6. All the plant extracts showed distinct degree of growth inhibition of the test pathogens at 5, 10, 15 and 20% concentrations. Among the five plant extracts, *Lippia alba* showed complete growth inhibition of *C. lindemuthianum* at 20% concentration which was followed by *Azadirachta indica* (47.59%), *Thuja occidentalis* (46.05%), *Heliotropium indicum* (24.67%) and *Michelia champaca* (23.89%) (Table 4). The inhibition of the pathogen increases with the increase of the concentration of the plant leaf extracts in culture medium. Choudhary *et al.* (2017) reported that leaf extract of *A. indica* significantly reduced (78.83%) the mycelial growth of *C. lindemuthianum* at 10 ppm.

Out of five plant extracts, *Lippia alba* showed complete radial growth inhibition of *D. sacchari* at 20% concentration which was followed by *A. indica* (50.09%), *Thuja occidentalis* (50%), *Heliotropium indicum* (40.49%) and *Michelia champaca* (28.19%) (Table 5). Miah *et al.* (2017) reported that BARI Gom-26 variety showed the lowest fungal infection (6%) owing to *A. indica* and *Thuja occidentalis* plant extract followed by *Citrus limon* (8%), *Allium sativum* (10%) and *Datura metel* (10%). Jadon and Shah (2012) found *A. indica* as the best mycelial growth inhibitor among the perennials against the *Drechslera bicolor*.

Table 4. Effect of plant leaf extracts on the radial growth of *Colletotrichum lindemuthianum* at different concentrations.

Name of plants	Per cent inhibition of radial growth at different concentrations (%)			
	5	10	15	20
Azadirachta indica	26.62[a]	31.87[b]	37.12[b]	47.59[b]
Heliotropium indicum	14.80[b]	17.92[c]	19.48[c]	24.67[c]
Lippia alba	39.68[b]	41.90[ab]	45.39[a]	100[a]
Michelia champaca	12.46[b]	14.80[c]	19.48[c]	23.89[c]
Thuja occidentalis	27.91[a]	40.69[a]	44.65[a]	46.05[b]
CV (%)	15.99	12.89	9.85	5.81

Means followed by the same letter within a column did not differ significantly at 5% level by DMRT.

Table 5. Effect of plant leaf extracts on the radial growth of *Drechslera sacchari* at different concentrations.

Name of plants	Per cent inhibition of radial growth at different concentrations (%)			
	5	10	15	20
Azadirachta indica	30.34[b]	36.80[b]	38.34[b]	50.09[b]
Heliotropium indicum	28.05[b]	34.84[b]	38.25[c]	40.49[d]
Lippia alba	47.07[a]	54.39[a]	62.23[a]	100[a]
Michelia champaca	16.86[c]	19.27[c]	23.61[d]	28.19[e]
Thuja occidentalis	27.78[b]	39.33[b]	45.11[b]	50.00[c]
CV (%)	9.13	9.48	5.28	3.32

Means followed by the same letter within a column did not differ significantly at 5% level by DMRT.

The highest inhibition of radial growth of *F. semitectum* was observed with *Lippia alba* (100%) at 20% concentration which was followed by *A. indica* (46.47%), *T. occidentalis* (41.46%), *M. champaca* (20.73%) and *H. indicum* (17.97%) (Table 6). Sinha and Varma

(2017) reported that the ethanolic, methanolic and aqueous extracts of *M. champaca* L. exhibited antioxidant and free radical activity.

Out of the five plant extracts *Lippia alba*, *Azadirachta indica* and *Thuja occidentalis* showed maximum radial growth inhibition of *C. lindemuthianum*, *D. sacchari* and *F. semitectum* at 20% concentration. But *Heliotropium indicum* and *Michelia champaca* showed minimum growth inhibition of the test pathogens.

Table 6. Effect of plant leaf extracts on the radial growth of *Fusarium semitectum* at different concentrations.

Name of plants	Per cent inhibition of radial growth at different concentrations (%)			
	5	10	15	20
Azadirachta indica.	29.05[a]	38.59[a]	40.87[a]	46.47[b]
Heliotropium indicum	7.83[d]	11.59[d]	14.49[d]	17.97[d]
Lippia alba	15.20[c]	39.18[a]	44.44[a]	100[a]
Michelia champaca	10.36[d]	14.14[c]	18.29[c]	20.73[d]
Thuja occidentalis	26.83[b]	31.71[b]	37.80[b]	41.46[c]
CV (%)	14.12	6.93	6.41	5.16

Means followed by the same letter within a column did not differ significantly at 5% level by DMRT.

Basella spp. is an important plant for its nutritional, medicinal point of view. So, the production of the vegetables by controlling various diseases it is necessary to identify the most prevalent pathogen causing leaf spot and to reduce the yield as well as post-harvest loss of the vegetables. The results of this investigation identified Nativo and Rovral as the best inhibiting fungicides against leaf spot of *Basellla* spp. Leaf extracts of *Lippia alba* and *Azadirachta indica* identified as an effective botanical against for further testing against pre-harvest diseases of *Basella* spp.

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FIRE RISK SITUATION ANALYSIS IN THE NIMTOLI AREA OF OLD DHAKA

MASABA TASNEEM SAHEBI[1], MD. MARUFUR RAHMAN*[1]
AND M. MAKSUDUR RAHMAN[2]
[1]Department of Disaster Science and Management, University of Dhaka, Dhaka-1000, Bangladesh
[2]Department of Geography and Environment, University of Dhaka, Dhaka-1000, Bangladesh

Abstract

Assessment of the fire risk situation of Nimtali area by using eight indicators related to the fire source, fire spreading and evacuation during fire incidents was conducted. The results revealed that none of the buildings has emergency exits, fire protection measures, fire hydrant and provision of a fire drill. The area is densely populated. Most of the buildings are mixed-used and have no space in between. Electrical cables are haphazardly hanging from poles. Access roads are incredibly narrow. By analyzing eight indicators, it is found that the entire Nimtoli area is still at risk of fire hazard. Within Nimtoli, 32% area is at high risk, and 45% is at moderate risk of fire hazard. Proper fire safety measures and safety inspection, regular maintenance of utility lines, awareness about fire hazards among the dwellers, proper implementation of Bangladesh national building code (BNBC) and regulation of mixed-use of buildings can drastically reduce the fire risk in the urban area of Bangladesh.

Key words: Fire hazard risk, Risk mapping, Nimtoli, Old Dhaka

Introduction

Fire incidents are one of the significant hazards in Bangladesh, particularly in the urban and industrial area. In 2019, the number of reported fire incidents was 24,078, and estimated damages were 330.04 million (in BDT), which caused 184 death and 560 injuries in Bangladesh (BFSCD 2020). Fire incidents in Bangladesh are increasing day by day (Fig. 1). High density of population, concentration of wealth and human activities (production, transport and service) are responsible for high risk of fire in the urban settlements compared to the rural settlement (Maniruzzaman and Haque 2007). The highest numbers of incidents occur in Dhaka city and its surrounding area. Electrical short circuits and fire from the burner are two leading causes of a fire incident in Dhaka as well as in Bangladesh (Islam and Adri 2008). Alam and Baroi (2004) found from their study that over 60% fire incident in Dhaka occur between noon to midnight and the dry season (December to March) is the riskiest period of the year (Nearly twice fire

*Corresponding author: maruf.dsm@du.ac.bd

incident occur compared to the wet season). Commercial and mixed landuse areas are more vulnerable to a fire incident in Dhaka (Rahman and Islam 2019). Based on a study on 31 slums in 2019, BFSCD (2020) reported that fire incidents cause a colossal amount of economic loss and claimed many lives; many people become homeless and lost their all belongings within a few hours, particularly those who are living in the slum areas.

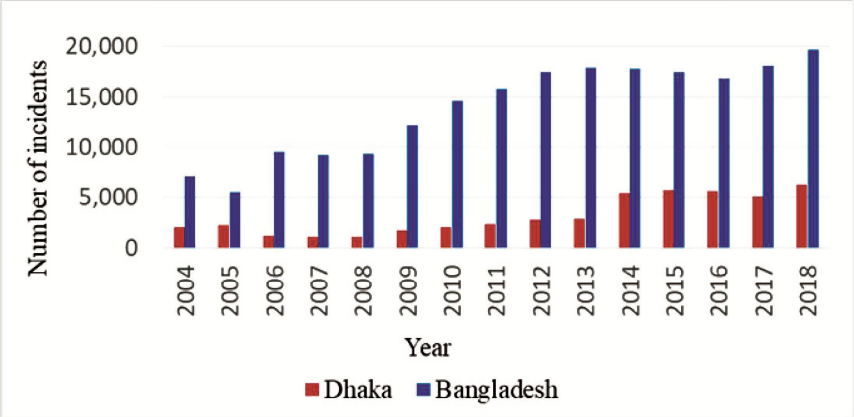

Fig. 1. Year-wise number of fire incidents in Dhaka and Bangladesh.
Source: BFSCD 2020, Tishi 2015.

Old Dhaka is prone to fire incidents due to mixed use of land, very high population density, unplanned and haphazard urban development, depots of chemicals in the residential area and lack of awareness of residents. A makeshift chemical warehouse in a residential building fuelled the fire of tragic Nimtoli incident on June 3, 2010, which claimed 124 lives (Molla 2019). Another tragic fire incident occurred in old Dhaka (Chawkbazar area) from a depot of chemicals on the night of February 20, 2019, which claimed 67, lives (Molla 2019). Awareness of city dwellers and activities of different authorities have increased to prevent fire immediately after a significant incident. With time, these activities are slowed down. As a result, another incident occurs. We face many fire incidents every year (Fig. 1), but the number of researches are very small in number on this issue. In this study, several physical indicators are analyzed to assess the present fire risk situation of the Nimtali area. This study also tries to find out what changes occurred since the devastating fire of 2010.

Study area: The study area is situated in ward number 33 (formerly 69; total area is 0.36 km^2) under the 4th zonal office of Dhaka South city corporation area (Fig. 2a). This ward

lies under Bangshal Thana, which was a part of Kotwali Thana. The population density in ward number 33 is 181,359 people per km^2. The number of households is 12,891 (Banglapedia 2015, BBS 2015). There are almost four hundred buildings in Nimtoli area. Many restaurants, tea stalls and Bakarkhani (a kind of backer shop) stores are situated in this area.

Methods: Both primary and secondary data are used for this study. Primary data collection methods include field observation, building inventory, landuse survey and interviews. A field survey has been conducted in 2018 for this study. Secondary data are collected from different published and unpublished sources, government and non-government organizations. In this study, non-participation observation method is used. It involves observing and recording events (photographic devices and field notes are used) on the spot, without any interaction with the community. During primary data collection, this study identifies risk factors/indicators for fire hazard analysis through field observation (Table 1). These factors are identified based on their ability to generate fire, the spread of fire quickly, constrain of quick evacuation and rescue. In-depth interviews have been conducted with urban planners of Bangladesh University of Engineering and Technology (BUET) and the University of Dhaka, experts from Bangladesh fire services and civil defence and residents of Nimtolia area. Fire safety measures, firefighting capabilities, and how fire risk can be reduced in the study area have been discussed with the interviewees. Based on experts' opinion, and the previous study of Rahman et al. (2017), weightage has been given to those indicators (Table 1). A scale of 8 to 1 has been assigned, eight equals to high susceptibility, and one equals to low susceptibility. Each risk factor is divided into several sub-indicators. Rank values from 0 to 4 have been assigned to those indicators to understand the risk index of each structure. Shapefiles of buildings, roads are exported from the open street map (OSM). The shapefiles contain some information about the structure type, material, level, quality. This information is validated during the primary data collection. Landuse survey has been carried out to collect data about landuse pattern, type of buildings, use of buildings, level (storey) of the building, interior information like the width of the staircase, collapsible gates etc. Width of the staircase has been measured manually using tape. Location of hazardous structures and utility facilities (transformers, electric poles) has been recorded through GPS. In the inventory; building material, building type, quality, use purpose, building level, the width of the staircase, access road next to structures, have been recorded. The study has used ArcGIS, Microsoft office packages to analyze the collected data. Finally, an individual risk map and aggregated risk map of the study area have been generated.

Table 1. Weightage and score of fire risk factors for structures.

Factors	Weightage*	Sub-factors	Score
Distance to hazardous structures	8	Hazardous structure	3
		Structures within 10 m of hazardous structure	2
		Structures within 15 m of hazardous structure	1
		Structures more than 15 m distant of hazardous structure	0
Space between adjacent structures	7	No space	2
		Space 0.5 -1.0 m	1
		Open space/unused space	0
Collapsible gate within structures	6	Structure without collapsible gate	1
		Structure with collapsible gate	2
Width of stair case of structures	5	< 2ft	4
		2 ft - 2.4 ft	3
		2.5 ft - 2.9 ft	2
		3 ft - 3.5 ft	1
		No stair case/>3.5 ft	0
Proximity to transformers	4	Structures within 3 m distance	3
		Structures within 5 m distance	2
		Structures within 10 m distance	1
		Structures more than 10 m distance	0
Distance from electric pole	3	Structures within 1 m distance	4
		Structures within 2 m distance	3
		Structures within 3m distance	2
		Structures within 4 m distance	1
		Structures more than 4 m distance	0
Proximity to roads	2	Roads within 10 m of structures	1
		Roads within 15 m of structures	2
		Roads within 20 m of structures	3
		Roads more than 20 m of structures	4
Accessibility to roads	1	Accessible	1
		Not accessible	2

*Weightages have been given based on experts' opinion, field observation and previous study of Rahman *et al.* 2017.

Results and Discussion

Landuse and building inventory: There are around 411 buildings (Table 2) in Nimtoli. Majority of the buildings are in dilapidated condition and have not built by following construction rules. About 66.4% of buildings are non-engineered buildings. Only ten buildings in Nimtoli are over six storeys (Table 2). The analysis shows that 48% of

structures are residential, 33% mixed, and the other 19% are used as commercial, industrial, public facilities.

Mixed-use (same structure/building used for a different purpose for example residential, commercial and restaurants are co-located in one building) of building escalates the risk of fire. The ground floor of mixed-use buildings are used as a storehouse of waste paper, old containers of plastic and tin, wasted electronic parts, plastic bags. These materials are

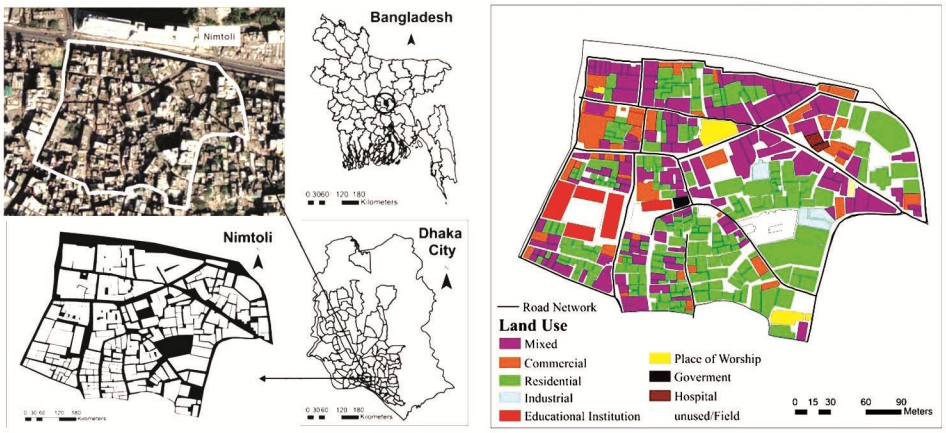

Fig. 2. a. Location and b. landuse pattern of structures in Nimtoli in old Dhaka.
Source: Open street map and field survey 2018.

Table 2. Story information of structures in Nimtoli.

Building level	No. of structure	Percentage
1 storey	114	27.74
2 storey	80	19.46
3-4 storey	105	25.55
5-6 storey	102	24.82
More than six storey	10	2.43
Total	411	100

Source: Field survey, open street map 2018.

highly sensitive to fire. Buildings used for commercial purpose are not equipped with fire protection measures. Many commercial buildings are used as shops, hotels, restaurants and a baker shop. The field survey observed that number of open spaces is minimal in Nimtoli area (Fig. 2b). It is important to note that there are no water bodies and fire

hydrants in Nimtoli area. Some roads are incredibly narrow. No emergency exit fund in the buildings of Nimtoli during the survey. Fire protection measure and equipment are not found inside any of these buildings. There are no provisions of a fire drill in this area. Previously, many buildings were used as the depot of combustible materials. During the field survey, no chemical depot could be identified in this area. Local residents claimed that all chemical storehouses had been shifted from Nimtali since 2010 incident.

Fire source and spreading of fire: The hazardous structures are termed to those which have the potential to ignite and spread fire to other structures. From the field observation, the structure used as a hotel, *bakarkhani* shop (backer shop), and tea stall has been marked as hazardous structures. These hazardous structures can ignite a fire in this area. Structures within 10 m, 15 m, over 15 m distance of hazardous structures have been demarcated and termed them as high, moderate, low risk structures. A score of 3-0 have been assigned to these sub-factors according to risk (Table 1.). Score 3 has been given to the hazardous structures itself, and 2 - 0 score has been applied to the other structures (Table 1) and a risk map (Fig. 3a) is produced. There are 5.3% structures used as a hotel, tea stall, *bakarkhani* shop in Nimtoli area. The risk map reveals the fact that 30.36% of structures are at high risk, 8.7% at moderate risk and 55.6% at low risk.

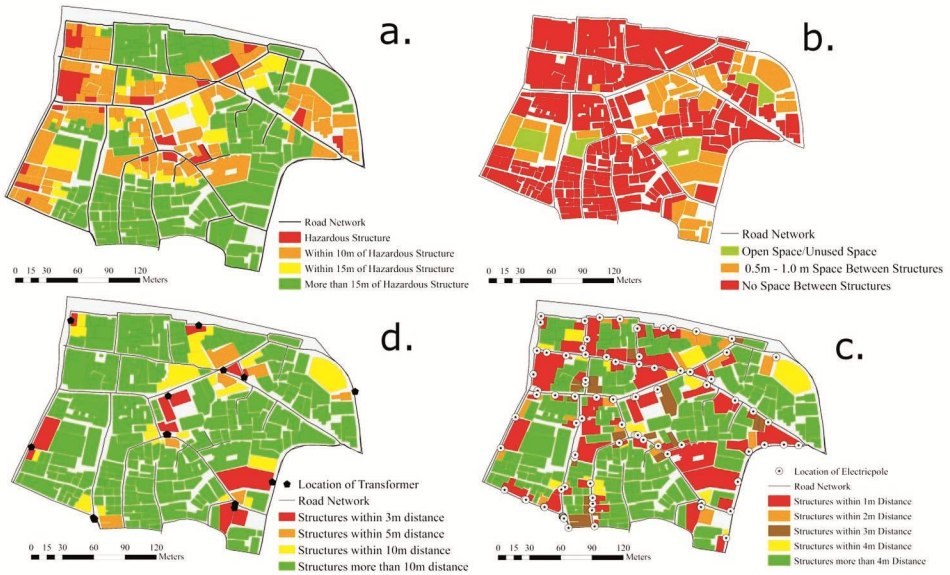

Fig. 3. Showing a. hazardous structures, b. space between structures c. location of electric poles and d. location of transformers in Nimtoli area.

Proper space between structures is essential to keep building safe from spreading of fire from other structure. The nearest buildings always have a higher probability of catching fire from the affected buildings than the distant one. Construction rules amended by the government of Bangladesh in 1996 imposes conditions on setbacks, site coverage, plot usage, which ensures building safety during different hazards (Rahman *et al.* 2017). Setback defines the optimum distance that a structure should maintain from the adjacent road and other structures. For Dhaka city, a minimum 1.5 m space in front, 1 m space in backside and 0.8 m space in both sides must be kept for a plot size of two *katha* (1440 ft^2) or less amount of land (GOB 2008). The study found that 90% of structures do not have any space between each other (Fig. 3b and Fig. 4). Only 10% have space 0.5 to 1 m (Fig. 3b) which has not followed the rules for all side of the plot. The structures with no space are scored as 2, and space with 0.5 - 1.0 m has been given score one (Table 1).

Fig. 4. Image of buildings without any space between each other (left image) and placement of transformer near residential building (right image) in Nimtoli area.

The electric poles have been marked in the study area during primary data collection (Fig. 3c and Fig. 4). Electric poles and cable can ignite the fire from a short circuit. Even if a fire occurs from other sources, electrical poles and cables can pose a risk for life and property. Electric cables are connected with individuals structures from the pole for electricity supply. A safe distance must be kept from an electric pole to avoid a fire incident. The buffer zones of 1, 2, 3 and 4 m from the electric pole are created to identify structures at risk for electric pole. The structure within 1, 2, 3, 4 and over 4 m are termed as very high, high, moderate, low, very low risk structures, and a score of 4 to 0 have been assigned (Table 1). Based on weights and score a risk map is produced (Fig. 3c). Underground electricity lines can increase fire safety and aesthetics of the area. Underground lines also can reduce damages of the electric supply system during the storm season.

Eleven transformers have been marked in the study area (Fig. 3d). The electric transformer is a dangerous source for a fire. A burst of a transformer can affect the nearest structures. The 2010 Nimtoli fire had started because of a transformer burst as claimed by some residents. To know the risk situation of the structures, several buffer zones are created from the transformer. During field observation, the study found that the location of transformers is very close to the buildings. So 3, 5, 10 m and more than 10 m buffer zone are created. Structures within 3 m, 5 m, 10 m and over 10 m have been marked as very high, high, moderate and low risk structures. Here rank value has been given from 3 (high risk) to 0 (low risk) (Table 1). All these information are used for producing a risk map for transformers (Fig. 3d). The study found that only 5.5% of structures are at high risk, 6% at moderate risk and 88.5% have a low risk because of the transformer.

Evacuations, rescue and firefighting: The proper width of the staircase is significant for smoother and rapid evacuation during a fire incident. Field inspection found that there is no emergency exit in any structure in Nimtoli. According to expert opinion, the width of the staircase must be at least 3.5 ft. Only 1.2% of structures have followed proper rules for the staircase. About 73.95% of structures have staircase width less than 3.5 ft; the rest of the structures (24.79%) are one-storied and do not have any staircase (Table 3). One storey building and the structures having staircase with width over 3.5 ft are termed as very low-risk structures, 3.0 - 3.5 ft staircase are termed as low risk, 2.5 - 2.9 ft as moderate risk, 2.0 - 2.4 ft are at high risk, less than 2 ft are grouped into a very high-risk structure. A score of 4 to 0 values have been assigned (Table 1) to the structures and a map is produced for this factor (Fig. 5a).

The structures having a collapsible gate act like an obstacle in the time of quick evacuation and rescue operation. During building inventory preparation, we observed that collapsible gates are kept under lock and key. In Nimtoli area gateman are not kept to maintain collapsible gates. The structures which have collapsible gate thus possess higher risk, and the structures without collapsible gate possess low risk. Based on expert opinion, score have been assign to 1 and 2 for structure with and without collapsible gates (Table 1). From the analysis, it is found that 42% of structures have a collapsible gate and 58% of structures do not have a collapsible gate (Fig. 5b).

Most of the access roads in Nimtoli area are narrow. According to the Dhaka metropolitan building construction rules 2008, every site has to be accessible to a minimum of 6 meter wide roads (GOB 2008). This minimum width ensures easy access to people and vehicle. At least 3.05 m (10 ft) road width is required for a small size fire

control vehicle. If the fire control vehicles are unable reach incident sites, then fire control pipe or pumps are used for firefighting. During the 2010 incident, narrow roads created an obstacle for fire services to access their fire control vehicle and slower down

Table 3. Staircase widths of the structures in Nimtoli.

Stair case width	Percentage
No stair case (One story building)	24.79
less than 2 ft	1.68
2.0 - 2.4 ft	15.13
2.5 - 2.9 ft	33.61
3.0 - 3.5 ft	23.53
More than 3.5 ft	1.26

Source: Field survey 2018.

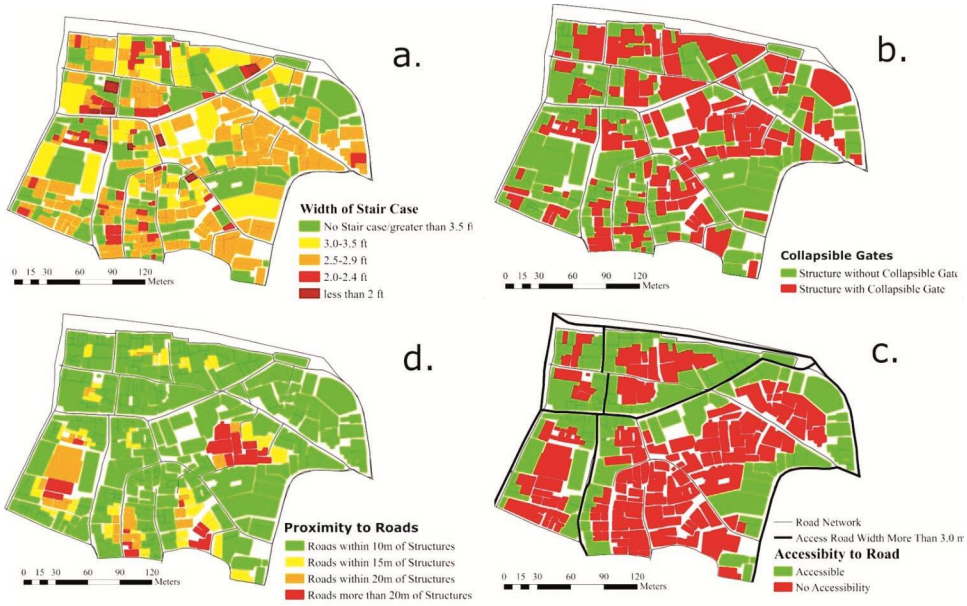

Fig. 5. Shows a. width of staircase, b. the collapsible gates, c. access roads width and d. the road proximity in Nimtoli area.

the firefighting process. The score has been assigned, and maps have been generated for this factor (Fig. 5c). Considering this, all structures accessible to the roads wider than 3.05 m have been identified. Analysis of this data shows that 67% of structures of the study area are not accessible to roads wider than 3.05 m.

Fire hydrant facilities, open space and water body are not available in Nimtoli area. As a result, firefighting, evacuation and first aid activities are hard to conduct here. People can be trapped in their dwellings or workplace, and the fire control vehicle may not reach to them because of the improper approach road. Accessibility is important during any emergency response. The structures which have access to roads within 10 m, 15 m, 20 m are termed as low, moderate and high risk structures. The structures which do not have any access road within 20 m are marked as very high-risk structures. Based on a score of 4 to 1 (Table 1), a risk map has been produced (Fig. 5d). The analysis shows that almost 74.21% of structures have access road within 10 m and only 6.74% structures do not have access road within 20 m.

Aggregate risk mapping: All the identified risk factors have been integrated. Based on weightage and ranking value (Table 1) fire hazard risk of the structures of the study area have been calculated as fire hazard risk = (8* score of distance to hazardous structures) + (7* space between adjacent structures) + (6* collapsible gate within structures) + (5* width of staircase of structures) + (4* structures proximity to transformers) + (3* distance from to electric pole) + (2* proximity to roads) + (1* accessibility to roads).

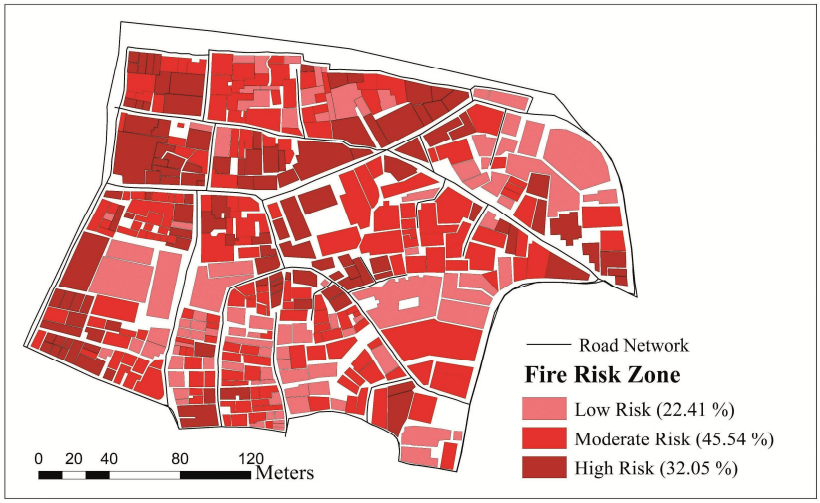

Fig. 6. Fire hazard risk map of Nimtoli.

All the structures have been categorized as severe, moderate and low risk in terms of eight risk factors (Table 4). The study found that 32% of structures are at high risk, 45.5% at moderate risk and rest of the structure at low risk (Fig. 6). The structure caught fire during the devastating 2010 Nimtoli tragedy still found as a high-risk structure for

fire incidents from the analysis. The previous study in Bangshal area also found that the area is at high risk of fire because of mixed landuse, very high density of population, narrow roads, and non-engineered old buildings, lack of water bodies, open spaces and fire protection measures (Jahan *et al.* 2011). The possible lowest and the highest score is 17 and 81, respectively (Table 4). Using this score, a risk appraisal is developed (Table 4).

Table 4. Fire hazard risk appraisal scale.

Risk appraisal	Range
Low risk	17 - 35
Moderate risk	36 - 51
High risk	52 - 81

Conclusion

In terms of fire safety, firefighting facilities, evacuation, rescue and first aid facilities, the Nimtoli area is still at risk of fire incidents. Mixed-use of structure and the business of wastage material must be regulated to reduce the fire risk of the study area. Electricity lines must be taken to the underground. Building code should be implemented strictly by government authority. Building owner and residents should ensure fire safety measure and firefighting drill to reduce fire risk in Nimtoli area. Identification of risky structure and issuance of warning is crucial. The government should take immediate legal action against those who are not taking any important initiatives after warnings from Bangladesh fire service and civil defence (BFSCD). Proper fire safety measures, regular maintenance of utility lines, awareness about fire hazards within the residents, proper implementation of Bangladesh national building code (BNBC), a regular safety inspection by Bangladesh fire service and civil defence, regulation of mixed-use of buildings can drastically reduce the fire risk in Nimtoli and other urban areas of Bangladesh.

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SOCIO-ECONOMIC AND DEMOGRAPHIC DETERMINANTS OF WOMEN PARTICIPATION IN LABOR FORCE IN RURAL BANGLADESH

SACCHIDANAND MAJUMDER*[1] AND SOMA DEY[2]
[1]*Department of Statistics, University of Chittagong, Bangladesh*
[2]*Faculty of Business Administration, BGC Trust University Bangladesh, BGC Biddyanagar, Chandanaish, Chittagong, Bangladesh*

Abstract

This attempt was made to investigate the socio-economic and demographic factors that influence women participation in labor force in rural Bangladesh using BDHS 2014 data. A total of 11,695 married women aged 15 - 49 in rural areas are selected for analysis. A logistic regression analysis is applied for determining the factors. The result shows that 32.2% rural women are currently employed and the remaining 67.8% are unemployed. The logistic model shows that women's age has a strong positive association with their participation in labor force. Participation of widowed and divorced/separated women in labor force is much higher as compared to married women. Enhanced education level of women and their husband has decreased women participation in labor force, but it gradually increased with increased education level of household head. Husband's occupation is a strong determinant of women participation in labor force. Women from middle income households are less interested to participate in labor force as compared to poor women. The result also reveals that with increased household size and number of children under age five, women participation in labor force is decreased. Again, with increased land and livestock ownership of household and NGO membership, women participation in labor force is highly increased.

Key words: Socio-economic determinants, Women participation, Logistic regression analysis

Introduction

In developing economies, women play an important role by contributing to household income, adding to the supply of labor for economic activities, and above all by empowering women (Rahman 2013).Women participation in labor force significantly contributes to socio-economic development because of a second source of household income and can help to reduce poverty. Women's employment is a critical factor in their progression towards economic independence and is considered as an indicator of their overall status in society (Mammen and Paxson 2008). Presently, women participation in labor force has become an essential element in the determination of the performance of economic development, both in Developed and in Developing countries (Che and Sundjo 2018).

*Corresponding author: mithu.m2011@gmail.com

In the Population and Housing Census 2011, it is mentioned that women constitute half in the population composition of Bangladesh and play a very significant role in the economy (BBS 2012). Bangladesh is a developing country in South Asian region with 161.3 million of total population, of which 80.3 million are female. Among the total population, 109.1 million are of age 15 or older which is 67.6% of total population, of whom 55.0 million (50.4%) are female. In rural areas, the total population of age 15 or older is 77.1 million (29.3 %) (BBS 2018). In 2016-2017, the employment rate of rural women of age 15 and over is 36.3% and it was 36.0% in 2010, 33.5% in 2013 and 35.6% in 2015-2016 (BBS 2018).

Women participation in labor force is influenced by a wide range of factors with socio-economic and demographic factors. Several studies found that women's age has positive significant effect on participation in labor force (Roy *et al.* 2015, Lisaniler and Bhatti 2005, Amin 1994). In contrary, another study mentioned that younger women (aged 15-24), are less likely to participate in the labor market (Faridi *et al*. 2009). Women's age is inversely related to participation rate in labor force in Kuwait (Aly and Quisi 1996).

Household size has a negative effect on women participation in labor force (Babalola and Akor 2013).Women who have a smaller family are more likely to engage in paid work and revealed that the number of children below age five has an insignificant impact on women employment in Bangladesh (Amin 2005). The number of children is positively significant with the women participation in labor force (Mincer 1962, Faridi *et al.* 2009) but, women with children aged 0-6 are less likely to participate in the labor market (Faridi *et al.* 2009). Several studies found that the number of children is inversely related with women participation in labor force (Aly and Quisi 1996, Maglad 1998, Sackey 2005). In Bangladesh, another study mentioned that having infants have a negative impact on women participation in labor force (Rahman 2006).

Women participation in labor force is strongly influenced by marital status (Grantham 2012). Divorced women are the most likely to seek employment, followed by married women, and lastly single women; reason being that these divorced women have little or no prospects of economic dependence (Mlatsheni and Leibbrandt 2001). Several studies found that marital status is positively related to women participation in labor force (Amin 1994, Faridi *et al.* 2009). In contrary, several other studies implied that marital status is inversely related with women labor force participation rate (Aly and Quisi 1996, Rahman 2006). Female headed household have a positive impact on the women participation in labor force (Amin 2005, Rahman 2006), but the household having educated household head have a negative impact on women participation in labor force (Rahman 2006).

Women tend to take part in the labor market more with higher levels of education. There is a stronger tendency for a more educated woman to remain economically active than a less educated woman (Oluwasey 2013). Another study showed that in Bangladesh, female with higher education have a positive impact on participation in labor force and low level of education has a negative impact on the female participant in labor market (Rahman 2006). Female literacy rates are important determinants of rural female work participation rates in the different districts of the rural areas of North Bengal region of the state of West Bengal in India (Rai 2017). Several studies also found that female education has positive impact on the women participation in labor force (Aly and Quisi 1996, Maglad 1998, Sackey 2005, Atieno 2006, Bbaale and Mpuga 2011, Babalola and Akor 2013). However, Roy *et al*. (2015) observed that education is negatively associated with women participation in labor force.

Women participation in labor force is strongly influenced by the occupation of the husband (Grantham 2012). A man with higher earnings through a higher level of education and occupation will prefer his wife to reduce her participation in labor force and to focus more on housework (Devereux 2004, Kalenkoski *et al.* 2009). A study portrayed that women whose husbands are economically active, are less likely to participate in the labor market (Faridi *et al.* 2009). Another study found that husband employment status has a negative effect on women participation in labor force (Babalola and Akor 2013).

Land holding, and husband's assets have negative effects on women participation in labor force (Khandker 1987). Land ownership has a negative impact on women participation in labor force and the rapid expansion of micro finance in rural areas has supported women's employment in poultry and livestock (Rahman 2006). Another study illustrated that home-based economic activities has pushed the women labor force participation in Bangladesh (Amin 2005). A study found that women participation in labor force is negatively related to asset ownership (Maglad 1998). On the other hand lower levels of household wealth and microcredit have a positive impact on women participation in labor force (Amin 2005).

Social factor, especially religion has tremendous effects on women participation in labor force. Several authors found that different religions have an influence on economic attitudes and women participation in labor force (Chadwick and Brent 1993, Guiso *et al.* 2003). In Nigeria, a study revealed that factors such as marital status, religion, and poverty rate were the significant determinants of female participation in labor force in the rural area (Iweagu *et al.* 2015).

The above discussed literature clearly shows that various factors have an important effect on women participation in labor force. Rural women's primary asset is their own labor, therefore one of the keys onto achieving the Sustainable Development Goals (SDGs), is to ensure more and better rural employment whether waged or self-employed enterprise (Fontana and Paciello 2010). This study is significant in that, it is tried to find out various important socio-economic and demographic factors which are significantly associated with women participation in labor force. So that, this study serves as an important contribution to make decision to increase women participation and development. The aim of this study is to investigate socio-economic and demographic factors that influence women participation in labor force in rural Bangladesh.

Materials and Methods

Data source: The data analyzed in this study are sourced from Demographic and Health Surveys (DHS) 2014 for Bangladesh under the authority of the National Institute of Population Research and Training (NIPORT), Ministry of Health and Family Welfare and implemented by Mitra and Associates, Dhaka. The dataset are available online at https://dhsprogram.com/data/available-datasets.cfm. The survey covered both rural and urban populations. The survey collected information relating to demographic and detailed information on asset ownership, access to public services and housing characteristics (BDHS 2014).

Sample size: The Bangladesh DHS, 2014 was based on a two-stage stratified sample of households. In the first stage, 600 Enumeration Areas (EAs) were selected with probability proportional to the EA size, with 207 clusters in urban areas and 393 in rural areas. A complete household listing operation was then carried out in all the selected EAs to provide a sampling frame for the second-stage selection of households. In the second stage of sampling, a systematic sample of 30 households on average was selected per EA to provide statistically reliable estimates of key demographic and health variables for the country as a whole, for urban and rural areas separately, and for each of the seven divisions. With this design, the survey selected 18,000 residential households to conduct the survey. Finally, 17,300 households (Urban: 5,930 and Rural: 11,370) were surveyed. In the BDHS, 2014, a total of 17,863 married women (urban: 6,167 and rural: 11,696) aged 15-49 were interviewed from 14,997 households out of 17,300 surveyed households (BDHS 2014). In this study, 11,695 married women aged 15-49 instead of total interviewed women 11,696 in rural areas are selected for analysis because of some missing observations of the selected variables in the study.

Variables: Women participation in labor force as dependent variable is measured based on individual question about current working status with two categories, employed and unemployed. So, to create the binary variable women participation in labor force, 1 = yes, if woman is currently employed and 0 = no, if woman is currently unemployed.

After reviewing some research paper related to women participation in labor force, some explanatory variables are included in the study to determinant the factor influencing women participation in labor force. The explanatory variables are women's age, women's education, women's marital status, age of household head, sex of household head, education level of household head, husband's education level, husband's occupation, household size, number of children under 5,wealth index, land ownership of household, livestock ownership of household, media exposure, NGO membership, and religion.

Methodologies: Bivariate analysis: Bivariate analysis was conducted to verify whether there is any association between dependent and independent variables. Chi-square test of independence is used to examine the effect of socio-economic and demographic characteristics on women participation in labor force. To identify the factors most strongly associated with it, the entre method of logistic regression model is performed.

Logistic regression analysis: The analysis uses logistic regression, which estimates model with a binary response and a set of explanatory variables. In logistic regression model, a dichotomous variable, women participation in labor force is introduced to determine whether woman is employed or not.

Here,

$$\text{Women participation in labor force} = \begin{cases} 0, & \text{if women is unemployed} \\ 1, & \text{if women is employed} \end{cases}$$

The logistic regression model is given by

$$logit\ (P_i) = \log\left(\frac{P_i}{1-P_i}\right) = \sum_i \beta_i X_i$$

where,

$$P_i = P\left(Y_{i=}\ \frac{1}{X_i}\right) = \frac{\exp(\sum \beta_i X_i)}{1+\exp(\sum \beta_i X_i)} = \text{Probability that the i}^{\text{th}}\text{ woman is employed}$$

Y = Woman participation status in labor force of i^{th} woman;

Y_i = 1 if woman is employed, and zero if woman is unemployed,

X_i = i^{th} predictor variable; and β_i = i^{th} parameter associated with X_i.

Results and Discussion

All the analyses are performed by SPSS (V21.0). Information are obtained from 11,695 ever married rural women with current age of 15-49. Among these selected women, 3,762 (32.2%) are currently employed and the remaining 7,933 (67.8%) are unemployed. In 2016-2017, the employment rate of rural women age of 15 and over is 36.3% (BBS 2018).

Bivariate analysis: In order to assess whether the covariates as significantly associated with women participantion in labor force or not, cross tabulation and Pearson Chi-square (χ^2) tests are performed first. Then those variables found to be significant in Chi-square test are used to construct logistic regression model.

The distribution of women by age and participation in labor force result shows that women who belong to age less than 35, 35-39, 40-44 and 45-49, about 28.7, 38.9, 39.2 and 37.3% are employed. In 2016-2017, the employment rate of rural women with age 15-24, 25-34, 35-44, 45-54, 55-64 and 65+ are 20.9, 44.0, 52.1, 44.7, 28.4 and 9.3%, respectively (BBS 2018). About 31.3% currently married women, 44.7% widowed, and 55.1% divorced or separated women are employed. The result portrays that women who have no education, primary, secondary, and higher education, about 39.6, 33.5, 26.2 and 29.8% are employed respectively. In 2016-2017, 38.2, 39.0, 33.1, 27.7, 51.2 and 18.6% rural women employed with no education, primary, secondary, tertiary and others education respectively (BBS 2018). It is also illustrated that the household's head who belongs to age less than 35, 35-44, 45-54, 55-59 and 60 and above, about 31.5, 36.6, 33.8, 29.2 and 25.3% women are employed. Further, women who are from male and female-headed household, about 32.1 and 32.3% women are employed. The result mentions that about 35.0, 31.7 and 29.3% women are employed who have non-educated, primary educated, secondary and higher educated household's head, respectively. It is showed that women whose husband have no education, primary, and secondary and higher education, 38.7, 32.5 and 26.4% women are employed respectively. It is found that about 21.3, 34.8, 35.9, 28.9 and 24.1% women are employed whose husband's occupation is unemployed, farmer, labor, business or service and others. Further, 47.7, 43.8, 36.6 and 28.5% women are employed whose household size is 1, 2, 3, 4 and 5 and more, respectively. Furthermore, about 36.2, 30.2, 22.7 and 16.4% women are employed who have no child, one child, 2 children, and 3 and more children aged under 5, respectively. The result also shows that women who belong to poor, middle income, and rich family, about 64.2, 68.8 and 73.3% are unemployed, respectively. It is observed that in respect of land ownership the household who have no land ownership, less than 1 acre, and 1 acre

and more, among these, 67.2, 66.2 and 71.6% women are unemployed, respectively. The households who have no livestock, 21.4% women are employed and 34.7% women are employed whose family has own livestock. It is found that women who expose to media and are not expose to media, 68.1 and 67.5% women are unemployed respectively. The study also mentions that women who have not NGO membership and have NGO membership, 27.9 and 40.5% women are employed, respectively. Finally, 68.7% muslim and 59.3% non-muslim women are unemployed.

The result of Pearsonχ^2 tests displays in the Table 1 that all the independent variables except sex of household head and media exposure shows unadjusted significant ($p<0.001$) association with women participation in labor force. Then, the logistic regression model is constructed using all these significant variables.

Table 1. Value of Pearson's χ^2 statistics on cross-classifying selected predictors with women participation in labor force.

Variables	Value of Pearson's χ^2	df	p value
Age of women	118.006	3	0.000
Marital status of women	85.876	2	0.000
Educational level of women	153.45	3	0.000
Age of household head	78.491	4	0.000
Sex of household head	0.019	1	0.891
Educational level of household head	30.889	2	0.000
Educational level of husband	140.078	2	0.000
Occupation of husband	80.393	4	0.000
Household size	119.941	3	0.000
No. of children aged under 5 in household	128.891	3	0.000
Wealth index	79.913	2	0.000
Land ownership of household	21.164	2	0.000
Livestock ownership of household	143.547	1	0.000
Media exposure	0.408	1	0.523
NGO membership	190.287	1	0.000
Religion	40.702	1	0.000

Logistic regression analysis: For the study purpose, entre method of binary logistic regression analysis is performed. Using BDHS 2014 data, the logistic regression model is estimated to determine the factors affecting women participation in labor force in rural Bangladesh. The results of the fitted logistic regression model is displayed in the Table 2.

Table 2. Results of logistic regression analysis with all independent variables.

Variables	Coeffi-cient (β)	Standard error of β	Degree of freedom (df)	p value	Odd ratio	95% C.I. for odd ratio	
						Lower	Upper
Age of women							
Aged less than 35 (RC)			3	0.000	1.000		
Aged 35 - 39	0.245	0.067	1	0.000	1.277	1.120	1.457
Aged 40 - 44	0.258	0.075	1	0.001	1.295	1.118	1.499
Aged 45 - 49	0.249	0.082	1	0.003	1.283	1.091	1.507
Marital status of women							
Married (RC)			2	0.000	1.000		
Widowed	0.282	0.117	1	0.016	1.326	1.055	1.666
Divorced or separated	0.966	0.145	1	0.000	2.629	1.979	3.491
Educational level of women							
No education (RC)			3	0.000	1.000		
Primary	–0.185	0.057	1	0.001	0.831	0.743	0.930
Secondary	–0.291	0.068	1	0.000	0.748	0.655	0.854
Higher	0.146	0.113	1	0.196	1.158	0.927	1.445
Age of household head							
Aged less than 35 (RC)			4	0.000	1.000		
Aged 35 - 44	0.139	.061	1	0.023	1.149	1.019	1.296
Aged 45 - 54	–0.114	.071	1	0.110	0.892	0.776	1.026
Aged 55 - 59	–0.243	.093	1	0.009	0.784	0.654	0.940
Aged 60 and above	–0.271	.078	1	0.001	0.762	0.654	0.889
Educational level of household head							
No Education (RC)			2	0.004	1.000		
Primary	0.038	0.075	1	0.614	1.039	0.896	1.204
Secondary and higher	0.255	0.085	1	0.003	1.290	1.093	1.523
Educational level of husband							
No Education (RC)			2	0.000	1.000		
Primary	–0.110	0.077	1	0.153	0.896	0.770	1.042
Secondary and higher	–0.449	0.086	1	0.000	0.638	0.539	0.756
Occupation of husband							
Unemployed (RC)			4	0.001	1.000		
Farmer	0.426	0.129	1	0.001	1.531	1.190	1.971
Labor	0.485	0.126	1	0.000	1.623	1.268	2.079
Business or service	0.358	0.125	1	0.004	1.430	1.120	1.826
Others	–0.110	0.457	1	0.809	0.896	0.366	2.192

(Contd.)

Household size							
1 member (RC)			3	0.000	1.000		
2 members	0.321	0.339	1	0.344	1.378	0.710	2.677
3-4 members	–0.042	.331	1	0.899	0.959	0.502	1.833
5 and more members	–0.292	.331	1	0.377	0.746	0.390	1.429
No. of Children aged under 5 in household							
No child (RC)			3	0.000	1.000		
1 child	–0.106	.048	1	0.028	0.900	0.819	0.989
2 children	–0.361	.081	1	0.000	0.697	0.595	0.817
3 and more children	–0.623	.180	1	0.001	0.536	0.377	0.764
Wealth index							
Poor (RC)			2	0.079	1.000		
Middle	–0.119	.054	1	0.028	0.888	0.798	0.988
Rich	–0.085	.061	1	0.166	0.919	0.815	1.036
Land ownership of household							
No land (RC)			2	0.031	1.000		
Less than 1 Acre	0.131	.051	1	0.011	1.140	1.031	1.260
1 acres and more	0.034	.065	1	0.599	1.035	0.911	1.175
Livestock ownership of household							
No (RC)					1.000		
Yes	0.764	.060	1	0.000	2.148	1.909	2.416
NGO membership							
No (RC)					1.000		
Yes	0.457	.044	1	0.000	1.579	1.449	1.720
Religion							
Muslim (RC)					1.000		
Non-muslim	0.320	.068	1	0.000	1.378	1.205	1.575

RC = Reference category.

The result of logistic regression analysis shows that all variables are found to have significant effects on women participation in labor force. Women's age has a strong positive association with women participation in labor force. Table 2 demonstrates that women with age 35-39, 40-44 and 45-49 are 1.277, 1.295 and 1.283 times more likely to have participation in labor force, respectively as compared to the women who belongs to age less than 35. This is because the early married women (age 15- less than 35) are dependent on their husbands and in the most cases, they are fully engaged with birth and take care of children and other domestic works. The result also portrays that widowed and divorced or separated women are 1.326 and 2.629 times more likely to participate in labor force as compared to currently married women. Because of that widowed and divorced or separated women are main income earner in the family in the most of cases.

The logistic model illustrates (Table 2) that women's education is negatively significant with women participation in labor force. Increased women's education level, women participation in labor force is gradually decreased. It is observed that women with primary and secondary education are 0.831 and 0.748 times less likely to participate in labor force as compared to women who has no education. It is noted that women's higher education has no significant ($p>0.10$) effects on women participation in labor force. This can be explained by the fact that there is not enough respectable job available for educated women and some cases, educated women are less interested to participate in job for better take care of their children and family in rural Bangladesh.

The study reveals that women whose household heads belong to age 35-44 are 1.149 times more and households' head belongs to age 54-59, and age 60 and above are 0.784 and 0.762 times less likely to participate in labor force respectively as compared to the household's head who belongs to age less than 35. The household's head who belongs to age 45-54 does not have significant impact ($p>0.10$) with women participation in labor force. It is also found that the women with secondary or higher educated household's head are 1.290 times more likely to participate in labor force as compared to women with non-educated household's head. The households head with primary education has no significant ($p>0.10$) effect on women participation in labor force.

The result of logistic model portrays that women with secondary or higher educated husband are 0.638 times less likely to participate in labor force as compared to women with non-educated husband. Women with primary educated husband have no significant ($p>0.10$) association with women participation in labor force. The multivariate analysis shows that husband's occupation is strongly associated with women participation in labor force. The odd ratio indicates that women whose husband's occupation is farmer, labor and business or service are 1.531, 1.623 and 1.430 times more likely to participate in labor force. Women whose husband's occupation is others (religious leader, beggar, etc.) are not significant ($p>0.10$) with women participation in labor force.

The result illustrates that household size is negatively significant with women participation in labor force. The result of logistic model portrays that number of children under age 5 have a negative significant association with women participation in labor force. The odds ratio shows that with increased number of children under five, women participation in labor force is gradually decreased.

There is no significant difference ($p>0.10$) between poor and rich class women. The odds ratio mentions that middle class women are 0.888 times less likely to participate in labor force ($p<0.05$) as compared to poor women. It is expected that poor women participated

in labor force to increase the family income. The study reveals that land ownership of household has significant association ($p>0.05$) with women participation in labor force. It is observed that women with less than 1 acre's land ownership household are 1.140 times more likely to participate in labor force as compared to women with no landowner household. The result of logistic model displays in the Table 2 that livestock ownership of household has a strong positive significant ($p<0.001$) relationship with women participation in labor force. The odds ratio shows that women whose household has own livestock are 2.148 times more likely to participate in labor force as compared to women whose household has no livestock ownership. Women with NGO membership are 1.579 times more likely to participate in labor force as compared to women without NGO-membership. This may happen because women with NGO membership are involved in various income generating activities (IGAs) through microcredit loan and other interventions. The logistic model also portrays that non-Muslim women are 1.378 times more likely to participate in labor force as compared to Muslim women.

From the results of logistic regression analysis, it is concluded that women's age has a strong positive association with women participation in labor force. Widowed and divorced/separated women participated more times in labor force as compared to married women. It is also found that with increased education level of women and their husband, women participation in labor force is decreased, but with increased education level of household head, women participation in labor force has gradually increased. Husband's occupation is also a strong significant determinant of women participation in labor force. Women with middle economic status are less interested in employment as compared to the women of poor economic status, but rich economic status has no significant impact on women participation in labor force. The result also shows that with increased household size and number of children under five, women participation in labor force is decreased. Again, with increased land ownership of household, livestock ownership of household and NGO membership, women participation in labor force is highly increased. Proper initiative need to be taken to increase women employment status in rural Bangladesh so that they can contribute to household income and poverty reeducation of household as well as socio-economic development of society.

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